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(54) Titre : PROCEDES DE PRODUCTION DE LYMPHOCYTES INFILTRANT LES TUMEURS ET LEURS UTILISATIONS EN IMMUNOTHERAPIE

(54) Title: PROCESSES FOR PRODUCTION OF TUMOR INFILTRATING LYMPHOCYTES AND USES OF THE SAME IN IMMUNOTHERAPY

(57) Abrégé/Abstract:
The present invention provides improved and/or shortened methods for expanding TILs and producing therapeutic populations of TILs, including novel methods for expanding TIL populations in a closed system that lead to improved efficacy, improved phenotype, and increased metabolic health of the TILs in a shorter time period, while allowing for reduced microbial contamination as well as decreased costs. Such TILs find use in therapeutic treatment regimens.

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Abstract:

The present invention provides improved and/or shortened methods for expanding TILs and producing therapeutic populations of TILs, including novel methods for expanding TIL populations in a closed system that lead to improved efficacy, improved phenotype, and increased metabolic health of the TILs in a shorter time period, while allowing for reduced microbial contamination as well as decreased costs. Such TILs find use in therapeutic treatment regimens.

PROCESSES FOR PRODUCTION OF TUMOR INFILTRATING LYMPHOCYTES AND USES OF THE SAME IN IMMUNOTHERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Patent Application No. 63/019,917, filed on May 4, 2020, U.S. Provisional Patent Application No. 63/023,666, filed May 12, 2020, U.S. Provisional Patent Application No. 63/146,405, filed February 5, 2021, and U.S. Provisional Patent Application No. 63/162,441, filed March 17, 2021, the disclosures of which are hereby incorporated by reference in their entireties for all purposes.

BACKGROUND OF THE INVENTION

[0002] Treatment of bulky, refractory cancers using adoptive transfer of tumor infiltrating lymphocytes (TILs) represents a powerful approach to therapy for patients with poor prognoses. Gattinoni, *et al.*, *Nat. Rev. Immunol.* **2006,** *6,* 383-393. A large number of TILs are required for successful immunotherapy, and a robust and reliable process is needed for commercialization. This has been a challenge to achieve because of technical, logistical, and regulatory issues with cell expansion. IL-2-based TIL expansion followed by a "rapid expansion process" (REP) has become a preferred method for TIL expansion because of its speed and efficiency. Dudley, *et al.*, *Science* **2002,** *298,* 850-54; Dudley, *et al.*, *J. Clin. Oncol.* **2005,** *23,* 2346-57; Dudley, *et al.*, *J. Clin. Oncol.* **2008**, *26,* 5233-39; Riddell, *et al.*, *Science* **1992,** *257,* 238-41; Dudley, *et al.*, *J. Immunother.* **2003**, *26,* 332-42. REP can result in a 1,000-fold expansion of TILs over a 14-day period, although it requires a large excess (*e.g.*, 200-fold) of irradiated allogeneic peripheral blood mononuclear cells (PBMCs, also known as mononuclear cells (MNCs)), often from multiple donors, as feeder cells, as well as anti-CD3 antibody (OKT3) and high doses of IL-2. Dudley, *et al., J. Immunother.* **2003,** *26,* 332-42. TILs that have undergone a REP procedure have produced successful adoptive cell therapy following host immunosuppression in patients with melanoma. Current infusion acceptance parameters rely on readouts of the composition of TILs (*e.g.*, CD28, CD8, or CD4 positivity) and on fold expansion and viability of the REP product.

[0003] Current TIL manufacturing processes are limited by length, cost, sterility concerns, and other factors described herein. There is an urgent need to provide TIL manufacturing

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processes and therapies based on such processes that are characterized by improved cost-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.

BRIEF SUMMARY OF THE INVENTION

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

[0005] The present invention 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 first TIL cell culture comprising a first cell culture medium, IL-2, and either:

i) a first culture supernatant obtained from a first culture of antigen-presenting feeder cells (APCs), wherein the first culture supernatant comprises OKT-3, or

ii) APCs and OKT-3,

wherein the priming first expansion is performed by culturing the first TIL cell culture in a first container comprising a first gas-permeable surface area for a first period of about 1 to 7 or 8 days to obtain a second population of TILs, and 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 first TIL cell culture with additional first cell culture medium, IL-2, and either:

i) a second culture supernatant obtained from a second culture of APCs, wherein the second culture supernatant comprises OKT-3, or

ii) APCs and OKT-3;

to form a second TIL cell culture, wherein the rapid second expansion is performed by culturing the second TIL cell culture for a second period of about 1 to

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11 days to obtain a third population of TILs, and wherein the third population of TILs is a therapeutic population of TILs; wherein the first TIL cell culture does not comprise both the first culture supernatant and APCs; wherein the second TIL cell culture does not comprise both the second culture supernatant and supplemental APCs; and wherein either the first TIL cell culture does not comprise APCs and/or the second TIL cell culture does not comprise supplemental APCs;

(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 some embodiments of the method, in the priming first expansion of step (b) the first TIL cell culture comprises the first culture supernatant, and wherein in the rapid second expansion of step (c) the first TIL cell culture is supplemented with OKT-3 and APCs to form the second TIL cell culture.

[0007] In some embodiments of the method, in the priming first expansion of step (b) the first TIL cell culture comprises OKT-3 and APCs, and wherein in the rapid second expansion of step (c) the first TIL cell culture is supplemented with the second culture supernatant to form the second TIL cell culture.

[0008] In some embodiments of the method, in the priming first expansion of step (b) the first TIL cell culture comprises the first culture supernatant, and wherein in the rapid second expansion of step (c) the first TIL cell culture is supplemented with the second culture supernatant to form the second TIL cell culture.

[0009] In some embodiments of the method, obtaining the first culture supernatant for use in step (b) comprises:

1) providing an APC cell culture medium comprising IL-2 and OKT-3;

2) culturing at least about $5x10^8$ APCs in the APC cell culture medium from 1) for about 3-4 days to generate the first culture supernatant; and

3) collecting the first culture supernatant from the cell culture in 2).

[0010] In some embodiments of the method, obtaining the second culture supernatant for use in step (c) comprises:

1) providing an APC cell culture medium comprising IL-2 and OKT-3;

2) culturing at least about $1x10^7$ APCs in the APC cell culture medium from 1) for about 3-4 days to generate the second culture supernatant; and

3) collecting the second culture supernatant from the cell culture in 2).

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[0011] In some embodiments of the method, the rapid second expansion of step (c) further comprises the step of:

i) supplementing the second TIL cell culture with additional IL-2 about 3 or 4 days after the initiation of the second period in step (c).

[0012] In some embodiments of the method, the APCs are exogenous to the subject.

[0013] In some embodiments of the method, the APCs are peripheral blood mononuclear cells (PBMCs).

[0014] In some embodiments of the method, the rapid second expansion of step (c) further comprises the steps of:

i) on or about 3 or 4 days after the initiation of the second period, transferring the second TIL cell culture from the first container into a plurality of second containers to form a subculture of the second TIL cell culture in each of the plurality of second containers; and

ii) culturing the subculture of the second TIL cell culture in each of the plurality of second containers for the remainder of the second period.

[0015] In some embodiments of the method, in step i) equal volumes of the second TIL cell culture are transferred into the plurality of second containers.

[0016] In some embodiments of the method, each of the second containers is equal in size to the first container.

[0017] In some embodiments of the method, each of the second containers is larger than the first container.

[0018] In some embodiments of the method, the second containers are equal in size.

[0019] In some embodiments of the method, the second containers are larger than the first container.

[0020] In some embodiments of the method, the second containers are smaller than the first container.

[0021] In some embodiments of the method, the first container is a G-Rex 100 flask.

[0022] In some embodiments of the method, the first container is a G-Rex 100 flask and each of the plurality of second containers is a G-Rex 100 flask.

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[0023] In some embodiments of the method, the plurality of second containers is selected from the group consisting of: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20 second containers.

[0024] In some embodiments of the method, the plurality of second containers is 5 second containers.

[0025] In some embodiments of the method, before step ii) the method further comprises supplementing each subculture of the second TIL cell culture with additional IL-2.

[0026] In some embodiments of the method, before step ii) the method further comprises supplementing each subculture of the second TIL cell culture with a second cell culture medium and IL-2.

[0027] In some embodiments of the method, the first cell culture medium and the second cell culture medium are the same.

[0028] In some embodiments of the method, the first cell culture medium and the second cell culture medium are different.

[0029] In some embodiments of the method, the first cell culture medium is DM1 and the second cell culture medium is DM2.

[0030] The present invention 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 either antigen presenting cells (APCs) and/or culture supernatant from a first culture of APCs comprising OKT-3 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, optionally OKT-3, and either

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antigen presenting cells (APCs) and/or culture supernatant from a second culture of APCs comprising OKT-3, 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.

[0031] The present invention 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 either antigen presenting cells (APCs) and/or culture supernatant from a first culture of APCs comprising OKT-3, 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, optionally OKT-3, and either antigen presenting cells (APCs) and/or culture supernatant from a second culture of APCs comprising OKT-3, 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).

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[0032] In some embodiments, "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 embodiments, '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").

[0033] In some embodiments 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).

[0034] The present invention 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 either antigen presenting cells (APCs) and/or culture supernatant from a first culture of APCs comprising OKT-3 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, optionally OKT-3, and either antigen presenting cells (APCs) and/or culture supernatant from a second culture of APCs comprising OKT-3, 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

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container comprising a second gas-permeable surface area; and

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

[0035] The present invention also 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 either antigen presenting cells (APCs) and/or culture supernatant from a first culture of APCs comprising OKT-3, 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, optionally OKT-3, and either antigen presenting cells (APCs) and/or culture supernatant from a second culture of APCs comprising OKT-3, 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).

[0036] In some embodiments 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).

[0037] In some embodiments, 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.

[0038] In some embodiments, 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.

[0039] In some embodiments, 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.

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[0040] In some embodiments, 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.

[0041] In some embodiments, 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.

[0042] In some embodiments, the number of APCs in the priming first expansion is selected from the range of about 1.0×10^6 APCs/cm^2 to about 4.5×10^6 APCs/cm^2, and wherein the number of APCs in the rapid second expansion is selected from the range of about 2.5×10^6 APCs/cm^2 to about 7.5×10^6 APCs/cm^2.

[0043] In some embodiments, the number of APCs in the priming first expansion is selected from the range of about 1.5×10^6 APCs/cm^2 to about 3.5×10^6 APCs/cm^2, and wherein the number of APCs in the rapid second expansion is selected from the range of about 3.5×10^6 APCs/cm^2 to about 6.0×10^6 APCs/cm^2.

[0044] In some embodiments, the number of APCs in the priming first expansion is selected from the range of about 2.0×10^6 APCs/cm^2 to about 3.0×10^6 APCs/cm^2, and wherein the number of APCs in the rapid second expansion is selected from the range of about 4.0×10^6 APCs/cm^2 to about 5.5×10^6 APCs/cm^2.

[0045] In some embodiments, the number of APCs in the priming first expansion is selected from the range of about 1×10^8 APCs to about 3.5×10^8 APCs, and wherein the number of APCs in the rapid second expansion is selected from the range of about 3.5×10^8 APCs to about 1×10^9 APCs.

[0046] In some embodiments, the number of APCs in the priming first expansion is selected from the range of about 1.5×10^8 APCs to about 3×10^8 APCs, and wherein the number of APCs in the rapid second expansion is selected from the range of about 4×10^8 APCs to about 7.5×10^8 APCs.

[0047] In some embodiments, the number of APCs in the priming first expansion is selected from the range of about 2×10^8 APCs to about 2.5×10^8 APCs, and wherein the number of APCs in the rapid second expansion is selected from the range of about 4.5×10^8 APCs to about 5.5×10^8 APCs.

[0048] In some embodiments, about 2.5×10^8 APCs are added to the priming first expansion and 5×10^8 APCs are added to the rapid second expansion.

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[0049] In some embodiments, 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.

[0050] In some embodiments, 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.

[0051] In some embodiments, 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.

[0052] In some embodiments, 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.

[0053] In some embodiments, 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.

[0054] In some embodiments, the second population of TILs is at least 50-fold greater in number than the first population of TILs.

[0055] In some embodiments, 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.

[0056] In some embodiments, 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.

[0057] In some embodiments, the plurality of separate containers comprises at least two separate containers.

[0058] In some embodiments, the plurality of separate containers comprises from two to twenty separate containers.

[0059] In some embodiments, the plurality of separate containers comprises from two to ten separate containers.

[0060] In some embodiments, the plurality of separate containers comprises from two to five separate containers.

[0061] In some embodiments, each of the separate containers comprises a first gas-permeable surface area.

[0062] In some embodiments, the multiple tumor fragments are distributed in a single container.

[0063] In some embodiments, the single container comprises a first gas-permeable surface area.

[0064] In some embodiments, 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.

[0065] In some embodiments, 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.

[0066] In some embodiments, 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.

[0067] In some embodiments, 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.

[0068] In some embodiments, 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.

[0069] In some embodiments, the rapid second expansion the APCs are layered onto the first gas-permeable surface area at a thickness of about 4 cell layers.

[0070] In some embodiments, 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.

[0071] In some embodiments, the second container is larger than the first container.

[0072] In some embodiments, the step of the priming first expansion the cell culture medium comprises antigen-presenting cells (APCs) and the APCs are layered onto the first

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gas-permeable surface area at an average thickness of about one cell layer to about three cell layers.

[0073] In some embodiments, 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.

[0074] In some embodiments, 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.

[0075] In some embodiments, 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.

[0076] In some embodiments, 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.

[0077] In some embodiments, 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.

[0078] In some embodiments, 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.

[0079] In some embodiments, each container comprises a first gas-permeable surface area.

[0080] In some embodiments, 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.

[0081] In some embodiments, 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.

[0082] In some embodiments, 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.

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[0083] In some embodiments, 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.

[0084] In some embodiments, 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.

[0085] In some embodiments, 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.

[0086] In some embodiments, 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.

[0087] In some embodiments, 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.

[0088] In some embodiments, 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.

[0089] In some embodiments, 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.

[0090] In some embodiments, 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.

[0091] In some embodiments, 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.

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[0092] In some embodiments, 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.

[0093] In some embodiments, 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.

[0094] In some embodiments, 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.

[0095] In some embodiments, 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.

[0096] In some embodiments, after 2 to 3 days in the step of the rapid second expansion, the cell culture medium is supplemented with additional IL-2.

[0097] In some embodiments, the method further comprises cryopreserving the harvested TIL population in the step of harvesting the therapeutic population of TILs using a cryopreservation process.

[0098] In some embodiments, the method further comprises the step of cryopreserving the infusion bag.

[0099] In some embodiments, the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.

[00100] In some embodiments, the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).

[00101] In some embodiments, the PBMCs are irradiated and allogeneic.

[00102] In some embodiments, 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 2.5×10^8.

[00103] In some embodiments, the step of the rapid second expansion the antigen-presenting cells (APCs) in the cell culture medium are peripheral blood mononuclear cells (PBMCs),

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

[00104] In some embodiments, the e antigen-presenting cells are artificial antigen-presenting cells.

[00105] In some embodiments, the harvesting in the step of harvesting the therapeutic population of TILs is performed using a membrane-based cell processing system.

[00106] In some embodiments, the harvesting in step harvesting the therapeutic population of TILs is performed using a LOVO cell processing system.

[00107] In some embodiments, 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.

[00108] In some embodiments, the multiple fragments comprise about 30 to about 60 fragments with a total volume of about 1300 mm^3 to about 1500 mm^3.

[00109] In some embodiments, the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3.

[00110] In some embodiments, the multiple fragments comprise about 50 fragments with a total mass of about 1 gram to about 1.5 grams.

[00111] In some embodiments, the cell culture medium is provided in a container selected from the group consisting of a G-container and a Xuri cellbag.

[00112] In some embodiments, the IL-2 concentration is about 10,000 IU/mL to about 5,000 IU/mL.

[00113] In some embodiments, the IL-2 concentration is about 6,000 IU/mL.

[00114] In some embodiments, the infusion bag in the step of transferring the harvested therapeutic population of TILs to an infusion bag is a HypoThermosol-containing infusion bag.

[00115] In some embodiments, the cryopreservation media comprises dimethlysulfoxide (DMSO).

[00116] In some embodiments, the cryopreservation media comprises 7% to 10% DMSO.

[00117] In some embodiments, 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.

[00118] In some embodiments, the first period in the step of the priming first expansion is performed within a period of 5 days, 6 days, or 7 days.

[00119] In some embodiments, the second period in the step of the rapid second expansion is performed within a period of 7 days, 8 days, or 9 days.

[00120] In some embodiments, 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.

[00121] In some embodiments, 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.

[00122] In some embodiments, 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.

[00123] In some embodiments, 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.

[00124] In some embodiments, 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.

[00125] In some embodiments, the steps the priming first expansion through the harvesting of the therapeutic population of TILs are performed within a period of about 16 days.

[00126] In some embodiments, 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.

[00127] In some embodiments, 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.

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[00128] In some embodiments, the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10}.

[00129] In some embodiments, 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.

[00130] In some embodiments, 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.

[00131] In some embodiments, 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.

[00132] In some embodiments, the therapeutic population of TILs from the step of the harvesting of the therapeutic population of TILs are infused into a patient.

[00133] Ther present invention 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 either antigen presenting cells (APCs) and/or culture supernatant from a first culture of APCs comprising OKT-3 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 either antigen presenting cells (APCs) and/or culture supernatant from a second culture of APCs comprising OKT-3, 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

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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.

[00134] In some embodiments, the number of TILs sufficient for administering a therapeutically effective dosage in step (f) is from about 2.3×10^{10} to about 13.7×10^{10}.

[00135] In some embodiments, the antigen presenting cells (APCs) are PBMCs.

[00136] In some embodiments, prior to administering a therapeutically effective dosage of TIL cells in step (f), a non-myeloablative lymphodepletion regimen has been administered to the patient.

[00137] In some embodiments, 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.

[00138] In some embodiments, 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).

[00139] In some embodiments, 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.

[00140] In some embodiments, the e third population of TILs in step (b) provides for increased efficacy, increased interferon-gamma production, and/or increased polyclonality.

[00141] In some embodiments, 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.

[00142] In some embodiments, 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

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relative to effector T cells and/or central memory T cells obtained from the second population of cells in step (b).

[00143] In some embodiments, the cancer is a solid tumor.

[00144] In some embodiments, 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, triple negative 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.

[00145] In some embodiments, the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

[00146] In some embodiments, the cancer is melanoma.

[00147] In some embodiments, the cancer is HNSCC.

[00148] In some embodiments, the cancer is a cervical cancer.

[00149] In some embodiments, the cancer is NSCLC.

[00150] In some embodiments, the cancer is glioblastoma (including GBM).

[00151] In some embodiments, the cancer is gastrointestinal cancer.

[00152] In some embodiments, the cancer is a hypermutated cancer.

[00153] In some embodiments, the the cancer is a pediatric hypermutated cancer.

[00154] In some embodiments, the container is a closed container.

[00155] In some embodiments, the container is a G-container.

[00156] In some embodiments, the container is a GREX-10.

[00157] In some embodiments, the closed container comprises a GREX-100.

[00158] In some embodiments, the closed container comprises a GREX-500.

[00159] The presenting invention also provides a therapeutic population of tumor infiltrating lymphocytes (TILs) made by the method as disclosed herein.

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[00160] The presenting invention 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.

[00161] In some embodiments, the therapeutic population of TILs as disclosed herein provide for increased interferon-gamma production.

[00162] In some embodiments, the therapeutic population of TILs as disclosed herein provide for increased polyclonality.

[00163] In some embodiments, the therapeutic population of TILs as disclosed herein provide for increased efficacy.

[00164] In some embodiments, 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.

[00165] In some embodiments, 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.

[00166] In some embodiments, 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.

[00167] In some embodiments, the the present invention 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).

[00168] In some embodiments, 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.

[00169] In some embodiments, 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.

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[00170] In some embodiments, the the present invention 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.

[00171] In some embodiments, 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.

[00172] In some embodiments, 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.

[00173] In some embodiments, the the present invention 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.

[00174] In some embodiments, 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.

[00175] In some embodiments, 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.

[00176] The present invention also provides a tumor infiltrating lymphocyte (TIL) composition comprising the therapeutic population of TILs as described herein and a pharmaceutically acceptable carrier.

[00177] The present invention also provides a sterile infusion bag comprising the TIL composition as described herein.

[00178] The present invention also provides a cryopreserved preparation of the therapeutic population of TILs as described herein.

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[00179] The present invention also provides a tumor infiltrating lymphocyte (TIL) composition comprising the therapeutic population of TILs as described herein and a cryopreservation media.

[00180] In some embodiments, the the cryopreservation media contains DMSO.

[00181] In some embodiments, the cryopreservation media contains 7-10% DMSO.

[00182] The present invention also provides a cryopreserved preparation of the TIL composition as described herein.

[00183] In some embodiments, the tumor infiltrating lymphocyte (TIL) composition as described herein is for use as a medicament.

[00184] In some embodiments, the tumor infiltrating lymphocyte (TIL) composition as described herein is for use in the treatment of a cancer.

[00185] In some embodiments, the tumor infiltrating lymphocyte (TIL) composition as described herein is for use in the treatment of a solid tumor cancer.

[00186] In some embodiments, 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, triple negative 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.

[00187] In some embodiments, 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.

[00188] In some embodiments, the TIL composition as described herein is for use in treatment of a cancer wherein cancer is melanoma.

[00189] In some embodiments, the TIL composition as described herein is for use in treatment of a cancer wherein cancer is HNSCC.

[00190] In some embodiments, the TIL composition as described herein is for use in treatment of a cancer wherein a cervical cancer.

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[00191] In some embodiments, the TIL composition as described herein is for use in treatment of a cancer wherein the cancer is NSCLC.

[00192] In some embodiments, the TIL composition as described herein is for use in treatment of a cancer wherein the cancer is glioblastoma (including GBM).

[00193] In some embodiments, the TIL composition as described herein is for use in treatment of a cancer wherein the cancer is gastrointestinal cancer.

[00194] In some embodiments, the TIL composition as described herein is for use in treatment of a cancer wherein the cancer is a hypermutated cancer.

[00195] In some embodiments, the TIL composition as described herein is for use in treatment of a cancer wherein the cancer is a pediatric hypermutated cancer.

[00196] In some embodiments, the present incention 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 embodiments, the cancer is a solid tumor. In some embodiments, 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, triple negative 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 embodiments, the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer. In some embodiments, the cancer is melanoma. In some embodiments, the cancer is HNSCC. In some embodiments, the cancer is a cervical cancer. In some embodiments, the cancer is NSCLC. In some embodiments, the cancer is glioblastoma (including GBM). In some embodiments, the cancer is gastrointestinal cancer. In some embodiments, the cancer is a hypermutated cancer. In some embodiments, the cancer is a pediatric hypermutated cancer.

[00197] In some embodiments, 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 embodiments, the cancer is a solid tumor. In some embodiments, 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, triple negative breast cancer,

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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 embodiments, the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

[00198] The present invention 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.

[00199] In some embodiments, the cancer is a solid tumor.

[00200] In some embodiments, 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, triple negative 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.

[00201] In some embodiments, the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer. In some embodiments, the cancer is melanoma. In some embodiments, the cancer is HNSCC. In some embodiments, the cancer is a cervical cancer. In some embodiments, the cancer is NSCLC. In some embodiments, the cancer is glioblastoma (including GBM). In some embodiments, the cancer is gastrointestinal cancer. In some embodiments, the cancer is a hypermutated cancer. In some embodiments, the cancer is a pediatric hypermutated cancer.

[00202] The present invention 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.

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[00203] In some embodiments, the priming first expansion of step (a) is performed during a period of up to 7 days.

[00204] In some embodiments, the rapid second expansion of step (b) is performed during a period of up to 11 days.

[00205] In some embodiments, the rapid second expansion of step (b) is performed during a period of up to 9 days.

[00206] In some embodiments, 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.

[00207] In some embodiments, the priming first expansion of step (a) is performed during a period of up to 8 days.

[00208] In some embodiments, the rapid second expansion of step (b) is performed during a period of up to 8 days.

[00209] In some embodiments, 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.

[00210] In some embodiments 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.

[00211] In some embodiments, the first culture medium comprises OKT-3, IL-2 and antigen-presenting cells (APCs).

[00212] In some embodiments 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).

[00213] In some embodiments 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) or culture supernatant from a first culture of APCs comprising OKT-3, 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

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and a second population of APCs or culture supernatant from a second culture of APCs comprising OKT-3, 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.

[00214] In some embodiments, 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.

[00215] In some embodiments, the number of APCs in the first population of APCs is about 2.5×10^8 and the number of APCs in the second population of APCs is about 5×10^8.

[00216] In some embodiments 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.

[00217] In some embodiments 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.

[00218] In some embodiments, 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.

[00219] In some embodiments, the APCs are peripheral blood mononuclear cells (PBMCs).

[00220] In some embodiments, the APCs comprise PBMCs, wherein the PBMCs are irradiated and exogenous to the donor of the first population of T cells.

[00221] In some embodiments, the T cells are tumor infiltrating lymphocytes (TILs).

[00222] In some embodiments, the T cells are marrow infiltrating lymphocytes (MILs).

[00223] In some embodiments, the T cells are peripheral blood lymphocytes (PBLs).

[00224] In some embodiments, the cell culture medium is a defined medium and/or a serum free medium.

[00225] In some embodiments, the defined medium comprises (optionally recombinant) transferrin, (optionally recombinant) insulin, and (optionally recombinant) albumin.

[00226] In some embodiments, the serum-free or defined medium comprises a basal cell medium and a serum supplement and/or a serum replacement.

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[00227] In some embodiments, 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.

[00228] In some embodiments, 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.

[00229] In some embodiments, the cell culture medium comprises one or more albumins or albumin substitutes.

[00230] In some embodiments, the cell culture medium comprises one or more amino acids.

[00231] In some embodiments, the cell culture medium comprises one or more vitamins, one or more transferrins or transferrin substitutes.

[00232] In some embodiments, the cell culture medium comprises one or more antioxidants, one or more insulins or insulin substitutes.

[00233] In some embodiments, the cell culture medium comprises one or more collagen precursors, one or more antibiotics, and one or more trace elements. In

[00234] In some embodiments, the cell culture medium comprises albumin.

[00235] In some embodiments, 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+}.

[00236] In some embodiments, the cell culture medium further comprises L-glutamine, sodium bicarbonate and/or 2-mercaptoethanol.

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[00237] In some embodiments, 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.

[00238] In some embodiments, 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.

[00239] In some embodiments, 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.

[00240] In some embodiments, the cell culture medium further comprises glutamine (*i.e.*, GlutaMAX®) at a concentration of about 2mM.

[00241] In some embodiments, 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.

[00242] In some embodiments, the cell culture medium further comprises 2-mercaptoethanol at a concentration of about 55mM.

[00243] In some embodiments, the cell culture medium comprises the defined media described in International PCT Publication No. WO/1998/030679.

[00244] In some embodiments, 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

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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.

[00245] In some embodiments, 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 4 provided herein.

[00246] In some embodiments, the osmolarity of the cell culture medium is between about 260 and 350 mOsmol.

[00247] In some embodiments, the cell culture medium further comprises about 3.7 g/L, or about 2.2 g/L sodium bicarbonate.

[00248] In some embodiments, 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).

[00249] In some embodiments, 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).

[00250] In some embodiments, the cell culture medium comprises CTS OpTmizer T-Cell Expansion SFM, 3% CTS Immune Cell Serum Replacement, 55mM BME, and optionally glutamine.

[00251] In some embodiments, 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.

[00252] In some embodiments, 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.

[00253] In some embodiments, the tumor sample is one or more small biopsies, core biopsies, or needle biopsies of the tumor in the subject.

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[00254] The present invention also provides 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 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.

[00255] The present invention also provides 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 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

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(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).

[00256] In some embodiments, after day 5 of the second period the culture is split into 2 or more subcultures, and each subculture is supplemented with an additional quantity of the third culture medium and cultured for about 6 days.

[00257] In some embodiments, after day 5 of the second period the culture is split into up to 5 subcultures.

[00258] In some embodiments, all steps in the method are completed in about 22 days.

[00259] The present invention also provides a method of expanding T cells comprising:

(i) performing a priming first expansion of a first population of T cells from a tumor sample 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.

[00260] In some embodiments, the tumor sample is obtained from a plurality of core biopsies.

[00261] In some embodiments, the plurality of core biopsies is selected from the group consisting of 2, 3, 4, 5, 6, 7, 8, 9 and 10 core biopsies.

[00262] The present invention 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.

[00263] In some embodiments, the defined medium or serum free medium comprises (optionally recombinant) transferrin, (optionally recombinant) insulin, and (optionally recombinant) albumin.

[00264] In some embodiments, the defined medium or serum free medium comprises a basal cell medium and a serum supplement and/or a serum replacement.

[00265] In some embodiments, 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.

[00266] In some embodiments, 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.

[00267] In some embodiments, the defined medium or serum free medium comprises one or more albumins or albumin substitutes.

[00268] In some embodiments, the defined medium or serum free medium comprises one or more amino acids.

[00269] In some embodiments, the defined medium or serum free medium comprises one or more vitamins, one or more transferrins or transferrin substitutes.

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[00270] In some embodiments, the defined medium or serum free medium comprises one or more antioxidants, one or more insulins or insulin substitutes.

[00271] In some embodiments, the defined medium or serum free medium comprises one or more collagen precursors, one or more antibiotics, and one or more trace elements.

[00272] In some embodiments, the defined medium or serum free medium comprises albumin.

[00273] In some embodiments, 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 $Zr^{4'}$.

[00274] In some embodiments, the defined medium or serum free medium further comprises L-glutamine, sodium bicarbonate and/or 2-mercaptoethanol.

[00275] In some embodiments, 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.

[00276] In some embodiments, 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.

[00277] In some embodiments, 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.

[00278] In some embodiments, the defined medium or serum free medium further comprises glutamine (*i.e.*, GlutaMAX®) at a concentration of about 2mM.

[00279] In some embodiments, 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

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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.

[00280] In some embodiments, the defined medium or serum free medium further comprises 2-mercaptoethanol at a concentration of about 55mM.

[00281] In some embodiments, the defined medium or serum free medium comprises the defined media described in International PCT Publication No. WO/1998/030679.

[00282] In some embodiments, 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.

[00283] In some embodiments, 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 4 provided herein.

[00284] In some embodiments, the osmolarity of the defined medium or serum free medium is between about 260 and 350 mOsmol.

[00285] In some embodiments, the defined medium or serum free medium further comprises about 3.7 g/L, or about 2.2 g/L sodium bicarbonate.

[00286] In some embodiments, 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).

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[00287] In some embodiments, 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).

[00288] In some embodiments, the cell culture medium comprises CTS OpTmizer T-Cell Expansion SFM, 3% CTS Immune Cell Serum Replacement, 55mM BME, and optionally glutamine.

[00289] In some embodiments, 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.

[00290] In some embodiments, 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.

[00291] In some embodiments, the population of TILs is a therapeutic population of TILs.

[00292] In some embodiments, 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.

[00293] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

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

obtaining a first population of TILs from a tumor resected from a patient by processing a tumor sample obtained from the patient into a tumor digest;

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

(c) performing a first expansion or a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, antigen presenting cells

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(APCs), and optionally OKT-3, to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 1 to 5 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 4 to 8 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

(f) harvesting the second plurality of subpopulations of TILs obtained from step (e), wherein the transition from step (e) to step (f) occurs without opening the system when optionally performed in a closed system; and

(g) transferring the harvested subpopulations of TILs from step (g) to one or more infusion bags, wherein the transition from step (f) to (g) occurs without opening the system when optionally performed in a closed system.

[00294] In some embodiments, the invention provides a tumor infiltrating lymphocyte (TIL) composition comprising a therapeutic population of infiltrating lymphocytes (TILs), wherein the TIL composition is produced by a method comprising:

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

obtaining a first population of TILs from a tumor resected from a patient by processing a tumor sample obtained from the patient into a tumor digest;

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

(c) performing a first expansion or a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, antigen presenting cells (APCs), and optionally OKT-3, to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 1 to 5 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 4 to 8 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

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(f) harvesting the second plurality of subpopulations of TILs obtained from step (e), wherein the transition from step (e) to step (f) occurs without opening the system when optionally performed in a closed system; and

(g) transferring the harvested subpopulations of TILs from step (g) to one or more infusion bags, wherein the transition from step (f) to (g) occurs without opening the system when optionally performed in a closed system.

[00295] In some embodiments, the TIL composition is a cryopreserved composition and wherein the method further comprises (h) cryopreserving the infusion bag comprising the harvested TIL population from step (g) using a cryopreservation process

[00296] In some embodiments, the invention provides a method for treating a subject with cancer, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

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

obtaining a first population of TILs from a tumor resected from a patient by processing a tumor sample obtained from the patient into a tumor digest;

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

(c) performing a first expansion or a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, antigen presenting cells (APCs), and optionally OKT-3, to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about to about 1 to 5 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing

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a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 4 to 8 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

(f) harvesting the second plurality of subpopulations of TILs obtained from step (e), wherein the transition from step (e) to step (f) occurs without opening the system when optionally performed in a closed system;

(g) transferring the harvested subpopulations of TILs from step (g) to one or more infusion bags, wherein the transition from step (f) to (g) occurs without opening the system when optionally performed in a closed system; and

(h) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (g) to the subject.

[00297] In some embodiments, the first expansion or the priming first expansion is performed for about 6-8 days.

[00298] In some embodiments, the rapid second expansionis performed for about 2-4 days.

[00299] In some embodiments, the third expansion is each performed for about 5-7 days.

[00300] In some embodiments, the first expansion or the priming first expansion is performed for about 7 days, the rapid second expansion is performed for about 3 days, and the third expansion is performed for about 6 days.

[00301] In some embodiments, steps (c)-(e) are performed in about 14-18 days.

[00302] In some embodiments, steps (c)-(e) are performed in about 16 days.

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[00303] In some embodiments, steps (c)-(e) are performed in about 18 days or less.

[00304] In some embodiments, steps (c)-(e) are performed in about 16 days or less.

[00305] In some embodiments, step (e) comprises seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container providing a third gas-permeable surface area at a seeding density of about $2x10^6$ cells/cm^2.

[00306] In some embodiments, 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, triple negative 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.

[00307] In some embodiments, the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

[00308] In some embodiments, the cancer is melanoma.

[00309] In some embodiments, the cancer is HNSCC.

[00310] In some embodiments, the cancer is a cervical cancer.

[00311] In some embodiments, the cancer is NSCLC.

[00312] In some embodiments, the cancer is glioblastoma (including GBM).

[00313] In some embodiments, the cancer is gastrointestinal cancer.

[00314] In some embodiments, the cancer is a hypermutated cancer.

[00315] In some embodiments, the cancer is a pediatric hypermutated cancer.

[00316] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

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

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

(c) performing a first expansion or a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, antigen presenting cells (APCs), and optionally OKT-3, to produce a second population of TILs, wherein the first expansion or the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

(f) harvesting the second plurality of subpopulations of TILs obtained from step (f), wherein the transition from step (e) to step (f) occurs without opening the system when optionally performed in a closed system; and

(g) transferring the harvested subpopulations of TILs from step (f) to one or more infusion bags, wherein the transition from step (f) to (g) occurs without opening the system when optionally performed in a closed system.

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[00317] In some embodiments, the invention provides a tumor infiltrating lymphocyte (TIL) composition comprising a therapeutic population of infiltrating lymphocytes (TILs), wherein the TIL composition is produced by a method comprising:

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

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

(c) performing a first expansion or a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, antigen presenting cells (APCs), and optionally OKT-3, to produce a second population of TILs, wherein the first expansion or the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (d)

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to step (e) occurs without opening the system when optionally performed in a closed system;

(f) harvesting the second plurality of subpopulations of TILs obtained from step (f), wherein the transition from step (e) to step (f) occurs without opening the system when optionally performed in a closed system; and

(g) transferring the harvested subpopulations of TILs from step (f) to one or more infusion bags, wherein the transition from step (f) to (g) occurs without opening the system when optionally performed in a closed system.

[00318] In some embodiments, the TIL composition is a cryopreserved composition and wherein the method further comprises (h) cryopreserving the infusion bag comprising the harvested TIL population from step (g) using a cryopreservation process

[00319] In some embodiments, the invention provides a method for treating a subject with cancer, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

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

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

(c) performing a first expansion or a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, antigen presenting cells (APCs), and optionally OKT-3, to produce a second population of TILs, wherein the first expansion or the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is

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performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

(f) harvesting the second plurality of subpopulations of TILs obtained from step (f), wherein the transition from step (e) to step (f) occurs without opening the system when optionally performed in a closed system;

(g) transferring the harvested subpopulations of TILs from step (f) to one or more infusion bags, wherein the transition from step (f) to (g) occurs without opening the system when optionally performed in a closed system; and

(h) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (g) to the subject.

[00320] In some embodiments, in step (a) the tumor sample obtained from the patient is processed into multiple tumor fragments by (i) cryopreserving the tumor sample to produce a cryopreserved tumor sample; (ii) thawing the cryopreserved tumor sample to produce a thawed tumor sample; and (iii) fragmenting the thawed tumor sample into multiple tumor fragments.

[00321] In some embodiments, in step (a) the tumor sample obtained from the patient is processed into a tumor digest by (i) cryopreserving the tumor sample to produce a cryopreserved tumor sample; (ii) thawing the cryopreserved tumor sample to produce a thawed tumor sample; and (iii) digesting the thawed tumor sample to produce a tumor digest.

[00322] In some embodiments, in step (a) the tumor sample obtained from the patient is processed into a tumor digest by (i) cryopreserving the tumor sample to produce a

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cryopreserved tumor sample; (ii) thawing the cryopreserved tumor sample to produce a thawed tumor sample; (iii) fragmenting the thawed tumor sample into multiple tumor fragments; and (iv) digesting the multiple tumor fragments to produce a tumor digest.

[00323] In some embodiments, step (e) comprises seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container providing a third gas-permeable surface area at a seeding density of about $2x10^6$ cells/cm^2.

[00324] In some embodiments, 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, triple negative 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.

[00325] In some embodiments, the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

[00326] In some embodiments, the cancer is melanoma.

[00327] In some embodiments, the cancer is HNSCC.

[00328] In some embodiments, the cancer is a cervical cancer.

[00329] In some embodiments, the cancer is NSCLC.

[00330] In some embodiments, the cancer is glioblastoma (including GBM).

[00331] In some embodiments, the cancer is gastrointestinal cancer.

[00332] In some embodiments, the cancer is a hypermutated cancer.

[00333] In some embodiments, the cancer is a pediatric hypermutated cancer.

[00334] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

(a) performing a first expansion or a priming first expansion by (i) thawing a cryopreserved tumor digest comprising a first population of TILs from a tumor that was resected from a subject, digested after the resection, and cryopreserved after the digestion, and (ii) culturing the first population of TILs in a cell culture medium comprising IL-2, antigen presenting cells (APCs), and optionally OKT-3, to produce

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a second population of TILs, wherein the first expansion or the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the the first expansion or priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, APCs, and optionally OKT-3, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) harvesting the second plurality of subpopulations of TILs obtained from step (c), wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system; and

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system.

[00335] In some embodiments, the invention provides a tumor infiltrating lymphocyte (TIL) composition comprising a therapeutic population of infiltrating lymphocytes (TILs), wherein the TIL composition is produced by a method comprising:

(a) performing a first expansion or a priming first expansion by (i) thawing a cryopreserved tumor comprising a first population of TILs from a tumor that was resected from a subject and cryopreserved after the resection, and (ii) culturing the

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first population of TILs in a cell culture medium comprising IL-2, antigen presenting cells (APCs), and optionally OKT-3, to produce a second population of TILs, wherein the first expansion or priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, APCs, optionally OKT-3, to produce a third population of TILs, wherein the second expansion is performed forabout 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) harvesting the second plurality of subpopulations of TILs obtained from step (c), wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system; and

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system.

[00336] In some embodiments, the TIL composition is a cryopreserved composition and wherein the method further comprises (f) cryopreserving the infusion bag comprising the harvested TIL population from step (e) using a cryopreservation process.

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[00337] In some embodiments, the invention provides for a method for treating a subject with cancer, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) performing a first expansion or priming first expansion by (i) thawing a cryopreserved tumor digest comprising a first population of TILs from a tumor that was resected from a subject, digested after the resection, and cryopreserved after the digestion, and (ii) culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the first expansion or priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) harvesting the second plurality of subpopulations of TILs obtained from step (c), wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

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(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system;

(f) administering a therapeutically effective dosage of the harvested population of TILs from the infusion bag in step (e) to the subject.

[00338] In some embodiments, step (a)(i) comprises thawing a cryopreserved tumor comprising a first population of TILs from a tumor that was resected from a subject and cryopreserved after the resection to produce a thawed tumor, and fragmenting the thawed tumor into multiple tumor fragments, and wherein step (a)(ii) comprises culturing the multiple tumor fragments comprising the first population of TILs.

[00339] In some embodiments, step (c) comprises seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container providing a third gas-permeable surface area at a seeding density of about $2x10^6$ cells/cm^2.

[00340] In some embodiments, the first expansion or priming first expansion is performed for about 6 to 8 days.

[00341] In some embodiments, the rapid second expansion is performed for about 6 to 8 days.

[00342] In some embodiments, the third expansion is performed for about 6 to 8 days.

[00343] In some embodiments, the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 18 to 24 days.

[00344] In some embodiments, the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 20 to 22 days.

[00345] In some embodiments, the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 21 days.

[00346] In some embodiments, the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 24 days or less.

[00347] In some embodiments, the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 22 days or less.

[00348] In some embodiments, the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 21 days or less.

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[00349] In some embodiments, 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, triple negative 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.

[00350] In some embodiments, the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

[00351] In some embodiments, the cancer is melanoma.

[00352] In some embodiments, the cancer is HNSCC.

[00353] In some embodiments, the cancer is a cervical cancer.

[00354] In some embodiments, the cancer is NSCLC.

[00355] In some embodiments, the cancer is glioblastoma (including GBM).

[00356] In some embodiments, the cancer is gastrointestinal cancer.

[00357] In some embodiments, the cancer is a hypermutated cancer.

[00358] In some embodiments, the cancer is a pediatric hypermutated cancer.

[00359] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

(a) performing a first expansion or a priming first expansion by culturing a tumor sample comprising a first population of TILs from a tumor resected from a patient in a cell culture medium comprising IL-2, antigen presenting cells (APCs), and optionally OKT-3, to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about to about 1 to 5 days to obtain the third population of TILs,

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wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 4 to 8 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) harvesting the second plurality of subpopulations of TILs obtained from step (c), wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system; and

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system.

[00360] In some embodiments, the invention provides a tumor infiltrating lymphocyte (TIL) composition comprising a therapeutic population of infiltrating lymphocytes (TILs), wherein the TIL composition is produced by a method comprising:

(a) performing a first expansion or a priming first expansion by culturing a tumor sample comprising a first population of TILs from a tumor resected from a patient in a cell culture medium comprising IL-2, antigen presenting cells (APCs), and optionally OKT-3, to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 1 to 5 days to obtain the third population of TILs, wherein the

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second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 4 to 8 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) harvesting the second plurality of subpopulations of TILs obtained from step (c), wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system; and

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system.

[00361] In some embodiments, the TIL composition is a cryopreserved composition and wherein the method further comprises (f) cryopreserving the infusion bag comprising the harvested TIL population from step (e) using a cryopreservation process.

[00362] In some embodiments, the invention provides a method for treating a subject with cancer, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) performing a first expansion or a priming first expansion by culturing a tumor sample comprising a first population of TILs from a tumor resected from a patient in a cell culture medium comprising IL-2, antigen presenting cells (APCs), and optionally OKT-3, to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

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(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 1 to 5 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 4 to 8 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) harvesting the second plurality of subpopulations of TILs obtained from step (c), wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system; and

(f) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (e) to the subject.

[00363] In some embodiments, before culturing in step (a) the tumor sample is fragmenting into multiple tumor fragments comprising the first population of TILs.

[00364] In some embodiments, before culturing in step (a) the tumor sample is digested to produce a tumor digest comprising the first population of TILs.

[00365] In some embodiments, the first expansion or priming first expansion is performed for about 6-8 days.

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[00366] In some embodiments, the rapid second expansionis performed for about 2-4 days.

[00367] In some embodiments, the third expansion is each performed for about 5-7 days.

[00368] In some embodiments, the first expansion or priming first expansion is performed for about 7 days, the rapid second expansion is performed for about 3 days, and the third expansion is performed for about 6 days.

[00369] In some embodiments, steps (a)-(c) are performed in about 14-18 days.

[00370] In some embodiments, steps (a)-(c) are performed in about 16 days.

[00371] In some embodiments, steps (a)-(c) are performed in about 18 days or less.

[00372] In some embodiments, steps (a)-(c) are performed in about 16 days or less.

[00373] In some embodiments, step (c) comprises seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container providing a third gas-permeable surface area at a seeding density of about $2x10^6$ cells/cm^2.

[00374] In some embodiments, 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, triple negative 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.

[00375] In some embodiments, the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

[00376] In some embodiments, the cancer is melanoma.

[00377] In some embodiments, the cancer is HNSCC.

[00378] In some embodiments, the cancer is a cervical cancer.

[00379] In some embodiments, the cancer is NSCLC.

[00380] In some embodiments, the cancer is glioblastoma (including GBM).

[00381] In some embodiments, the cancer is gastrointestinal cancer.

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[00382] In some embodiments, the cancer is a hypermutated cancer.

[00383] In some embodiments, the cancer is a pediatric hypermutated cancer.

[00384] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

(a) performing a first expansion or a priming first expansion by culturing a tumor sample comprising a first population of TILs from a tumor resected from a patient in a cell culture medium comprising IL-2, antigen presenting cells (APCs), and optionally OKT-3, to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, APCs, and optionally OKT-3, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) harvesting the second plurality of subpopulations of TILs obtained from step (f), wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system; and

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(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system.

[00385] In some embodiments, the invention provides a tumor infiltrating lymphocyte (TIL) composition comprising a therapeutic population of infiltrating lymphocytes (TILs), wherein the TIL composition is produced by a method comprising:

(a) performing a first expansion or a priming first expansion by culturing a tumor sample comprising a first population of TILs from a tumor resected from a patient in a cell culture medium comprising IL-2, antigen presenting cells (APCs), and optionally OKT-3, to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, APCs, and optionally OKT-3, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) harvesting the second plurality of subpopulations of TILs obtained from step (f), wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system; and

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(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system.

[00386] In some embodiments, the TIL composition is a cryopreserved composition and wherein the method further comprises (f) cryopreserving the infusion bag comprising the harvested TIL population from step (e) using a cryopreservation process.

[00387] In some embodiments, the invention provides a method for treating a subject with cancer, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) performing a first expansion or a priming first expansion by culturing a tumor sample comprising a first population of TILs from a tumor resected from a patient in a cell culture medium comprising IL-2, and antigen presenting cells (APCs), and optionally OKT-3, to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, APCs, and optionally OKT-3, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

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(d) harvesting the second plurality of subpopulations of TILs obtained from step (f), wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system; and

(f) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (e) to the subject.

[00388] In some embodiments, before culturing in step (a) the tumor sample is fragmenting into multiple tumor fragments comprising the first population of TILs.

[00389] In some embodiments, before culturing in step (a) the tumor sample is digested to produce a tumor digest comprising the first population of TILs.

[00390] In some embodiments, the first expansion or priming first expansion is performed for about 6 to 8 days.

[00391] In some embodiments, the rapid second expansion is performed for about 6 to 8 days.

[00392] In some embodiments, the third expansion is performed for about 6 to 8 days.

[00393] In some embodiments, the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 18 to 24 days.

[00394] In some embodiments, the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 20 to 22 days.

[00395] In some embodiments, the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 21 days.

[00396] In some embodiments, the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 24 days or less.

[00397] In some embodiments, the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 22 days or less.

[00398] In some embodiments, the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 21 days or less.

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[00399] In some embodiments, step (c) comprises seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container providing a third gas-permeable surface area at a seeding density of about $2x10^6$ cells/cm^2.

[00400] In some embodiments, 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, triple negative 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.

[00401] In some embodiments, the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

[00402] In some embodiments, the cancer is melanoma.

[00403] In some embodiments, the cancer is HNSCC.

[00404] In some embodiments, the cancer is a cervical cancer.

[00405] In some embodiments, the cancer is NSCLC.

[00406] In some embodiments, the cancer is glioblastoma (including GBM).

[00407] In some embodiments, the cancer is gastrointestinal cancer.

[00408] In some embodiments, the cancer is a hypermutated cancer.

[00409] In some embodiments, the cancer is a pediatric hypermutated cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[00410] **Figure 1A-1G: 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 18-days process). **B)** Exemplary Process Gen3 chart providing an overview of Steps A through F (approximately 14-days to 18-days process). **C)** Chart providing three exemplary Gen 3 processes with an overview of Steps A through F (approximately 14-days to 18-days process) for each of the three process variations. **D)** Exemplary Modified Gen 2-like process providing an overview of Steps A through F (approximately 22-days process). **E)**

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Exemplary Second Generation Process Gen3 chart providing an overview of Steps A through F (approximately 14-days to 18-days process). **F)** Exemplary Second Generation Process Gen3 chart providing an overview of Steps A through F (approximately 14-days to 18-days process). **G)** Exemplary Second Generation Process Gen3 chart providing an overview of Steps A through F (approximately 14-days to 18-days process).

[00411] Figure 2: Provides an experimental flow chart for comparability between Gen 2 (process 2A) versus Gen 3.

[00412] Figure 3: Shows a comparison between various Gen 2 (2A process) and the Gen 3.1 process embodiment.

[00413] Figure 4: Table describing various features of embodiments of the Gen 2, Gen 2.1 and Gen 3.0 process.

[00414] Figure 5: Overview of the media conditions for an embodiment of the Gen 3 process, referred to as Gen 3.1.

[00415] Figure 6: Schematic of an exemplary embodiment of the Gen 3 process (a 16-day process).

[00416] Figure 7: Schematic of an exemplary embodiment for expanding TILs from hematopoietic malignancies using the Gen 3 process. At Day 0, a T cell fraction (CD3+, CD45+) is isolated from an apheresis product enriched for lymphocytes, whole blood, or tumor digest (fresh or thawed) using positve or negative selection methods, *i.e.*, removing the T-cells using a T-cell marker (CD2, CD3, etc., or removing other cells leaving T-cells), or gradient centrifugation.

[00417] Figure 8: Schematic of an exemplary embodiment of the Gen 3 process (a 16-day process).

[00418] Figure 9: Schematic of an exemplary embodiment of the Gen 3.1 Test (Gen 3.1 optimized) process (a 16-17 day process).

[00419] Figure 10A-10B: Schematics of exemplary embodiment of the Gen 3 process (a 16-day process).

[00420] Figure 11: Schematic of an exemplary embodiment of the Gen 3 process (a 16/17 day process) preparation timeline.

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[00421] Figure 12: Schematic of an exemplary embodiment of the Gen 3 process (a 14-16 day process).

[00422] Figure 13A-13B: Schematic of an exemplary embodiment of the Gen 3 process (a 16 day process).

[00423] Figure 14: Comparison of Gen 2, Gen 2.1 and an embodiment of the Gen 3 process (a 16 day process).

[00424] Figure 15: Gen 3 embodiment flow chart comparison (Gen 3.0, Gen 3.1 control, Gen 3.1 Test).

[00425] Figure 16: Shown are the components of an exemplary embodiment of the Gen 3 process (Gen 3-Optimized, a 16-17 day process).

[00426] Figure 17: Provides the structures I-A and I-B, the cylinders refer to individual polypeptide binding domains. Structures I-A and I-B comprise three linearly-linked TNFRSF binding domains derived from *e.g.*, 4-1BBL or an antibody that binds 4-1BB, which fold to form a trivalent protein, which is then linked to a second trivalent protein through IgG1-Fc (including CH3 and CH2 domains) is then used to link two of the trivalent proteins together through disulfide bonds (small elongated ovals), stabilizing the structure and providing an agonists capable of bringing together the intracellular signaling domains of the six receptors and signaling proteins to form a signaling complex. The TNFRSF binding domains denoted as cylinders may be scFv domains comprising, *e.g.*, a VH and a VL chain connected by a linker that may comprise hydrophilic residues and Gly and Ser sequences for flexibility, as well as Glu and Lys for solubility.

[00427] Figure 18: Overview of Gen 2 and Gen 3 processes using biopsy samples.

[00428] Figure 19: Exemplary embodiment of Gen 3 processes.

[00429] Figure 20: Exemplary embodiment of current Gen 3 process.

[00430] Figure 21: Feeder proposal conditions in exemplary Gen 3 and three exemplary Second Generation Gen 3 processes.

[00431] Figure 22: Exemplary embodiments of Gen 2 and Gen 3 processes using various starting materials.

[00432] Figure 23: %CD3+CD45+ core versus resection samples by processes as exemplified in Figure 18.

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[00433] Figure 24: IFNγ data from core versus resection samples by processes as exemplified in Figure 18.

[00434] Figure 25: Summary of total viable cells and product attributes by processes as exemplified in Figure 18.

[00435] Figure 26: Extended phenotype characteristics related to purity, identity, and memory by processes as exemplified in Figure 18. Note: < 3% of B cells or Monocytes or NK cells were detected.

[00436] Figure 27: Phenotypic comparison of processes as exemplified in Figure 18

[00437] Figure 28A-28B: Extended phenotype characteristics related to differentiation, activation, and exhaustion by processes as exemplified in Figure 18.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

[00438] SEQ ID NO:1 is the amino acid sequence of the heavy chain of muromonab.

[00439] SEQ ID NO:2 is the amino acid sequence of the light chain of muromonab.

[00440] SEQ ID NO:3 is the amino acid sequence of a recombinant human IL-2 protein.

[00441] SEQ ID NO:4 is the amino acid sequence of aldesleukin.

[00442] SEQ ID NO:5 is the amino acid sequence of a recombinant human IL-4 protein.

[00443] SEQ ID NO:6 is the amino acid sequence of a recombinant human IL-7 protein.

[00444] SEQ ID NO:7 is the amino acid sequence of a recombinant human IL-15 protein.

[00445] SEQ ID NO:8 is the amino acid sequence of a recombinant human IL-21 protein.

[00446] SEQ ID NO:9 is the amino acid sequence of human 4-1BB.

[00447] SEQ ID NO:10 is the amino acid sequence of murine 4-1BB.

[00448] SEQ ID NO:11 is the heavy chain for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00449] SEQ ID NO:12 is the light chain for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

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[00450] SEQ ID NO:13 is the heavy chain variable region (VH) for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00451] SEQ ID NO:14 is the light chain variable region (VL) for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00452] SEQ ID NO:15 is the heavy chain CDRl for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00453] SEQ ID NO:16 is the heavy chain CDR2 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00454] SEQ ID NO:17 is the heavy chain CDR3 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00455] SEQ ID NO:18 is the light chain CDR1 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00456] SEQ ID NO:19 is the light chain CDR2 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00457] SEQ ID NO:20 is the light chain CDR3 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00458] SEQ ID NO:21 is the heavy chain for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00459] SEQ ID NO:22 is the light chain for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00460] SEQ ID NO:23 is the heavy chain variable region (V_H) for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00461] SEQ ID NO:24 is the light chain variable region (V_L) for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00462] SEQ ID NO:25 is the heavy chain CDR1 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00463] SEQ ID NO:26 is the heavy chain CDR2 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

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[00464] SEQ ID NO:27 is the heavy chain CDR3 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00465] SEQ ID NO:28 is the light chain CDR1 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00466] SEQ ID NO:29 is the light chain CDR2 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00467] SEQ ID NO:30 is the light chain CDR3 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00468] SEQ ID NO:31 is an Fc domain for a TNFRSF agonist fusion protein.

[00469] SEQ ID NO:32 is a linker for a TNFRSF agonist fusion protein.

[00470] SEQ ID NO:33 is a linker for a TNFRSF agonist fusion protein.

[00471] SEQ ID NO:34 is a linker for a TNFRSF agonist fusion protein.

[00472] SEQ ID NO:35 is a linker for a TNFRSF agonist fusion protein.

[00473] SEQ ID NO:36 is a linker for a TNFRSF agonist fusion protein.

[00474] SEQ ID NO:37 is a linker for a TNFRSF agonist fusion protein.

[00475] SEQ ID NO:38 is a linker for a TNFRSF agonist fusion protein.

[00476] SEQ ID NO:39 is a linker for a TNFRSF agonist fusion protein.

[00477] SEQ ID NO:40 is a linker for a TNFRSF agonist fusion protein.

[00478] SEQ ID NO:41 is a linker for a TNFRSF agonist fusion protein.

[00479] SEQ ID NO:42 is an Fc domain for a TNFRSF agonist fusion protein.

[00480] SEQ ID NO:43 is a linker for a TNFRSF agonist fusion protein.

[00481] SEQ ID NO:44 is a linker for a TNFRSF agonist fusion protein.

[00482] SEQ ID NO:45 is a linker for a TNFRSF agonist fusion protein.

[00483] SEQ ID NO:46 is a 4-1BB ligand (4-1BBL) amino acid sequence.

[00484] SEQ ID NO:47 is a soluble portion of 4-1BBL polypeptide.

[00485] SEQ ID NO:48 is a heavy chain variable region (V_H) for the 4-1BB agonist antibody 4B4-1-1 version 1.

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[00486] SEQ ID NO:49 is a light chain variable region (V_L) for the 4-1BB agonist antibody 4B4-1-1 version 1.

[00487] SEQ ID NO:50 is a heavy chain variable region (V_H) for the 4-1BB agonist antibody 4B4-1-1 version 2.

[00488] SEQ ID NO:51 is a light chain variable region (V_L) for the 4-1BB agonist antibody 4B4-1-1 version 2.

[00489] SEQ ID NO:52 is a heavy chain variable region (V_H) for the 4-1BB agonist antibody H39E3-2.

[00490] SEQ ID NO:53 is a light chain variable region (V_L) for the 4-1BB agonist antibody H39E3-2.

[00491] SEQ ID NO:54 is the amino acid sequence of human OX40.

[00492] SEQ ID NO:55 is the amino acid sequence of murine OX40.

[00493] SEQ ID NO:56 is the heavy chain for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00494] SEQ ID NO:57 is the light chain for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00495] SEQ ID NO:58 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00496] SEQ ID NO:59 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00497] SEQ ID NO:60 is the heavy chain CDRl for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00498] SEQ ID NO:61 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00499] SEQ ID NO:62 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00500] SEQ ID NO:63 is the light chain CDR1 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

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[00501] SEQ ID NO:64 is the light chain CDR2 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00502] SEQ ID NO:65 is the light chain CDR3 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00503] SEQ ID NO:66 is the heavy chain for the OX40 agonist monoclonal antibody 11D4.

[00504] SEQ ID NO:67 is the light chain for the OX40 agonist monoclonal antibody 11D4.

[00505] SEQ ID NO:68 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 11D4.

[00506] SEQ ID NO:69 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 11D4.

[00507] SEQ ID NO:70 is the heavy chain CDRl for the OX40 agonist monoclonal antibody 11D4.

[00508] SEQ ID NO:71 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody 11D4.

[00509] SEQ ID NO:72 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody 11D4.

[00510] SEQ ID NO:73 is the light chain CDR1 for the OX40 agonist monoclonal antibody 11D4.

[00511] SEQ ID NO:74 is the light chain CDR2 for the OX40 agonist monoclonal antibody 11D4.

[00512] SEQ ID NO:75 is the light chain CDR3 for the OX40 agonist monoclonal antibody 11D4.

[00513] SEQ ID NO:76 is the heavy chain for the OX40 agonist monoclonal antibody 18D8.

[00514] SEQ ID NO:77 is the light chain for the OX40 agonist monoclonal antibody 18D8.

[00515] SEQ ID NO:78 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 18D8.

[00516] SEQ ID NO:79 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 18D8.

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[00517] SEQ ID NO:80 is the heavy chain CDRl for the OX40 agonist monoclonal antibody 18D8.

[00518] SEQ ID NO:81 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody 18D8.

[00519] SEQ ID NO:82 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody 18D8.

[00520] SEQ ID NO:83 is the light chain CDR1 for the OX40 agonist monoclonal antibody 18D8.

[00521] SEQ ID NO:84 is the light chain CDR2 for the OX40 agonist monoclonal antibody 18D8.

[00522] SEQ ID NO:85 is the light chain CDR3 for the OX40 agonist monoclonal antibody 18D8.

[00523] SEQ ID NO:86 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody Hu119-122.

[00524] SEQ ID NO:87 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody Hu119-122.

[00525] SEQ ID NO:88 is the heavy chain CDRl for the OX40 agonist monoclonal antibody Hu119-122.

[00526] SEQ ID NO:89 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody Hu119-122.

[00527] SEQ ID NO:90 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody Hu119-122.

[00528] SEQ ID NO:91 is the light chain CDR1 for the OX40 agonist monoclonal antibody Hu119-122.

[00529] SEQ ID NO:92 is the light chain CDR2 for the OX40 agonist monoclonal antibody Hu119-122.

[00530] SEQ ID NO:93 is the light chain CDR3 for the OX40 agonist monoclonal antibody Hu119-122.

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[00531] SEQ ID NO:94 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody Hu106-222.

[00532] SEQ ID NO:95 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody Hu106-222.

[00533] SEQ ID NO:96 is the heavy chain CDRl for the OX40 agonist monoclonal antibody Hu106-222.

[00534] SEQ ID NO:97 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody Hu106-222.

[00535] SEQ ID NO:98 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody Hu106-222.

[00536] SEQ ID NO:99 is the light chain CDR1 for the OX40 agonist monoclonal antibody Hu106-222.

[00537] SEQ ID NO:100 is the light chain CDR2 for the OX40 agonist monoclonal antibody Hu106-222.

[00538] SEQ ID NO:101 is the light chain CDR3 for the OX40 agonist monoclonal antibody Hu106-222.

[00539] SEQ ID NO:102 is an OX40 ligand (OX40L) amino acid sequence.

[00540] SEQ ID NO:103 is a soluble portion of OX40L polypeptide.

[00541] SEQ ID NO:104 is an alternative soluble portion of OX40L polypeptide.

[00542] SEQ ID NO:105 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 008.

[00543] SEQ ID NO:106 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 008.

[00544] SEQ ID NO:107 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 011.

[00545] SEQ ID NO:108 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 011.

[00546] SEQ ID NO:109 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 021.

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[00547] SEQ ID NO:110 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 021.

[00548] SEQ ID NO:111 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 023.

[00549] SEQ ID NO:112 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 023.

[00550] SEQ ID NO:113 is the heavy chain variable region (V_H) for an OX40 agonist monoclonal antibody.

[00551] SEQ ID NO:114 is the light chain variable region (V_L) for an OX40 agonist monoclonal antibody.

[00552] SEQ ID NO:115 is the heavy chain variable region (V_H) for an OX40 agonist monoclonal antibody.

[00553] SEQ ID NO:116 is the light chain variable region (V_L) for an OX40 agonist monoclonal antibody.

[00554] SEQ ID NO:117 is the heavy chain variable region (V_H) for a humanized OX40 agonist monoclonal antibody.

[00555] SEQ ID NO:118 is the heavy chain variable region (V_H) for a humanized OX40 agonist monoclonal antibody.

[00556] SEQ ID NO:119 is the light chain variable region (V_L) for a humanized OX40 agonist monoclonal antibody.

[00557] SEQ ID NO:120 is the light chain variable region (V_L) for a humanized OX40 agonist monoclonal antibody.

[00558] SEQ ID NO:121 is the heavy chain variable region (V_H) for a humanized OX40 agonist monoclonal antibody.

[00559] SEQ ID NO:122 is the heavy chain variable region (V_H) for a humanized OX40 agonist monoclonal antibody.

[00560] SEQ ID NO:123 is the light chain variable region (V_L) for a humanized OX40 agonist monoclonal antibody.

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[00561] SEQ ID NO:124 is the light chain variable region (V_L) for a humanized OX40 agonist monoclonal antibody.

[00562] SEQ ID NO:125 is the heavy chain variable region (V_H) for an OX40 agonist monoclonal antibody.

[00563] SEQ ID NO:126 is the light chain variable region (V_L) for an OX40 agonist monoclonal antibody.

[00564] SEQ ID NO:127-462 are currently not assigned.

[00565] SEQ ID NO:463 is the heavy chain amino acid sequence of the PD-1 inhibitor nivolumab.

[00566] SEQ ID NO:464 is the light chain amino acid sequence of the PD-1 inhibitor nivolumab.

[00567] SEQ ID NO:465 is the heavy chain variable region (V_H) amino acid sequence of the PD-1 inhibitor nivolumab.

[00568] SEQ ID NO:466 is the light chain variable region (V_L) amino acid sequence of the PD-1 inhibitor nivolumab.

[00569] SEQ ID NO:467 is the heavy chain CDR1 amino acid sequence of the PD-1 inhibitor nivolumab.

[00570] SEQ ID NO:468 is the heavy chain CDR2 amino acid sequence of the PD-1 inhibitor nivolumab.

[00571] SEQ ID NO:469 is the heavy chain CDR3 amino acid sequence of the PD-1 inhibitor nivolumab.

[00572] SEQ ID NO:470 is the light chain CDR1 amino acid sequence of the PD-1 inhibitor nivolumab.

[00573] SEQ ID NO:471 is the light chain CDR2 amino acid sequence of the PD-1 inhibitor nivolumab.

[00574] SEQ ID NO:472 is the light chain CDR3 amino acid sequence of the PD-1 inhibitor nivolumab.

[00575] SEQ ID NO:473 is the heavy chain amino acid sequence of the PD-1 inhibitor pembrolizumab.

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[00576] SEQ ID NO:474 is the light chain amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00577] SEQ ID NO:475 is the heavy chain variable region (V_H) amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00578] SEQ ID NO:476 is the light chain variable region (V_L) amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00579] SEQ ID NO:477 is the heavy chain CDR1 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00580] SEQ ID NO:478 is the heavy chain CDR2 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00581] SEQ ID NO:479 is the heavy chain CDR3 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00582] SEQ ID NO:480 is the light chain CDR1 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00583] SEQ ID NO:481 is the light chain CDR2 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00584] SEQ ID NO:482 is the light chain CDR3 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00585] SEQ ID NO:483 is the heavy chain amino acid sequence of the PD-L1 inhibitor durvalumab.

[00586] SEQ ID NO:484 is the light chain amino acid sequence of the PD-L1 inhibitor durvalumab.

[00587] SEQ ID NO:485 is the heavy chain variable region (V_H) amino acid sequence of the PD-L1 inhibitor durvalumab.

[00588] SEQ ID NO:486 is the light chain variable region (V_L) amino acid sequence of the PD-L1 inhibitor durvalumab.

[00589] SEQ ID NO:487 is the heavy chain CDR1 amino acid sequence of the PD-L1 inhibitor durvalumab.

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[00590] SEQ ID NO:488 is the heavy chain CDR2 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00591] SEQ ID NO:489 is the heavy chain CDR3 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00592] SEQ ID NO:490 is the light chain CDR1 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00593] SEQ ID NO:491 is the light chain CDR2 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00594] SEQ ID NO:492 is the light chain CDR3 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00595] SEQ ID NO:493 is the heavy chain amino acid sequence of the PD-L1 inhibitor avelumab.

[00596] SEQ ID NO:494 is the light chain amino acid sequence of the PD-L1 inhibitor avelumab.

[00597] SEQ ID NO:495 is the heavy chain variable region (V_H) amino acid sequence of the PD-L1 inhibitor avelumab.

[00598] SEQ ID NO:496 is the light chain variable region (V_L) amino acid sequence of the PD-L1 inhibitor avelumab.

[00599] SEQ ID NO:497 is the heavy chain CDR1 amino acid sequence of the PD-L1 inhibitor avelumab.

[00600] SEQ ID NO:498 is the heavy chain CDR2 amino acid sequence of the PD-L1 inhibitor avelumab.

[00601] SEQ ID NO:499 is the heavy chain CDR3 amino acid sequence of the PD-L1 inhibitor avelumab.

[00602] SEQ ID NO:500 is the light chain CDR1 amino acid sequence of the PD-L1 inhibitor avelumab.

[00603] SEQ ID NO:501 is the light chain CDR2 amino acid sequence of the PD-L1 inhibitor avelumab.

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[00604] SEQ ID NO:502 is the light chain CDR3 amino acid sequence of the PD-L1 inhibitor avelumab.

[00605] SEQ ID NO:503 is the heavy chain amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00606] SEQ ID NO:504 is the light chain amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00607] SEQ ID NO:505 is the heavy chain variable region (V_H) amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00608] SEQ ID NO:506 is the light chain variable region (V_L) amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00609] SEQ ID NO:507 is the heavy chain CDR1 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00610] SEQ ID NO:508 is the heavy chain CDR2 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00611] SEQ ID NO:509 is the heavy chain CDR3 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00612] SEQ ID NO:510 is the light chain CDR1 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00613] SEQ ID NO:511 is the light chain CDR2 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00614] SEQ ID NO:512 is the light chain CDR3 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00615] SEQ ID NO:513 is the heavy chain amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00616] SEQ ID NO:514 is the light chain amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00617] SEQ ID NO:515 is the heavy chain variable region (V_H) amino acid sequence of the CTLA-4 inhibitor ipilimumab.

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[00618] SEQ ID NO:516 is the light chain variable region (V_L) amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00619] SEQ ID NO:517 is the heavy chain CDR1 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00620] SEQ ID NO:518 is the heavy chain CDR2 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00621] SEQ ID NO:519 is the heavy chain CDR3 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00622] SEQ ID NO:520 is the light chain CDR1 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00623] SEQ ID NO:521 is the light chain CDR2 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00624] SEQ ID NO:522 is the light chain CDR3 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00625] SEQ ID NO:523 is the heavy chain amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00626] SEQ ID NO:524 is the light chain amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00627] SEQ ID NO:525 is the heavy chain variable region (V_H) amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00628] SEQ ID NO:526 is the light chain variable region (V_L) amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00629] SEQ ID NO:527 is the heavy chain CDR1 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00630] SEQ ID NO:528 is the heavy chain CDR2 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00631] SEQ ID NO:529 is the heavy chain CDR3 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

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[00632] SEQ ID NO:530 is the light chain CDR1 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00633] SEQ ID NO:531 is the light chain CDR2 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00634] SEQ ID NO:532 is the light chain CDR3 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00635] SEQ ID NO:533 is the heavy chain amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00636] SEQ ID NO:534 is the light chain amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00637] SEQ ID NO:535 is the heavy chain variable region (V_H) amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00638] SEQ ID NO:536 is the light chain variable region (V_L) amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00639] SEQ ID NO:537 is the heavy chain CDR1 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00640] SEQ ID NO:538 is the heavy chain CDR2 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00641] SEQ ID NO:539 is the heavy chain CDR3 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00642] SEQ ID NO:540 is the light chain CDR1 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00643] SEQ ID NO:541 is the light chain CDR2 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00644] SEQ ID NO:542 is the light chain CDR3 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00645] SEQ ID NO:543 is the IL-2 sequence.

[00646] SEQ ID NO:544 is an IL-2 mutein sequence.

[00647] SEQ ID NO:545 is an IL-2 mutein sequence.

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[00648] SEQ ID NO:546 is the HCDR1_IL-2 for IgG.IL2R67A.H1.

[00649] SEQ ID NO:547 is the HCDR2 for IgG.IL2R67A.H1.

[00650] SEQ ID NO:548 is the HCDR3 for IgG.IL2R67A.H1.

[00651] SEQ ID NO:549 is the HCDR1_IL-2 kabat for IgG.IL2R67A.H1.

[00652] SEQ ID NO:550 is the HCDR2 kabat for IgG.IL2R67A.H1.

[00653] SEQ ID NO:551 is the HCDR3 kabat for IgG.IL2R67A.H1.

[00654] SEQ ID NO:552 is the HCDR1_IL-2 clothia for IgG.IL2R67A.H1.

[00655] SEQ ID NO:553 is the HCDR2 clothia for IgG.IL2R67A.H1.

[00656] SEQ ID NO:554 is the HCDR3 clothia for IgG.IL2R67A.H1.

[00657] SEQ ID NO:555 is the HCDR1_IL-2 IMGT for IgG.IL2R67A.H1.

[00658] SEQ ID NO:556 is the HCDR2 IMGT for IgG.IL2R67A.H1.

[00659] SEQ ID NO:557 is the IICDR3 IMGT for IgG.IL2R67A.II1.

[00660] SEQ ID NO:558 is the VH chain for IgG.IL2R67A.H1.

[00661] SEQ ID NO:559 is the heavy chain for IgG.IL2R67A.H1.

[00662] SEQ ID NO:560 is the LCDR1 kabat for IgG.IL2R67A.H1.

[00663] SEQ ID NO:561 is the LCDR2 kabat for IgG.IL2R67A.H1.

[00664] SEQ ID NO:562 is the LCDR3 kabat for IgG.IL2R67A.H1.

[00665] SEQ ID NO:563 is the LCDR1 chothia for IgG.IL2R67A.H1.

[00666] SEQ ID NO:564 is the LCDR2 chothia for IgG.IL2R67A.H1.

[00667] SEQ ID NO:565 is the LCDR3 chothia for IgG.IL2R67A.H1.

[00668] SEQ ID NO:566 is the VL chain.

[00669] SEQ ID NO:567 is the light chain.

[00670] SEQ ID NO:568 is the light chain.

[00671] SEQ ID NO:569 is the light chain.

[00672] SEQ ID NO: 570 is an IL-2 form.

[00673] SEQ ID NO: 571 is an IL-2 form.

[00674] SEQ ID NO: 572 is an IL-2 form.

[00675] SEQ ID NO: 573 is a mucin domain polypeptide.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

[00676] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All patents and publications referred to herein are incorporated by reference in their entireties.

[00677] The term "*in vivo*" refers to an event that takes place in a subject's body.

[00678] The term "*in vitro*" refers to an event that takes places outside of a subject's body. In vitro assays encompass cell-based assays in which cells alive or dead are employed and may also encompass a cell-free assay in which no intact cells are employed.

[00679] The term "*ex vivo*" refers to an event which involves treating or performing a procedure on a cell, tissue and/or organ which has been removed from a subject's body. Aptly, the cell, tissue and/or organ may be returned to the subject's body in a method of surgery or treatment.

[00680] The term "rapid expansion" means an increase in the number of antigen-specific TILs of at least about 3-fold (or 4-, 5-, 6-, 7-, 8-, or 9-fold) over a period of a week, more preferably at least about 10-fold (or 20-, 30-, 40-, 50-, 60-, 70-, 80-, or 90-fold) over a period of a week, or most preferably at least about 100-fold over a period of a week. A number of rapid expansion protocols are outlined below.

[00681] By "tumor infiltrating lymphocytes" or "TILs" herein is meant a population of cells originally obtained as white blood cells that have left the bloodstream of a subject and migrated into a tumor. TILs include, but are not limited to, $CD8^+$ cytotoxic T cells (lymphocytes), Th1 and Th17 $CD4^+$ T cells, natural killer cells, dendritic cells and M1 macrophages. TILs include both primary and secondary TILs. "Primary TILs" are those that are obtained from patient tissue samples as outlined herein (sometimes referred to as "freshly obtained" or "freshly isolated"), and "secondary TILs" are any TIL cell populations that have been expanded or proliferated as discussed herein, including, but not limited to bulk TILs and

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expanded TILs ("REP TILs" or "post-REP TILs"). TIL cell populations can include genetically modified TILs.

[00682] By "population of cells" (including TILs) herein is meant a number of cells that share common traits. In general, populations generally range from 1×10^6 to 1×10^{10} in number, with different TIL populations comprising different numbers. For example, initial growth of primary TILs in the presence of IL-2 results in a population of bulk TILs of roughly 1×10^8 cells. REP expansion is generally done to provide populations of 1.5×10^9 to 1.5×10^{10} cells for infusion. In some embodiemtns, REP expansion is done to provide populations of $2.3 \times 10^{10} - 13.7 \times 10^{10}$.

[00683] By "cryopreserved TILs" herein is meant that TILs, either primary, bulk, or expanded (REP TILs), are treated and stored in the range of about -150°C to -60°C. General methods for cryopreservation are also described elsewhere herein, including in the Examples. For clarity, "cryopreserved TILs" are distinguishable from frozen tissue samples which may be used as a source of primary TILs.

[00684] By "thawed cryopreserved TILs" herein is meant a population of TILs that was previously cryopreserved and then treated to return to room temperature or higher, including but not limited to cell culture temperatures or temperatures wherein TILs may be administered to a patient.

[00685] TILs can generally be defined either biochemically, using cell surface markers, or functionally, by their ability to infiltrate tumors and effect treatment. TILs can be generally categorized by expressing one or more of the following biomarkers: CD4, CD8, TCR αβ, CD27, CD28, CD56, CCR7, CD45Ra, CD95, PD-1, and CD25. Additionally, and alternatively, TILs can be functionally defined by their ability to infiltrate solid tumors upon reintroduction into a patient. TILs may further be characterized by potency – for example, TILs may be considered potent if, for example, interferon (IFN) release is greater than about 50 pg/mL, greater than about 100 pg/mL, greater than about 150 pg/mL, or greater than about 200 pg/mL. TILs may be considered potent if, for example, interferon (IFNγ) release is greater than about 50 pg/mL, greater than about 100 pg/mL, greater than about 150 pg/mL, or greater than about 200 pg/mL, greater than about 300 pg/mL, greater than about 400 pg/mL, greater than about 500 pg/mL, greater than about 600 pg/mL, greater than about 700 pg/mL, greater than about 800 pg/mL, greater than about 900 pg/mL, greater than about 1000 pg/mL.

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[00686] The term "cryopreservation media" or "cryopreservation medium" refers to any medium that can be used for cryopreservation of cells. Such media can include media comprising 7% to 10% DMSO. Exemplary media include CryoStor CS10, Hyperthermasol, as well as combinations thereof. The term "CS10" refers to a cryopreservation medium which is obtained from Stemcell Technologies or from Biolife Solutions. The CS10 medium may be referred to by the trade name "CryoStor® CS10". The CS10 medium is a serum-free, animal component-free medium which comprises DMSO.

[00687] The term "central memory T cell" refers to a subset of T cells that in the human are CD45R0+ and constitutively express CCR7 ($CCR7^{hi}$) and CD62L ($CD62^{hi}$). The surface phenotype of central memory T cells also includes TCR, CD3, CD127 (IL-7R), and IL-15R. Transcription factors for central memory T cells include BCL-6, BCL-6B, MBD2, and BMI1. Central memory T cells primarily secret IL-2 and CD40L as effector molecules after TCR triggering. Central memory T cells are predominant in the CD4 compartment in blood, and in the human are proportionally enriched in lymph nodes and tonsils.

[00688] The term "effector memory T cell" refers to a subset of human or mammalian T cells that, like central memory T cells, are CD45R0+, but have lost the constitutive expression of CCR7 ($CCR7^{lo}$) and are heterogeneous or low for CD62L expression ($CD62L^{lo}$). The surface phenotype of central memory T cells also includes TCR, CD3, CD127 (IL-7R), and IL-15R. Transcription factors for central memory T cells include BLIMP1. Effector memory T cells rapidly secret high levels of inflammatory cytokines following antigenic stimulation, including interferon-γ, IL-4, and IL-5. Effector memory T cells are predominant in the CD8 compartment in blood, and in the human are proportionally enriched in the lung, liver, and gut. CD8+ effector memory T cells carry large amounts of perforin.

[00689] The term "closed system" refers to a system that is closed to the outside environment. Any closed system appropriate for cell culture methods can be employed with the methods of the present invention. Closed systems include, for example, but are not limited to closed G-containers. Once a tumor segment is added to the closed system, the system is not opened to the outside environment until the TILs are ready to be administered to the patient.

[00690] The terms "fragmenting," "fragment," and "fragmented," as used herein to describe processes for disrupting a tumor, includes mechanical fragmentation methods such as

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crushing, slicing, dividing, and morcellating tumor tissue as well as any other method for disrupting the physical structure of tumor tissue.

[00691] The term "fine needle aspirate" or FNA refers to a type of biopsy procedure that can be employed for sampling or diagnostic procedures, including tumor sampling, in which a sample is taken but the tumor is not removed or resected. In fine needle aspiration, a hollow needle, for example 25-18 gauge, is inserted into the tumor or into an area containing the tumor and fluid and cells (including tissue) are obtained for further analysis or expansion, as described herein. With an FNA, the cells are removed without preserving the histological architecture of the tissue cells. An FNA can comprise TILs. In some instances, a fine needle aspiration biopsy is performed using an ultrasound-guided fine needle aspiration biopsy needle. FNA needles are commercially available from Becton Dickinson, Covidien, and the like.

[00692] The term "core biopsy" or "core needle biopsy" refers to a type of biopsy procedure that can be employed for sampling or diagnostic procedures, including tumor sampling, in which a sample is taken but the tumor is not removed or resected. In a core biopsy, a hollow needle, for example 16-11 gauge, is inserted into the tumor or into an area containing the tumor and fluid and cells (including tissue) are obtained for further analysis or expansion, as described herein. With a core biopsy, the cells can be removed with some preservation of the histological architecture of the tissue cells, given the larger needle size as compared to a FNA. The core biopsy needle is generally of a gauge size that is able to preserve at least some portion of the histological architecture of the tumor. A core biopsy can comprise TILs. In some instances, a core needle biopsy is performed using a biopsy instrument, a vacuum-assisted core-needle biopsy instrument, a steretactically guided core-needle biopsy instrument, an ultrasound-guided core-needle biopsy instrument, an MRI-guided core-needle biopsy instrument commercially available from Bard Medical, Becton Dickinson, and the like.

[00693] The terms "peripheral blood mononuclear cells" and "PBMCs" refers to a peripheral blood cell having a round nucleus, including lymphocytes (T cells, B cells, NK cells) and monocytes. When used as antigen-presenting cells (PBMCs are a type of antigen-presenting cell), the peripheral blood mononuclear cells are irradiated allogeneic peripheral blood mononuclear cells.

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[00694] The terms "peripheral blood lymphocytes" and "PBLs" refer to T cells expanded from peripheral blood. In some embodiments, PBLs are separated from whole blood or apheresis product from a donor. In some embodiments, PBLs are separated from whole blood or apheresis product from a donor by positive or negative selection of a T cell phenotype, such as the T cell phenotype of CD3+ CD45+.

[00695] The term "anti-CD3 antibody" refers to an antibody or variant thereof, *e.g.*, a monoclonal antibody and including human, humanized, chimeric or murine antibodies which are directed against the CD3 receptor in the T cell antigen receptor of mature T cells. Anti-CD3 antibodies include OKT-3, also known as muromonab. Anti-CD3 antibodies also include the UHCT1 clone, also known as T3 and CD3ε. Other anti-CD3 antibodies include, for example, otelixizumab, teplizumab, and visilizumab.

[00696] The term "OKT-3" (also referred to herein as "OKT3") refers to a monoclonal antibody or biosimilar or variant thereof, including human, humanized, chimeric, or murine antibodies, directed against the CD3 receptor in the T cell antigen receptor of mature T cells, and includes commercially-available forms such as OKT-3 (30 ng/mL, MACS GMP CD3 pure, Miltenyi Biotech, Inc., San Diego, CA, USA) and muromonab or variants, conservative amino acid substitutions, glycoforms, or biosimilars thereof. The amino acid sequences of the heavy and light chains of muromonab are given in Table 1 (SEQ ID NO:1 and SEQ ID NO:2). A hybridoma capable of producing OKT-3 is deposited with the American Type Culture Collection and assigned the ATCC accession number CRL 8001. A hybridoma capable of producing OKT-3 is also deposited with European Collection of Authenticated Cell Cultures (ECACC) and assigned Catalogue No. 86022706.

TABLE 1. Amino acid sequences of muromonab (exemplary OKT-3 antibody).

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:1 Muromonab heavy chain	QVQLQQSGAE LARPGASVKM SCKASGYTFT RYTMHWVKQR PGQGLEWIGY INPSRGYTNY NQKFKDKATL TTDKSSSTAY MQLSSLTSED SAVYYCARYY DDHYCLDYWG QGTTLTVSSA KTTAPSVYPL APVCGGTTGS SVTLGCLVKG YFPEPVTLTW NSGSLSSGVH TFPAVLQSDL YTLSSSVTVT SSTWPSQSIT CNVAHPASST KVDKKIEPRP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK	60 120 180 240 300 360 420 450
SEQ ID NO:2 Muromonab light chain	QIVLTQSPAI MSASPGEKVT MTCSASSSVS YMNWYQQKSG TSPKRWIYDT SKLASGVPAH FRGSGSGTSY SLTISGMEAE DAATYYCQQW SSNPFTFGSG TKLEINRADT APTVSIFPPS SEQLTSGGAS VVCFLNNFYP KDINVKWKID GSERQNGVLN SWTDQDSKDS TYSMSSTLTL TKDEYERHNS YTCEATHKTS TSPIVKSFNR NEC	60 120 180 213

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[00697] The term "IL-2" (also referred to herein as "IL2") refers to the T cell growth factor known as interleukin-2, and includes all forms of IL-2 including human and mammalian forms, conservative amino acid substitutions, glycoforms, biosimilars, and variants thereof. IL-2 is described, *e.g.*, in Nelson, *J. Immunol.* **2004,** *172,* 3983-88 and Malek, *Annu. Rev. Immunol.* **2008,** *26,* 453-79, the disclosures of which are incorporated by reference herein. The amino acid sequence of recombinant human IL-2 suitable for use in the invention is given in Table 2 (SEQ ID NO:3). For example, the term IL-2 encompasses human, recombinant forms of IL-2 such as aldesleukin (PROLEUKIN, available commercially from multiple suppliers in 22 million IU per single use vials), as well as the form of recombinant IL-2 commercially supplied by CellGenix, Inc., Portsmouth, NH, USA (CELLGRO GMP) or ProSpec-Tany TechnoGene Ltd., East Brunswick, NJ, USA (Cat. No. CYT-209-b) and other commercial equivalents from other vendors. Aldesleukin (des-alanyl-1, serine-125 human IL-2) is a nonglycosylated human recombinant form of IL-2 with a molecular weight of approximately 15 kDa. The amino acid sequence of aldesleukin suitable for use in the invention is given in Table 2 (SEQ ID NO:4). The term IL-2 also encompasses pegylated forms of IL-2, as described herein, including the pegylated IL2 prodrug bempegaldesleukin (NKTR-214, pegylated human recombinant IL-2 as in SEQ ID NO:4 in which an average of 6 lysine residues are N^6 substituted with [(2,7-bis{[methylpoly(oxyethylene)]carbamoyl}-9H-fluoren-9-yl)methoxy]carbonyl), which is available from Nektar Therapeutics, South San Francisco, CA, USA, or which may be prepared by methods known in the art, such as the methods described in Example 19 of International Patent Application Publication No. WO 2018/132496 A1 or the method described in Example 1 of U.S. Patent Application Publication No. US 2019/0275133 A1, the disclosures of which are incorporated by reference herein. Bempegaldesleukin (NKTR-214) and other pegylated IL-2 molecules suitable for use in the invention are described in U.S. Patent Application Publication No. US 2014/0328791 A1 and International Patent Application Publication No. WO 2012/065086 A1, the disclosures of which are incorporated by reference herein. Alternative forms of conjugated IL-2 suitable for use in the invention are described in U.S. Patent Nos. 4,766,106, 5,206,344, 5,089,261 and 4,902,502, the disclosures of which are incorporated by reference herein. Formulations of IL-2 suitable for use in the invention are described in U.S. Patent No. 6,706,289, the disclosure of which is incorporated by reference herein.

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[00698] In some embodiments, an IL-2 form suitable for use in the present invention is THOR-707, available from Synthorx, Inc. The preparation and properties of THOR-707 and additional alternative forms of IL-2 suitable for use in the invention are described in U.S. Patent Application Publication Nos. US 2020/0181220 A1 and US 2020/0330601 A1, the disclosures of which are incorporated by reference herein. In some embodiments, and IL-2 form suitable for use in the invention is an interleukin 2 (IL-2) conjugate comprising: an isolated and purified IL-2 polypeptide; and a conjugating moiety that binds to the isolated and purified IL-2 polypeptide at an amino acid position selected from K35, T37, R38, T41, F42, K43, F44, Y45, E61, E62, E68, K64, P65, V69, L72, and Y107, wherein the numbering of the amino acid residues corresponds to SEQ ID NO:5. In some embodiments, the amino acid position is selected from T37, R38, T41, F42, F44, Y45, E61, E62, E68, K64, P65, V69, L72, and Y107. In some embodiments, the amino acid position is selected from T37, R38, T41, F42, F44, Y45, E61, E62, E68, P65, V69, L72, and Y107. In some embodiments, the amino acid position is selected from T37, T41, F42, F44, Y45, P65, V69, L72, and Y107. In some embodiments, the amino acid position is selected from R38 and K64. In some embodiments, the amino acid position is selected from E61, E62, and E68. In some embodiments, the amino acid position is at E62. In some embodiments, the amino acid residue selected from K35, T37, R38, T41, F42, K43, F44, Y45, E61, E62, E68, K64, P65, V69, L72, and Y107 is further mutated to lysine, cysteine, or histidine. In some embodiments, the amino acid residue is mutated to cysteine. In some embodiments, the amino acid residue is mutated to lysine. In some embodiments, the amino acid residue selected from K35, T37, R38, T41, F42, K43, F44, Y45, E61, E62, E68, K64, P65, V69, L72, and Y107 is further mutated to an unnatural amino acid. In some embodiments, the unnatural amino acid comprises N6-azidoethoxy-L-lysine (AzK), N6-propargylethoxy-L-lysine (PraK), BCN-L-lysine, norbornene lysine, TCO-lysine, methyltetrazine lysine, allyloxycarbonyllysine, 2-amino-8-oxononanoic acid, 2-amino-8-oxooctanoic acid, p-acetyl-L-phenylalanine, p-azidomethyl-L-phenylalanine (pAMF), p-iodo-L-phenylalanine, m-acetylphenylalanine, 2-amino-8-oxononanoic acid, p-propargyloxyphenylalanine, p-propargyl-phenylalanine, 3-methyl-phenylalanine, L-Dopa, fluorinated phenylalanine, isopropyl-L-phenylalanine, p-azido-L-phenylalanine, p-acyl-L-phenylalanine, p-benzoyl-L-phenylalanine, p-bromophenylalanine, p-amino-L-phenylalanine, isopropyl-L-phenylalanine, O-allyltyrosine, O-methyl-L-tyrosine, O-4-allyl-L-tyrosine, 4-propyl-L-tyrosine, phosphonotyrosine, tri-O-acetyl-GlcNAcp-serine, L-phosphoserine, phosphonoserine, L-3-(2-naphthyl)alanine, 2-amino-3-((2-((3-(benzyloxy)-3-oxopropyl)amino)ethyl)selanyl)propanoic acid, 2-amino-3-(phenylselanyl)propanoic, or

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selenocysteine. In some embodiments, the IL-2 conjugate has a decreased affinity to IL-2 receptor α (IL-2Rα) subunit relative to a wild-type IL-2 polypeptide. In some embodiments, the decreased affinity is about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or greater than 99% decrease in binding affinity to IL-2Rα relative to a wild-type IL-2 polypeptide. In some embodiments, the decreased affinity is about 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 30-fold, 50-fold, 100-fold, 200-fold, 300-fold, 500-fold, 1000-fold, or more relative to a wild-type IL-2 polypeptide. In some embodiments, the conjugating moiety impairs or blocks the binding of IL-2 with IL-2Rα. In some embodiments, the conjugating moiety comprises a water-soluble polymer. In some embodiments, the additional conjugating moiety comprises a water-soluble polymer. In some embodiments, each of the water-soluble polymers independently comprises polyethylene glycol (PEG), poly(propylene glycol) (PPG), copolymers of ethylene glycol and propylene glycol, poly(oxyethylated polyol), poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxyalkylmethacrylamide), poly(hydroxyalkylmethacrylate), poly(saccharides), poly(α-hydroxy acid), poly(vinyl alcohol), polyphosphazene, polyoxazolines (POZ), poly(N-acryloylmorpholine), or a combination thereof. In some embodiments, each of the water-soluble polymers independently comprises PEG. In some embodiments, the PEG is a linear PEG or a branched PEG. In some embodiments, each of the water-soluble polymers independently comprises a polysaccharide. In some embodiments, the polysaccharide comprises dextran, polysialic acid (PSA), hyaluronic acid (HA), amylose, heparin, heparan sulfate (HS), dextrin, or hydroxyethyl-starch (HES). In some embodiments, each of the water-soluble polymers independently comprises a glycan. In some embodiments, each of the water-soluble polymers independently comprises polyamine. In some embodiments, the conjugating moiety comprises a protein. In some embodiments, the additional conjugating moiety comprises a protein. In some embodiments, each of the proteins independently comprises an albumin, a transferrin, or a transthyretin. In some embodiments, each of the proteins independently comprises an Fc portion. In some embodiments, each of the proteins independently comprises an Fc portion of IgG. In some embodiments, the conjugating moiety comprises a polypeptide. In some embodiments, the additional conjugating moiety comprises a polypeptide. In some embodiments, each of the polypeptides independently comprises a XTEN peptide, a glycine-rich homoamino acid polymer (HAP), a PAS polypeptide, an elastin-like polypeptide (ELP), a CTP peptide, or a gelatin-like protein (GLK) polymer. In some embodiments, the isolated and purified IL-2 polypeptide is modified by glutamylation. In some embodiments, the conjugating moiety is directly bound to the isolated and purified

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IL-2 polypeptide. In some embodiments, the conjugating moiety is indirectly bound to the isolated and purified IL-2 polypeptide through a linker. In some embodiments, the linker comprises a homobifunctional linker. In some embodiments, the homobifunctional linker comprises Lomant's reagent dithiobis (succinimidylpropionate) DSP, 3′3′-dithiobis(sulfosuccinimidyl proprionate) (DTSSP), disuccinimidyl suberate (DSS), bis(sulfosuccinimidyl)suberate (BS), disuccinimidyl tartrate (DST), disulfosuccinimidyl tartrate (sulfo DST), ethylene glycobis(succinimidylsuccinate) (EGS), disuccinimidyl glutarate (DSG), N,N′-disuccinimidyl carbonate (DSC), dimethyl adipimidate (DMA), dimethyl pimelimidate (DMP), dimethyl suberimidate (DMS), dimethyl-3,3′-dithiobispropionimidate (DTBP), 1,4-di-(3′-(2′-pyridyldithio)propionamido)butane (DPDPB), bismaleimidohexane (BMH), aryl halide-containing compound (DFDNB), such as e.g. 1,5-difluoro-2,4-dinitrobenzene or 1,3-difluoro-4,6-dinitrobenzene, 4,4′-difluoro-3,3′-dinitrophenylsulfone (DFDNPS), bis-[β-(4-azidosalicylamido)ethyl]disulfide (BASED), formaldehyde, glutaraldehyde, 1,4-butanediol diglycidyl ether, adipic acid dihydrazide, carbohydrazide, o-toluidine, 3,3′-dimethylbenzidine, benzidine, α,α′-p-diaminodiphenyl, diiodo-p-xylene sulfonic acid, N,N′-ethylene-bis(iodoacetamide), or N,N′-hexamethylene-bis(iodoacetamide). In some embodiments, the linker comprises a heterobifunctional linker. In some embodiments, the heterobifunctional linker comprises N-succinimidyl 3-(2-pyridyldithio)propionate (sPDP), long-chain N-succinimidyl 3-(2-pyridyldithio)propionate (LC-sPDP), water-soluble-long-chain N-succinimidyl 3-(2-pyridyldithio) propionate (sulfo-LC-sPDP), succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)toluene (sMPT), sulfosuccinimidyl-6-[α-methyl-α-(2-pyridyldithio)toluamido]hexanoate (sulfo-LC-sMPT), succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sMCC), sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-sMCC), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBs), m-maleimidobenzoyl-N-hydroxysulfosuccinimide ester (sulfo-MBs), N-succinimidyl(4-iodoacteyl)aminobenzoate (sIAB), sulfosuccinimidyl(4-iodoacteyl)aminobenzoate (sulfo-sIAB), succinimidyl-4-(p-maleimidophenyl)butyrate (sMPB), sulfosuccinimidyl-4-(p-maleimidophenyl)butyrate (sulfo-sMPB), N-(γ-maleimidobutyryloxy)succinimide ester (GMBs), N-(γ-maleimidobutyryloxy) sulfosuccinimide ester (sulfo-GMBs), succinimidyl 6-((iodoacetyl)amino)hexanoate (sIAX), succinimidyl 6-[6-(((iodoacetyl)amino)hexanoyl)amino]hexanoate (slAXX), succinimidyl 4-(((iodoacetyl)amino)methyl)cyclohexane-1-carboxylate (sIAC), succinimidyl 6-(((((4-iodoacetyl)amino)methyl)cyclohexane-1-carbonyl)amino) hexanoate (sIACX), p-nitrophenyl iodoacetate (NPIA), carbonyl-reactive and sulfhydryl-reactive cross-linkers such as 4-(4-N-

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maleimidophenyl)butyric acid hydrazide (MPBH), 4-(N-maleimidomethyl)cyclohexane-1-carboxyl-hydrazide-8 (M2C2H), 3-(2-pyridyldithio)propionyl hydrazide (PDPH), N-hydroxysuccinimidyl-4-azidosalicylic acid (NHs-AsA), N-hydroxysulfosuccinimidyl-4-azidosalicylic acid (sulfo-NHs-AsA), sulfosuccinimidyl-(4-azidosalicylamido)hexanoate (sulfo-NHs-LC-AsA), sulfosuccinimidyl-2-(p-azidosalicylamido)ethyl-1,3′-dithiopropionate (sAsD), N-hydroxysuccinimidyl-4-azidobenzoate (HsAB), N-hydroxysulfosuccinimidyl-4-azidobenzoate (sulfo-HsAB), N-succinimidyl-6-(4′-azido-2′-nitrophenyl amino)hexanoate (sANPAH), sulfosuccinimidyl-6-(4′-azido-2′-nitrophenylamino)hexanoate (sulfo-sANPAH), N-5-azido-2-nitrobenzoyloxysuccinimide (ANB-NOs), sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3′-dithiopropionate (sAND), N-succinimidyl-4(4-azidophenyl)1,3′-dithiopropionate (sADP), N-sulfosuccinimidyl(4-azidophenyl)-1,3′-dithiopropionate (sulfo-sADP), sulfosuccinimidyl 4-(ρ-azidophenyl)butyrate (sulfo-sAPB), sulfosuccinimidyl 2-(7-azido-4-methylcoumarin-3-acetamide)ethyl-1,3′-dithiopropionate (sAED), sulfosuccinimidyl 7-azido-4-methylcoumain-3-acetate (sulfo-sAMCA), p-nitrophenyl diazopyruvate (pNPDP), p-nitrophenyl-2-diazo-3,3,3-trifluoropropionate (PNP-DTP), 1-(ρ-azidosalicylamido)-4-(iodoacetamido)butane (AsIB), N-[4-(ρ-azidosalicylamido)butyl]-3′-(2′-pyridyldithio) propionamide (APDP), benzophenone-4-iodoacetamide, p-azidobenzoyl hydrazide (ABH), 4-(ρ-azidosalicylamido)butylamine (AsBA), or p-azidophenyl glyoxal (APG). In some embodiments, the linker comprises a cleavable linker, optionally comprising a dipeptide linker. In some embodiments, the dipeptide linker comprises Val-Cit, Phe-Lys, Val-Ala, or Val-Lys. In some embodiments, the linker comprises a non-cleavable linker. In some embodiments, the linker comprises a maleimide group, optionally comprising maleimidocaproyl (mc), succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sMCC), or sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-sMCC). In some embodiments, the linker further comprises a spacer. In some embodiments, the spacer comprises p-aminobenzyl alcohol (PAB), p-aminobenzyoxycarbonyl (PABC), a derivative, or an analog thereof. In some embodiments, the conjugating moiety is capable of extending the serum half-life of the IL-2 conjugate. In some embodiments, the additional conjugating moiety is capable of extending the serum half-life of the IL-2 conjugate. In some embodiments, the IL-2 form suitable for use in the invention is a fragment of any of the IL-2 forms described herein. In some embodiments, the IL-2 form suitable for use in the invention is pegylated as disclosed in U.S. Patent Application Publication No. US 2020/0181220 A1 and U.S. Patent Application Publication No. US 2020/0330601 A1. In some embodiments, the IL-2 form suitable for use in the invention is an IL-2 conjugate comprising: an IL-2

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polypeptide comprising an N6-azidoethoxy-L-lysine (AzK) covalently attached to a conjugating moiety comprising a polyethylene glycol (PEG), wherein: the IL-2 polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO:5; and the AzK substitutes for an amino acid at position K35, F42, F44, K43, E62, P65, R38, T41, E68, Y45, V69, or L72 in reference to the amino acid positions within SEQ ID NO:5. In some embodiments, the IL-2 polypeptide comprises an N-terminal deletion of one residue relative to SEQ ID NO:5. In some embodiments, the IL-2 form suitable for use in the invention lacks IL-2R alpha chain engagement but retains normal binding to the intermediate affinity IL-2R beta-gamma signaling complex. In some embodiments, the IL-2 form suitable for use in the invention is an IL-2 conjugate comprising: an IL-2 polypeptide comprising an N6-azidoethoxy-L-lysine (AzK) covalently attached to a conjugating moiety comprising a polyethylene glycol (PEG), wherein: the IL-2 polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:5; and the AzK substitutes for an amino acid at position K35, F42, F44, K43, E62, P65, R38, T41, E68, Y45, V69, or L72 in reference to the amino acid positions within SEQ ID NO:5. In some embodiments, the IL-2 form suitable for use in the invention is an IL-2 conjugate comprising: an IL-2 polypeptide comprising an N6-azidoethoxy-L-lysine (AzK) covalently attached to a conjugating moiety comprising a polyethylene glycol (PEG), wherein: the IL-2 polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO:5; and the AzK substitutes for an amino acid at position K35, F42, F44, K43, E62, P65, R38, T41, E68, Y45, V69, or L72 in reference to the amino acid positions within SEQ ID NO:5. In some embodiments, the IL-2 form suitable for use in the invention is an IL-2 conjugate comprising: an IL-2 polypeptide comprising an N6-azidoethoxy-L-lysine (AzK) covalently attached to a conjugating moiety comprising a polyethylene glycol (PEG), wherein: the IL-2 polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO:5; and the AzK substitutes for an amino acid at position K35, F42, F44, K43, E62, P65, R38, T41, E68, Y45, V69, or L72 in reference to the amino acid positions within SEQ ID NO:570.

[00699] In some embodiments, an IL-2 form suitable for use in the invention is nemvaleukin alfa, also known as ALKS-4230 (SEQ ID NO:571), which is available from Alkermes, Inc. Nemvaleukin alfa is also known as human interleukin 2 fragment (1-59), variant (Cys^{125}>Ser^{51}), fused via peptidyl linker (^{60}GG^{61}) to human interleukin 2 fragment (62-132), fused via peptidyl linker (^{133}GSGGGS^{138}) to human interleukin 2 receptor α-chain fragment (139-303), produced in Chinese hamster ovary (CHO) cells, glycosylated; human

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interleukin 2 (IL-2) (75-133)-peptide [Cys^{125}(51)>Ser]-mutant (1-59), fused via a G_2 peptide linker (60-61) to human interleukin 2 (IL-2) (4-74)-peptide (62-132) and via a GSG_3S peptide linker (133-138) to human interleukin 2 receptor α-chain (IL2R subunit alpha, IL2Rα, IL2RA) (1-165)-peptide (139-303), produced in Chinese hamster ovary (CHO) cells, glycoform alfa. The amino acid sequence of nemvaleukin alfa is given in SEQ ID NO:571. In some embodiments, nemvaleukin alfa exhibits the following post-translational modifications: disulfide bridges at positions: 31-116, 141-285, 184-242, 269-301, 166-197 or 166-199, 168-199 or 168-197 (using the numbering in SEQ ID NO: 571), and glycosylation sites at positions: N187, N206, T212 using the numbering in SEQ ID NO:571. The preparation and properties of nemvaleukin alfa, as well as additional alternative forms of IL-2 suitable for use in the invention, is described in U.S. Patent Application Publication No. US 2021/0038684 A1 and U.S. Patent No. 10,183,979, the disclosures of which are incorporated by reference herein. In some embodiments, an IL-2 form suitable for use in the invention is a protein having at least 80%, at least 90%, at least 95%, or at least 90% sequence identity to SEQ ID NO: 571. In some embodiments, an IL-2 form suitable for use in the invention has the amino acid sequence given in SEQ ID NO: 571 or conservative amino acid substitutions thereof. In some embodiments, an IL-2 form suitable for use in the invention is a fusion protein comprising amino acids 24-452 of SEQ ID NO:572, or variants, fragments, or derivatives thereof. In some embodiments, an IL-2 form suitable for use in the invention is a fusion protein comprising an amino acid sequence having at least 80%, at least 90%, at least 95%, or at least 90% sequence identity to amino acids 24-452 of SEQ ID NO: 572, or variants, fragments, or derivatives thereof. Other IL-2 forms suitable for use in the present invention are described in U.S. Patent No. 10,183,979, the disclosures of which are incorporated by reference herein. Optionally, in some embodiments, an IL-2 form suitable for use in the invention is a fusion protein comprising a first fusion partner that is linked to a second fusion partner by a mucin domain polypeptide linker, wherein the first fusion partner is IL-1Rα or a protein having at least 98% amino acid sequence identity to IL-1Rα and having the receptor antagonist activity of IL-Rα, and wherein the second fusion partner comprises all or a portion of an immunoglobulin comprising an Fc region, wherein the mucin domain polypeptide linker comprises SEQ ID NO:573 or an amino acid sequence having at least 90% sequence identity to SEQ ID NO:573 and wherein the half-life of the fusion protein is improved as compared to a fusion of the first fusion partner to the second fusion partner in the absence of the mucin domain polypeptide linker.

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TABLE 2. Amino acid sequences of interleukins.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:3 recombinant human IL-2 (rhIL-2)	MAPTSSSTKK TQLQLEHLLL DLQMILNGIN NYKNPKLTRM LTFKFYMPKK ATELKHLQCL EEELKPLEEV LNLAQSKNFH LRPRDLISNI NVIVLELKGS ETTFMCEYAD ETATIVEFLN RWITFCQSII STLT	60 120 134
SEQ ID NO:4 Aldesleukin	PTSSSTKKTQ LQLEHLLLDL QMILNGINNY KNPKLTRMLT FKFYMPKKAT ELKHLQCLEE ELKPLEEVLN LAQSKNFHLR PRDLISNINV IVLELKGSET TFMCEYADET ATIVEFLNRW ITFSQSIIST LT	60 120 132
SEQ ID NO:5 recombinant human IL-4 (rhIL-4)	MHKCDITLQE IIKTLNSLTE QKTLCTELTV TDIFAASKNT TEKETFCRAA TVLRQFYSHH EKDTRCLGAT AQQFHRHKQL IRFLKRLDRN LWGLAGLNSC PVKEANQSTL ENFLERLKTI MREKYSKCSS	60 120 130
SEQ ID NO:6 recombinant human IL-7 (rhIL-7)	MDCDIEGKDG KQYESVLMVS IDQLLDSMKE IGSNCLNNEF NFFKRHICDA NKEGMFLFRA ARKLRQFLKM NSTGDFDLHL LKVSEGTTIL LNCTGQVKGR KPAALGEAQP TKSLEENKSL KEQKKLNDLC FLKRLLQEIK TCWNKILMGT KEH	60 120 153
SEQ ID NO:7 recombinant human IL-15 (rhIL-15)	MNWVNVISDL KKIEDLIQSM HIDATLYTES DVHPSCKVTA MKCFLLELQV ISLESGDASI HDTVENLIIL ANNSLSSNGN VTESGCKECE ELEEKNIKEF LQSFVHIVQM FINTS	60 115
SEQ ID NO:8 recombinant human IL-21 (rhIL-21)	MQDRHMIRMR QLIDIVDQLK NYVNDLVPEF LPAPEDVETN CEWSAFSCFQ KAQLKSANTG NNERIINVSI KKLKRKPPST NAGRRQKHRL TCPSCDSYEK KPPKEFLERF KSLLQKMIHQ HLSSRTHGSE DS	60 120 132
SEQ ID NO: 570 IL-2 form	APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR WITFCQSIIS TLT	60 120 133
SEQ ID NO: 571 IL-2 form	SKNFHLRPRD LISNINVIVL ELKGSETTFM CEYADETATI VEFLNRWITF SQSIISTLTG GSSSTKKTQL QLEHLLLDLQ MILNGINNYK NPKLTRMLTF KFYMPKKATE LKHLQCLEEE LKPLEEVLNL AQGSGGGSEL CDDDPPEIPH ATFKAMAYKE GTMLNCECKR GFRRIKSGSL YMLCTGNSSH SSWDNQCQCT SSATRNTTKQ VTPQPEEQKE RKTTEMQSPM QPVDQASLPC HCREPPPWEN EATERIYHFV VGQMVYYQCV QGYRALHRGP AESVCKMTHG KTRWTQPQLI CTG	60 120 180 240 300 303
SEQ ID NO: 572 IL-2 form	MDAMKRGLCC VLLLCGAVFV SARRPSGRKS SKMQAFRIWD VNQKTFYLRN NQLVAGYLQG PNVNLEEKID VVPIEPHALF LGIHGGKMCL SCVKSGDETR LQLEAVNITD LSENRKQDKR FAFIRSDSGP TTSFESAACP GWFLCTAMEA DQPVSLTNMP DEGVMVTKFY FQEDESGSGG ASSPSSASSD GPHPVITPSR ASSPSSASSD GPHPVITPSR PPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK	60 120 180 240 300 360 420 453
SEQ ID NO: 573 mucin domain polypeptide	SESSASSDGP HPVITP	16

[00700] In some embodiments, an IL-2 form suitable for use in the invention includes an antibody cytokine engrafted protein that comprises a heavy chain variable region (V_H), comprising complementarity determining regions HCDR1, HCDR2, HCDR3; a light chain variable region (V_L), comprising LCDR1, LCDR2, LCDR3; and an IL-2 molecule or a fragment thereof engrafted into a CDR of the V_H or the V_L, wherein the antibody cytokine engrafted protein preferentially expands T effector cells over regulatory T cells. In some embodiments, the antibody cytokine engrafted protein comprises a heavy chain variable region (V_H), comprising complementarity determining regions HCDR1, HCDR2, HCDR3; a light chain variable region (V_L), comprising LCDR1, LCDR2, LCDR3; and an IL-2 molecule or a fragment thereof engrafted into a CDR of the V_H or the V_L, wherein the IL-2 molecule is a mutein, and wherein the antibody cytokine engrafted protein preferentially expands T

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effector cells over regulatory T cells. In some embodiments, the IL-2 regimen comprises administration of an antibody described in U.S. Patent Application Publication No. US 2020/0270334 A1, the disclosures of which are incorporated by reference herein. In some embodiments, the antibody cytokine engrafted protein comprises a heavy chain variable region (VH), comprising complementarity determining regions HCDR1, HCDR2, HCDR3; a light chain variable region (VL), comprising LCDR1, LCDR2, LCDR3; and an IL-2 molecule or a fragment thereof engrafted into a CDR of the V_H or the V_L, wherein the IL-2 molecule is a mutein, wherein the antibody cytokine engrafted protein preferentially expands T effector cells over regulatory T cells, and wherein the antibody further comprises an IgG class heavy chain and an IgG class light chain selected from the group consisting of: a IgG class light chain comprising SEQ ID NO:569 and a IgG class heavy chain comprising SEQ ID NO:568; a IgG class light chain comprising SEQ ID NO:567 and a IgG class heavy chain comprising SEQ ID NO:559; a IgG class light chain comprising SEQ ID NO:569 and a IgG class heavy chain comprising SEQ ID NO:559; and a IgG class light chain comprising SEQ ID NO:37 and a IgG class heavy chain comprising SEQ ID NO:568.

[00701] In some embodiments, an IL-2 molecule or a fragment thereof is engrafted into HCDR1 of the V_H, wherein the IL-2 molecule is a mutein. In some embodiments, an IL-2 molecule or a fragment thereof is engrafted into HCDR2 of the V_H, wherein the IL-2 molecule is a mutein. In some embodiments, an IL-2 molecule or a fragment thereof is engrafted into HCDR3 of the V_H, wherein the IL-2 molecule is a mutein. In some embodiments, an IL-2 molecule or a fragment thereof is engrafted into LCDR1 of the V_L, wherein the IL-2 molecule is a mutein. In some embodiments, an IL-2 molecule or a fragment thereof is engrafted into LCDR2 of the V_L, wherein the IL-2 molecule is a mutein. In some embodiments, an IL-2 molecule or a fragment thereof is engrafted into LCDR3 of the V_L, wherein the IL-2 molecule is a mutein.

[00702] The insertion of the IL-2 molecule can be at or near the N-terminal region of the CDR, in the middle region of the CDR or at or near the C-terminal region of the CDR. In some embodiments, the antibody cytokine engrafted protein comprises an IL-2 molecule incorporated into a CDR, wherein the IL2 sequence does not frameshift the CDR sequence. In some embodiments, the antibody cytokine engrafted protein comprises an IL-2 molecule incorporated into a CDR, wherein the IL-2 sequence replaces all or part of a CDR sequence. The replacement by the IL-2 molecule can be the N-terminal region of the CDR, in the middle region of the CDR or at or near the C-terminal region the CDR. A replacement by the

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IL-2 molecule can be as few as one or two amino acids of a CDR sequence, or the entire CDR sequences.

[00703] In some embodiments, an IL-2 molecule is engrafted directly into a CDR without a peptide linker, with no additional amino acids between the CDR sequence and the IL-2 sequence. In some embodiments, an IL-2 molecule is engrafted indirectly into a CDR with a peptide linker, with one or more additional amino acids between the CDR sequence and the IL-2 sequence.

[00704] In some embodiments, the IL-2 molecule described herein is an IL-2 mutein. In some instances, the IL-2 mutein comprising an R67A substitution. In some embodiments, the IL-2 mutein comprises the amino acid sequence SEQ ID NO:544 or SEQ ID NO:545. In some embodiments, the IL-2 mutein comprises an amino acid sequence in Table 1 in U.S. Patent Application Publication No. US 2020/0270334 A1, the disclosure of which is incorporated by reference herein.

[00705] In some embodiments, the antibody cytokine engrafted protein comprises an HCDR1 selected from the group consisting of SEQ ID NO:546, SEQ ID NO:549, SEQ ID NO:552 and SEQ ID NO:555. In some embodiments, the antibody cytokine engrafted protein comprises an HCDR1 selected from the group consisting of SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:543 and SEQ ID NO:546. In some embodiments, the antibody cytokine engrafted protein comprises an HCDR1 selected from the group consisting of HCDR2 selected from the group consisting of SEQ ID NO:547, SEQ ID NO:550, SEQ ID NO:553, and SEQ ID NO:556. In some embodiments, the antibody cytokine engrafted protein comprises an HCDR3 selected from the group consisting of SEQ ID NO:548, SEQ ID NO:551, SEQ ID NO:554, and SEQ ID NO:557. In some embodiments, the antibody cytokine engrafted protein comprises a V_H region comprising the amino acid sequence of SEQ ID NO:558. In some embodiments, the antibody cytokine engrafted protein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:559. In some embodiments, the antibody cytokine engrafted protein comprises a V_L region comprising the amino acid sequence of SEQ ID NO:566. In some embodiments, the antibody cytokine engrafted protein comprises a light chain comprising the amino acid sequence of SEQ ID NO:567. In some embodiments, the antibody cytokine engrafted protein comprises a V_H region comprising the amino acid sequence of SEQ ID NO:28 and a V_L region comprising the amino acid sequence of SEQ ID NO:566. In some embodiments, the antibody cytokine engrafted protein comprises a heavy chain region comprising the amino acid sequence of SEQ ID NO:559 and

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a light chain region comprising the amino acid sequence of SEQ ID NO:567. In some embodiments, the antibody cytokine engrafted protein comprises a heavy chain region comprising the amino acid sequence of SEQ ID NO:559 and a light chain region comprising the amino acid sequence of SEQ ID NO:569. In some embodiments, the antibody cytokine engrafted protein comprises a heavy chain region comprising the amino acid sequence of SEQ ID NO:568 and a light chain region comprising the amino acid sequence of SEQ ID NO:567. In some embodiments, the antibody cytokine engrafted protein comprises a heavy chain region comprising the amino acid sequence of SEQ ID NO:568 and a light chain region comprising the amino acid sequence of SEQ ID NO:569. In some embodiments, the antibody cytokine engrafted protein comprises IgG.IL2F71A.H1 or IgG.IL2R67A.H1 of U.S. Patent Application Publication No. 2020/0270334 A1, or variants, derivatives, or fragments thereof, or conservative amino acid substitutions thereof, or proteins with at least 80%, at least 90%, at least 95%, or at least 98% sequence identity thereto. In some embodiments, the antibody components of the antibody cytokine engrafted protein described herein comprise immunoglobulin sequences, framework sequences, or CDR sequences of palivizumab. In some embodiments, the antibody cytokine engrafted protein described herein has a longer serum half-life that a wild-type IL-2 molecule such as, but not limited to, aldesleukin or a comparable molecule.

Table 3: Sequences of exemplary palivizumab antibody-IL-2 engrafted proteins

Identifier US 2020/0270334	SEQ ID NO:	Sequence
SEQ ID NO:2 IL-2	SEQ ID NO:543 IL-2	MYRMQLLSCI ALSLALVTNS APTSSSTKKT QLQLEHLLLD LQMILNGINN 50 YKNPKLTRML TFKFYMPKKA TELKHLQCLE EELKPLEEVL NLAQSKNFHL 100 RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR WITFCQSIIS 150 TLT 153
SEQ ID NO:4 IL-2 mutein	SEQ ID NO:544 IL-2 mutein	APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTAML TFKFYMPKKA 50 TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE 100 TTFMCEYADE TATIVEFLNR WITFCQSIIS TLT 133
SEQ ID NO:6 IL-2 mutein	SEQ ID NO:545 IL-2 mutein	APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TAKFYMPKKA 50 TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE 100 TTFMCEYADE TATIVEFLNR WITFCQSIIS TLT 133
IgG.IL2R67A. H1	IgG.IL2 R67A.H1	
SEQ ID NO:7 HCDR1_IL-2	SEQ ID NO:546 HCDR1_I L-2	GFSLAPTSSS TKKTQLQLEH LLLDLQMILN GINNYKNPKL TAMLTFKFYM 50 PKKATELKHL QCLEEELKPL EEVLNLAQSK NFHLRPRDLI SNINVIVLEL 100 KGSETTFMCE YADETATIVE FLNRWITFCQ SIISTLTSTS GMSVG 145
SEQ ID NO:8 HCDR2	SEQ ID NO:547 HCDR2	DIWWDDKKDY NPSLKS 16

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SEQ ID NO:9 HCDR3	SEQ ID NO:548 HCDR3	SMITNWYFDV 10
SEQ ID NO:10 HCDR1_IL-2 kabat	SEQ ID NO:549 HCDR1_IL-2 kabat	TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE 100 TTFMCEYADE TATIVEFLNR WITFCQSIIS TLTSTSGMSV G 141
SEQ ID NO:11 HCDR2 kabat	SEQ ID NO:550 HCDR2 kabat	DIWWDDKKDY NPSLKS 16
SEQ ID NO:12 HCDR3 kabat	SEQ ID NO:551 HCDR3 kabat	SMITNWYFDV 10
SEQ ID NO:13 HCDR1_IL-2 clothia	SEQ ID NO:552 HCDR1_IL-2 clothia	GFSLAPTSSS TKKTQLQLEH LLLDLQMILN GINNYKNPKL TAMLTFKFYM 50 PKKATELKHL QCLEEELKPL EEVLNLAQSK NFHLRPRDLI SNINVIVLEL 100 KGSETTFMCE YADETATIVE FLNRWITFCQ SIISTLTSTS GM 142
SEQ ID NO:14 HCDR2 clothia	SEQ ID NO:553 HCDR2 clothia	WWDDK 5
SEQ ID NO:15 HCDR3 clothia	SEQ ID NO:554 HCDR3 clothia	SMITNWYFDV 10
SEQ ID NO:16 HCDR1_IL-2 IMGT	SEQ ID NO:555 HCDR1_IL-2 IMGT	GFSLAPTSSS TKKTQLQLEH LLLDLQMILN GINNYKNPKL TAMLTFKFYM 50 PKKATELKHL QCLEEELKPL EEVLNLAQSK NFHLRPRDLI SNINVIVLEL 100 KGSETTFMCE YADETATIVE FLNRWITFCQ SIISTLTSTS GMS 143
SEQ ID NO:17 HCDR2 IMGT	SEQ ID NO:556 HCDR2 IMGT	IWWDDKK 7
SEQ ID NO:18 HCDR3 IMGT	SEQ ID NO:557 HCDR3 IMGT	ARSMITNWYF DV 12
SEQ ID NO:19 VH	SEQ ID NO:558 VH	QVTLRESGPA LVKPTQTLTL TCTFSGFSLA PTSSSTKKTQ LQLEHLLLDL 50 QMILNGINNY KNPKLTAMLT FKFYMPKKAT ELKHLQCLEE ELKPLEEVLN 100 LAQSKNFHLR PRDLISNINV IVLELKGSET TFMCEYADET ATIVEFLNRW 150 ITFCQSIIST LTSTSGMSVG WIRQPPGKAL EWLADIWWDD KKDYNPSLKS 200 RLTISKDTSK NQVVLKVTNM DPADTATYYC ARSMITNWYF DVWGAGTTVT 250 VSS 253
SEQ ID NO:21 Heavy chain	SEQ ID NO:559 Heavy chain	QMILNGINNY KNPKLTAMLT FKFYMPKKAT ELKHLQCLEE ELKPLEEVLN 100 LAQSKNFHLR PRDLISNINV IVLELKGSET TFMCEYADET ATIVEFLNRW 150 ITFCQSIIST LTSTSGMSVG WIRQPPGKAL EWLADIWWDD KKDYNPSLKS 200 RLTISKDTSK NQVVLKVTNM DPADTATYYC ARSMITNWYF DVWGAGTTVT 250 VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV TVSWNSGALT 300 SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKR 350 VEPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV 400 AVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL 450

		NGKEYKCKVS NKALAAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS 500 LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK 550 SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK 583
SEQ ID NO:26 LCDR1 kabat	SEQ ID NO:560 LCDR1 kabat	KAQLSVGYMH 10
SEQ ID NO:27 LCDR2 kabat	SEQ ID NO:561 LCDR2 kabat	DTSKLAS 7
SEQ ID NO:28 LCDR3 kabat	SEQ ID NO:562 LCDR3 kabat	FQGSGYPFT 9
SEQ ID NO:29 LCDR1 chothia	SEQ ID NO:563 LCDR1 chothia	QLSVGY 6
SEQ ID NO:30 LCDR2 chothia	SEQ ID NO:564 LCDR2 chothia	DTS 3
SEQ ID NO:31 LCDR3 chothia	SEQ ID NO:565 LCDR3 chothia	GSGYPF 6
SEQ ID NO:35 VL	SEQ ID NO:566 VL	DIQMTQSPST LSASVGDRVT ITCKAQLSVG YMHWYQQKPG KAPKLLIYDT 50 SKLASGVPSR FSGSGSGTEF TLTISSLQPD DFATYYCFQG SGYPFTFGGG 100 TKLEIK 106
SEQ ID NO:37 Light chain	SEQ ID NO:567 Light chain	DIQMTQSPST LSASVGDRVT ITCKAQLSVG YMHWYQQKPG KAPKLLIYDT 50 SKLASGVPSR FSGSGSGTEF TLTISSLQPD DFATYYCFQG SGYPFTFGGG 100 TKLEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD 150 NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL 200 SSPVTKSFNR GEC 213
SEQ ID NO:53 Light chain	SEQ ID NO:568 Light chain	QVTLRESGPA LVKPTQTLTL TCTFSGFSLA PTSSSTKKTQ LQLEHLLLDL 50 QMILNGINNY KNPKLTRMLT AKFYMPKKAT ELKHLQCLEE ELKPLEEVLN 100 LAQSKNFHLR PRDLISNINV IVLELKGSET TFMCEYADET ATIVEFLNRW 150 ITFCQSIIST LTSTSGMSVG WIRQPPGKAL EWLADIWWDD KKDYNPSLKS 200 RLTISKDTSK NQVVLKVTNM DPADTATYYC ARSMITNWYF DVWGAGTTVT 250 VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV TVSWNSGALT 300 SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKR 350 VEPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV 400 AVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL 450 NGKEYKCKVS NKALAAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS 500 LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK 550 SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK 583
SEQ ID NO:69 Light chain	SEQ ID NO:569 Light chain	DIQMTQSPST LSASVGDRVT ITCKAQLSVG YMHWYQQKPG KAPKLLIYDT 50 SKLASGVPSR FSGSGSGTEF TLTISSLQPD DFATYYCFQG SGYPFTFGGG 100 TKLEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD 150 NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL 200 SSPVTKSFNR GEC 213

[00706] The term "IL-4" (also referred to herein as "IL4") refers to the cytokine known as interleukin 4, which is produced by Th2 T cells and by eosinophils, basophils, and mast cells. IL-4 regulates the differentiation of naïve helper T cells (Th0 cells) to Th2 T cells. Steinke and Borish, *Respir. Res.* **2001,** *2,* 66-70. Upon activation by IL-4, Th2 T cells subsequently produce additional IL-4 in a positive feedback loop. IL-4 also stimulates B cell proliferation and class II MHC expression, and induces class switching to IgE and IgG_1 expression from B cells. Recombinant human IL-4 suitable for use in the invention is commercially available from multiple suppliers, including ProSpec-Tany TechnoGene Ltd., East Brunswick, NJ, USA (Cat. No. CYT-211) and ThermoFisher Scientific, Inc., Waltham, MA, USA (human IL-15 recombinant protein, Cat. No. Gibco CTP0043). The amino acid sequence of recombinant human IL-4 suitable for use in the invention is given in Table 2 (SEQ ID NO:5).

[00707] The term "IL-7" (also referred to herein as "IL7") refers to a glycosylated tissue-derived cytokine known as interleukin 7, which may be obtained from stromal and epithelial cells, as well as from dendritic cells. Fry and Mackall, *Blood* **2002,** *99,* 3892-904. IL-7 can stimulate the development of T cells. IL-7 binds to the IL-7 receptor, a heterodimer consisting of IL-7 receptor alpha and common gamma chain receptor, which in a series of signals important for T cell development within the thymus and survival within the periphery. Recombinant human IL-7 suitable for use in the invention is commercially available from multiple suppliers, including ProSpec-Tany TechnoGene Ltd., East Brunswick, NJ, USA (Cat. No. CYT-254) and ThermoFisher Scientific, Inc., Waltham, MA, USA (human IL-15 recombinant protein, Cat. No. Gibco PHC0071). The amino acid sequence of recombinant human IL-7 suitable for use in the invention is given in Table 2 (SEQ ID NO:6).

[00708] The term "IL-15" (also referred to herein as "IL15") refers to the T cell growth factor known as interleukin-15, and includes all forms of IL-2 including human and mammalian forms, conservative amino acid substitutions, glycoforms, biosimilars, and variants thereof. IL-15 is described, *e.g.*, in Fehniger and Caligiuri, *Blood* **2001,** *97,* 14-32, the disclosure of which is incorporated by reference herein. IL-15 shares β and γ signaling receptor subunits with IL-2. Recombinant human IL-15 is a single, non-glycosylated polypeptide chain containing 114 amino acids (and an N-terminal methionine) with a molecular mass of 12.8 kDa. Recombinant human IL-15 is commercially available from multiple suppliers, including ProSpec-Tany TechnoGene Ltd., East Brunswick, NJ, USA (Cat. No. CYT-230-b) and ThermoFisher Scientific, Inc., Waltham, MA, USA (human IL-15

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recombinant protein, Cat. No. 34-8159-82). The amino acid sequence of recombinant human IL-15 suitable for use in the invention is given in Table 2 (SEQ ID NO:7).

[00709] The term "IL-21" (also referred to herein as "IL21") refers to the pleiotropic cytokine protein known as interleukin-21, and includes all forms of IL-21 including human and mammalian forms, conservative amino acid substitutions, glycoforms, biosimilars, and variants thereof. IL-21 is described, *e.g.*, in Spolski and Leonard, *Nat. Rev. Drug. Disc.* **2014,** *13,* 379-95, the disclosure of which is incorporated by reference herein. IL-21 is primarily produced by natural killer T cells and activated human $CD4^+$ T cells. Recombinant human IL-21 is a single, non-glycosylated polypeptide chain containing 132 amino acids with a molecular mass of 15.4 kDa. Recombinant human IL-21 is commercially available from multiple suppliers, including ProSpec-Tany TechnoGene Ltd., East Brunswick, NJ, USA (Cat. No. CYT-408-b) and ThermoFisher Scientific, Inc., Waltham, MA, USA (human IL-21 recombinant protein, Cat. No. 14-8219-80). The amino acid sequence of recombinant human IL-21 suitable for use in the invention is given in Table 2 (SEQ ID NO:8).

[00710] When "an anti-tumor effective amount", "a tumor-inhibiting effective amount", or "therapeutic amount" is indicated, the precise amount of the compositions of the present invention to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject). It can generally be stated that a pharmaceutical composition comprising the tumor infiltrating lymphocytes (*e.g.* secondary TILs or genetically modified cytotoxic lymphocytes) described herein may be administered at a dosage of 10^4 to 10^{11} cells/kg body weight (*e.g.*, 10^5 to 10^6, 10^5 to 10^{10}, 10^5 to 10^{11}, 10^6 to 10^{10}, 10^6 to 10^{11},10^7 to 10^{11}, 10^7 to 10^{10}, 10^8 to 10^{11}, 10^8 to 10^{10}, 10^9 to 10^{11}, or 10^9 to 10^{10} cells/kg body weight), including all integer values within those ranges. Tumor infiltrating lymphocytes (inlcuding in some cases, genetically modified cytotoxic lymphocytes) compositions may also be administered multiple times at these dosages. The tumor infiltrating lymphocytes (including in some cases, genetically modified cytotoxic lymphocytes) can be administered by using infusion techniques that are commonly known in immunotherapy (see, *e.g.*, Rosenberg et al., *New Eng. J. of Med.* 319: 1676, 1988). The optimal dosage and treatment regime for a particular patient can readily be determined by one skilled in the art of medicine by monitoring the patient for signs of disease and adjusting the treatment accordingly.

[00711] The term "hematological malignancy", "hematologic malignancy" or terms of correlative meaning refer to mammalian cancers and tumors of the hematopoietic and

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lymphoid tissues, including but not limited to tissues of the blood, bone marrow, lymph nodes, and lymphatic system. Hematological malignancies are also referred to as "liquid tumors." Hematological malignancies include, but are not limited to, acute lymphoblastic leukemia (ALL), chronic lymphocytic lymphoma (CLL), small lymphocytic lymphoma (SLL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), acute monocytic leukemia (AMoL), Hodgkin's lymphoma, and non-Hodgkin's lymphomas. The term "B cell hematological malignancy" refers to hematological malignancies that affect B cells.

[00712] The term "solid tumor" refers to an abnormal mass of tissue that usually does not contain cysts or liquid areas. Solid tumors may be benign or malignant. The term "solid tumor cancer" refers to malignant, neoplastic, or cancerous solid tumors. Solid tumor cancers include, but are not limited to, sarcomas, carcinomas, and lymphomas, such as cancers of the lung, breast, prostate, colon, rectum, and bladder. The tissue structure of solid tumors includes interdependent tissue compartments including the parenchyma (cancer cells) and the supporting stromal cells in which the cancer cells are dispersed and which may provide a supporting microenvironment.

[00713] The term "liquid tumor" refers to an abnormal mass of cells that is fluid in nature. Liquid tumor cancers include, but are not limited to, leukemias, myelomas, and lymphomas, as well as other hematological malignancies. TILs obtained from liquid tumors may also be referred to herein as marrow infiltrating lymphocytes (MILs). TILs obtained from liquid tumors, including liquid tumors circulating in peripheral blood, may also be referred to herein as PBLs. The terms MIL, TIL, and PBL are used interchangeably herein and differ only based on the tissue type from which the cells are derived.

[00714] The term "microenvironment," as used herein, may refer to the solid or hematological tumor microenvironment as a whole or to an individual subset of cells within the microenvironment. The tumor microenvironment, as used herein, refers to a complex mixture of "cells, soluble factors, signaling molecules, extracellular matrices, and mechanical cues that promote neoplastic transformation, support tumor growth and invasion, protect the tumor from host immunity, foster therapeutic resistance, and provide niches for dominant metastases to thrive," as described in Swartz, *et al.*, *Cancer Res.*, **2012,** *72*, 2473. Although tumors express antigens that should be recognized by T cells, tumor clearance by the immune system is rare because of immune suppression by the microenvironment.

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[00715] In some embodiments, the invention includes a method of treating a cancer with a population of TILs, wherein a patient is pre-treated with non-myeloablative chemotherapy prior to an infusion of TILs according to the invention. In some embodiments, the population of TILs may be provided wherein a patient is pre-treated with nonmyeloablative chemotherapy prior to an infusion of TILs according to the present invention. In some embodiments, the non-myeloablative chemotherapy is cyclophosphamide 60 mg/kg/d for 2 days (days 27 and 26 prior to TIL infusion) and fludarabine 25 mg/m2/d for 5 days (days 27 to 23 prior to TIL infusion). In some embodiments, the non-myeloablative chemotherapy is cyclophosphamide 60 mg/kg/d for 2 days (days 27 and 26 prior to TIL infusion) and fludarabine 25 mg/m2/d for 3 days (days 27 to 25 prior to TIL infusion). In some embodiments, the non-myeloablative chemotherapy is cyclophosphamide 60 mg/kg/d for 2 days (days 27 and 26 prior to TIL infusion) followed by fludarabine 25 mg/m2/d for 3 days (days 25 to 23 prior to TIL infusion). In some embodiments, after non-myeloablative chemotherapy and TIL infusion (at day 0) according to the invention, the patient receives an intravenous infusion of IL-2 intravenously at 720,000 IU/kg every 8 hours to physiologic tolerance.

[00716] Experimental findings indicate that lymphodepletion prior to adoptive transfer of tumor-specific T lymphocytes plays a key role in enhancing treatment efficacy by eliminating regulatory T cells and competing elements of the immune system ("cytokine sinks"). Accordingly, some embodiments of the invention utilize a lymphodepletion step (sometimes also referred to as "immunosuppressive conditioning") on the patient prior to the introduction of the rTILs of the invention.

[00717] The terms "co-administration," "co-administering," "administered in combination with," "administering in combination with," "simultaneous," and "concurrent," as used herein, encompass administration of two or more active pharmaceutical ingredients (in a preferred embodiment of the present invention, for example, at least one potassium channel agonist in combination with a plurality of TILs) to a subject so that both active pharmaceutical ingredients and/or their metabolites are present in the subject at the same time. Co-administration includes simultaneous administration in separate compositions, administration at different times in separate compositions, or administration in a composition in which two or more active pharmaceutical ingredients are present. Simultaneous administration in separate compositions and administration in a composition in which both agents are present are preferred.

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[00718] The term "effective amount" or "therapeutically effective amount" refers to that amount of a compound or combination of compounds as described herein that is sufficient to effect the intended application including, but not limited to, disease treatment. A therapeutically effective amount may vary depending upon the intended application (in vitro or in vivo), or the subject and disease condition being treated (*e.g.*, the weight, age and gender of the subject), the severity of the disease condition, or the manner of administration. The term also applies to a dose that will induce a particular response in target cells (*e.g.*, the reduction of platelet adhesion and/or cell migration). The specific dose will vary depending on the particular compounds chosen, the dosing regimen to be followed, whether the compound is administered in combination with other compounds, timing of administration, the tissue to which it is administered, and the physical delivery system in which the compound is carried.

[00719] The terms "treatment", "treating", "treat", and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment", as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, *i.e.*, arresting its development or progression; and (c) relieving the disease, *i.e.*, causing regression of the disease and/or relieving one or more disease symptoms. "Treatment" is also meant to encompass delivery of an agent in order to provide for a pharmacologic effect, even in the absence of a disease or condition. For example, "treatment" encompasses delivery of a composition that can elicit an immune response or confer immunity in the absence of a disease condition, *e.g.*, in the case of a vaccine.

[00720] The term "heterologous" when used with reference to portions of a nucleic acid or protein indicates that the nucleic acid or protein comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, *e.g.*, a promoter from one source and a coding region from another source, or coding regions from different sources. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (*e.g.*, a fusion protein).

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[00721] The terms "sequence identity," "percent identity," and "sequence percent identity" (or synonyms thereof, *e.g.*, "99% identical") in the context of two or more nucleic acids or polypeptides, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid substitutions as part of the sequence identity. The percent identity can be measured using sequence comparison software or algorithms or by visual inspection. Various algorithms and software are known in the art that can be used to obtain alignments of amino acid or nucleotide sequences. Suitable programs to determine percent sequence identity include for example the BLAST suite of programs available from the U.S. Government's National Center for Biotechnology Information BLAST web site. Comparisons between two sequences can be carried using either the BLASTN or BLASTP algorithm. BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. ALIGN, ALIGN-2 (Genentech, South San Francisco, California) or MegAlign, available from DNASTAR, are additional publicly available software programs that can be used to align sequences. One skilled in the art can determine appropriate parameters for maximal alignment by particular alignment software. In certain embodiments, the default parameters of the alignment software are used.

[00722] As used herein, the term "variant" encompasses but is not limited to proteins, antibodies or fusion proteins which comprise an amino acid sequence which differs from the amino acid sequence of a reference protein, antibody or fusion protein by way of one or more substitutions, deletions and/or additions at certain positions within or adjacent to the amino acid sequence of the reference antibody, protein, or fusion protein. The variant may comprise one or more conservative substitutions in its amino acid sequence as compared to the amino acid sequence of a reference antibody. Conservative substitutions may involve, *e.g.*, the substitution of similarly charged or uncharged amino acids. The variant retains the ability to specifically bind to the antigen of the reference antibody, protein, or fusion protein. The term variant also includes pegylated antibodies or proteins.

[00723] By "tumor infiltrating lymphocytes" or "TILs" herein is meant a population of cells originally obtained as white blood cells that have left the bloodstream of a subject and migrated into a tumor. TILs include, but are not limited to, $CD8^+$ cytotoxic T cells (lymphocytes), Th1 and Th17 $CD4^+$ T cells, natural killer cells, dendritic cells and M1 macrophages. TILs include both primary and secondary TILs. "Primary TILs" are those that

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are obtained from patient tissue samples as outlined herein (sometimes referred to as "freshly obtained" or "freshly isolated"), and "secondary TILs" are any TIL cell populations that have been expanded or proliferated as discussed herein, including, but not limited to bulk TILs, expanded TILs ("REP TILs") as well as "reREP TILs" as discussed herein. reREP TILs can include for example second expansion TILs or second additional expansion TILs (such as, for example, those described in Step D of Figure 1, including TILs referred to as reREP TILs).

[00724] TILs can generally be defined either biochemically, using cell surface markers, or functionally, by their ability to infiltrate tumors and effect treatment. TILs can be generally categorized by expressing one or more of the following biomarkers: CD4, CD8, TCR αβ, CD27, CD28, CD56, CCR7, CD45Ra, CD95, PD-1, and CD25. Additionally, and alternatively, TILs can be functionally defined by their ability to infiltrate solid tumors upon reintroduction into a patient. TILS may further be characterized by potency – for example, TILs may be considered potent if, for example, interferon (IFN) release is greater than about 50 pg/mL, greater than about 100 pg/mL, greater than about 150 pg/mL, or greater than about 200 pg/mL. TILs may be considered potent if, for example, interferon (IFNγ) release is greater than about 50 pg/mL, greater than about 100 pg/mL, greater than about 150 pg/mL, or greater than about 200 pg/mL, greater than about 300 pg/mL, greater than about 400 pg/mL, greater than about 500 pg/mL, greater than about 600 pg/mL, greater than about 700 pg/mL, greater than about 800 pg/mL, greater than about 900 pg/mL, greater than about 1000 pg/mL.

[00725] The terms "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" are intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and inert ingredients. The use of such pharmaceutically acceptable carriers or pharmaceutically acceptable excipients for active pharmaceutical ingredients is well known in the art. Except insofar as any conventional pharmaceutically acceptable carrier or pharmaceutically acceptable excipient is incompatible with the active pharmaceutical ingredient, its use in the therapeutic compositions of the invention is contemplated. Additional active pharmaceutical ingredients, such as other drugs, can also be incorporated into the described compositions and methods.

[00726] The terms "about" and "approximately" mean within a statistically meaningful range of a value. Such a range can be within an order of magnitude, preferably within 50%, more preferably within 20%, more preferably still within 10%, and even more preferably within 5% of a given value or range. The allowable variation encompassed by the terms

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"about" or "approximately" depends on the particular system under study, and can be readily appreciated by one of ordinary skill in the art. Moreover, as used herein, the terms "about" and "approximately" mean that dimensions, sizes, formulations, parameters, shapes and other quantities and characteristics are not and need not be exact, but may be approximate and/or larger or smaller, as desired, reflecting tolerances, conversion factors, rounding off, measurement error and the like, and other factors known to those of skill in the art. In general, a dimension, size, formulation, parameter, shape or other quantity or characteristic is "about" or "approximate" whether or not expressly stated to be such. It is noted that embodiments of very different sizes, shapes and dimensions may employ the described arrangements.

[00727] The transitional terms "comprising," "consisting essentially of," and "consisting of," when used in the appended claims, in original and amended form, define the claim scope with respect to what unrecited additional claim elements or steps, if any, are excluded from the scope of the claim(s). The term "comprising" is intended to be inclusive or open-ended and does not exclude any additional, unrecited element, method, step or material. The term "consisting of" excludes any element, step or material other than those specified in the claim and, in the latter instance, impurities ordinary associated with the specified material(s). The term "consisting essentially of" limits the scope of a claim to the specified elements, steps or material(s) and those that do not materially affect the basic and novel characteristic(s) of the claimed invention. All compositions, methods, and kits described herein that embody the present invention can, in alternate embodiments, be more specifically defined by any of the transitional terms "comprising," "consisting essentially of," and "consisting of."

II. TIL Manufacturing Processes (Embodiments of Gen 3 Processes, optionally including Defined Media)

[00728] In addition to the methods descibred herein, International Application No. PCT/US2019/059718 is incorporated by reference herein in its entirety for all purposes. Without being limited to any particular theory, it is believed that the priming first expansion that primes an activation of T cells followed by the rapid second expansion that boosts the activation of T cells as described in the methods of the invention allows the preparation of expanded T cells that retain a "younger" phenotype, and as such the expanded T cells of the invention are expected to exhibit greater cytotoxicity against cancer cells than T cells expanded by other methods. In particular, it is believed that an activation of T cells that is

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primed by exposure to an anti-CD3 antibody (*e.g.* OKT-3), IL-2 and optionally antigen-presenting cells (APCs) or to IL-2 and a first culture supernatant obtained from a first culture of APCs supplemented with an anti-CD3 antibody (*e.g.* OKT-3), and then boosted by subsequent exposure to additional anti-CD-3 antibody (*e.g.* OKT-3), IL-2 and APCs or to additional IL-2 and a second culture supernatant obtained from a second culture of APCs supplemented with an anti-CD3 antibody (*e.g.* OKT-3), as taught by the methods of the invention limits or avoids the maturation of T cells in culture, yielding a population of T cells with a less mature phenotype, which T cells are less exhausted by expansion in culture and exhibit greater cytotoxicity against cancer cells. In some embodiments, the step of rapid second expansion is split into a plurality of steps to achieve a scaling up of the culture by: (a) performing the rapid second expansion by culturing T cells in a small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 3 to 4 days, and then (b) effecting the transfer of the T cells in the small scale culture to a second container larger than the first container, *e.g.*, a G-REX 500MCS container, and culturing the T cells from the small scale culture in a larger scale culture in the second container for a period of about 4 to 7 days. In some embodiments, the step of rapid expansion is split into a plurality of steps to achieve a scaling out of the culture by: (a) performing the rapid second expansion by culturing T cells in a first small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 3 to 4 days, and then (b) effecting the transfer and apportioning of the T cells from the first small scale culture into and amongst at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 second containers that are equal in size to the first container, wherein in each second container the portion of the T cells from first small scale culture transferred to such second container is cultured in a second small scale culture for a period of about 4 to 7 days. In some embodiments, the step of rapid expansion is split into a plurality of steps to achieve a scaling out and scaling up of the culture by: (a) performing the rapid second expansion by culturing T cells in a small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 3 to 4 days, and then (b) effecting the transfer and apportioning of the T cells from the small scale culture into and amongst at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 second containers that are larger in size than the first container, *e.g.*, G-REX 500MCS containers, wherein in each second container the portion of the T cells from the small scale culture transferred to such second container is cultured in a larger scale culture for a period of about 4 to 7 days. In some embodiments, the step of rapid expansion is split into a plurality of steps to achieve a scaling out and scaling up of the culture by: (a) performing the rapid second expansion by culturing T

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cells in a small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 4 days, and then (b) effecting the transfer and apportioning of the T cells from the small scale culture into and amongst 2, 3 or 4 second containers that are larger in size than the first container, *e.g.*, G-REX 500MCS containers, wherein in each second container the portion of the T cells from the small scale culture transferred to such second container is cultured in a larger scale culture for a period of about 5 days.

[00729] In some embodiments, the rapid second expansion is performed after the activation of T cells effected by the priming first expansion begins to decrease, abate, decay or subside.

[00730] In some embodiments, the rapid second expansion is performed after the activation of T cells effected by the priming first expansion has decreased by at or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%.

[00731] In some embodiments, the rapid second expansion is performed after the activation of T cells effected by the priming first expansion has decreased by a percentage in the range of at or about 1% to 100%.

[00732] In some embodiments, the rapid second expansion is performed after the activation of T cells effected by the priming first expansion has decreased by a percentage in the range of at or about 1% to 10%, 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50%, 50% to 60%, 60% to 70%, 70% to 80%, 80% to 90%, or 90% to 100%.

[00733] In some embodiments, the rapid second expansion is performed after the activation of T cells effected by the priming first expansion has decreased by at least at or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99%.

[00734] In some embodiments, the rapid second expansion is performed after the activation of T cells effected by the priming first expansion has decreased by up to at or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55,

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56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%.

[00735] In some embodiments, the decrease in the activation of T cells effected by the priming first expansion is determined by a reduction in the amount of interferon gamma released by the T cells in response to stimulation with antigen.

[00736] In some embodiments, the priming first expansion of T cells is performed during a period of up to at or about 7 days or about 8 days.

[00737] In some embodiments, the priming first expansion of T cells is performed during a period of up to at or about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, or 8 days.

[00738] In some embodiments, the priming first expansion of T cells is performed during a period of 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, or 8 days.

[00739] In some embodiments, the rapid second expansion of T cells is performed during a period of up to at or about 11 days.

[00740] In some embodiments, the rapid second expansion of T cells is performed during a period of up to at or about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days or 11 days.

[00741] In some embodiments, the rapid second expansion of T cells is performed during a period of 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days or 11 days.

[00742] In some embodiments, the priming first expansion of T cells is performed during a period of from at or about 1 day to at or about 7 days and the rapid second expansion of T cells is performed during a period of from at or about 1 day to at or about 11 days.

[00743] In some embodiments, the priming first expansion of T cells is performed during a period of up to at or about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, or 8 days and the rapid second expansion of T cells is performed during a period of up to at or about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days or 11 days.

[00744] In some embodiments, the priming first expansion of T cells is performed during a period of from at or about 1 day to at or about 8 days and the rapid second expansion of T cells is performed during a period of from at or about 1 day to at or about 9 days.

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[00745] In some embodiments, the priming first expansion of T cells is performed during a period of 8 days and the rapid second expansion of T cells is performed during a period of 9 days.

[00746] In some embodiments, the priming first expansion of T cells is performed during a period of from at or about 1 day to at or about 7 days and the rapid second expansion of T cells is performed during a period of from at or about 1 day to at or about 9 days.

[00747] In some embodiments, the priming first expansion of T cells is performed during a period of 7 days and the rapid second expansion of T cells is performed during a period of 9 days.

[00748] In some embodiments, the T cells are tumor infiltrating lymphocytes (TILs).

[00749] In some embodiments, the T cells are marrow infiltrating lymphocytes (MILs).

[00750] In some embodiments, the T cells are peripheral blood lymphocytes (PBLs).

[00751] In some embodiments, the T cells are obtained from a donor suffering from a cancer.

[00752] In some embodiments, the T cells are TILs obtained from a tumor excised from a patient suffering from a cancer.

[00753] In some embodiments, the T cells are MILs obtained from bone marrow of a patient suffering from a hematologic malignancy.

[00754] In some embodiments, the T cells are PBLs obtained from peripheral blood mononuclear cells (PBMCs) from a donor. In some embodiments, the donor is suffering from a cancer. In some embodiments, the cancer is the cancer is selected from the group consisting of melanoma, ovarian cancer, endometrial cancer, thyroid cancer, colorectal 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 embodiments, 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, triple negative 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 embodments,

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the donor is suffering from a tumor. In some embodiments, the tumor is a liquid tumor. In some embodiments, the tumor is a solid tumor. In some embodiments, the donor is suffering from a hematologic malignancy.

[00755] In certain aspects of the present disclosure, immune effector cells, *e.g.*, T cells, can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as FICOLL separation. In one preferred aspect, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In one aspect, the cells collected by apheresis may be washed to remove the plasma fraction and, optionally, to place the cells in an appropriate buffer or media for subsequent processing steps. In one embodiment, the cells are washed with phosphate buffered saline (PBS). In an alternative embodiment, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations. In one aspect, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL gradient or by counterflow centrifugal elutriation.

[00756] In some embodiments, the T cells are PBLs separated from whole blood or apheresis product enriched for lymphocytes from a donor. In some embodiments, the donor is suffering from a cancer. In some embodiments, the cancer is the cancer is selected from the group consisting of melanoma, ovarian cancer, endometrial cancer, thyroid cancer, colorectal 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 embodiments, 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, triple negative 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 embodments, the donor is suffering from a tumor. In some embodiments, the tumor is a liquid tumor. In some embodiments, the tumor is a solid tumor. In some embodiments, the donor is suffering from a hematologic malignancy. In some embodiments, the PBLs are isolated from whole blood or apheresis product enriched for lymphocytes by using positive or negative selection

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methods, *i.e.*, removing the PBLs using a marker(s), *e.g.*, CD3+ CD45+, for T cell phenotype, or removing non-T cell phenotype cells, leaving PBLs. In other embodiments, the PBLs are isolated by gradient centrifugation. Upon isolation of PBLs from donor tissue, the priming first expansion of PBLs can be initiated by seeding a suitable number of isolated PBLs (in some embodiments, approximately 1×10^7 PBLs) in the priming first expansion culture according to the priming first expansion step of any of the methods described herein.

[00757] An exemplary TIL process known as process 3 (also referred to herein as GEN 3 or Gen 3) containing some of these features is depicted in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), and some of the advantages of this embodiment of the present invention over process 2A are described in Figures 1, and 2 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). Two embodiments of process 3 are shown in Figures 1 and 30 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). Process 2A or Gen 2 is also described in U.S. Patent Publication Nos. 2018/0280436 and 2019/0231820, incorporated by reference herein in their entireties.

[00758] As discussed and generally outlined herein, TILs are taken from a patient sample and manipulated to expand their number prior to transplant into a patient using the TIL expansion process described herein and referred to as Gen 3. In some embodiments, the TILs may be optionally genetically manipulated as discussed below. In some embodiments, the TILs may be cryopreserved prior to or after expansion. Once thawed, they may also be restimulated to increase their metabolism prior to infusion into a patient.

[00759] In some embodiments, the priming first expansion (including processes referred herein as the pre-Rapid Expansion (Pre-REP), as well as processes shown in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) as Step B) is shortened to 1 to 8 days and the rapid second expansion (including processes referred to herein as Rapid Expansion Protocol (REP) as well as processes shown in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) as Step D) is shortened to 1 to 9 days, as discussed in detail below as well as in the examples and figures. In some embodiments, the priming first expansion (including processes referred herein as the pre-Rapid Expansion (Pre-REP), as well as processes shown in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) as Step B) is shortened to 1 to 8 days and the rapid second expansion (including processes referred to herein as Rapid Expansion Protocol (REP) as well as

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processes shown in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) as Step D) is shortened to 1 to 8 days, as discussed in detail below as well as in the examples and figures. In some embodiments, the priming first expansion (including processes referred herein as the pre-Rapid Expansion (Pre-REP), as well as processes shown in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) as Step B) is shortened to 1 to 7 days and the rapid second expansion (including processes referred to herein as Rapid Expansion Protocol (REP) as well as processes shown in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) as Step D) is shortened to 1 to 9 days, as discussed in detail below as well as in the examples and figures. In some embodiments, the priming first expansion (including processes referred herein as the pre-Rapid Expansion (Pre-REP), as well as processes shown in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) as Step B) is 1 to 7 days and the rapid second expansion (including processes referred to herein as Rapid Expansion Protocol (REP) as well as processes shown in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) as Step D) is 1 to 10 days, as discussed in detail below as well as in the examples and figures. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is shortened to 8 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is 7 to 9 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is 8 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is 8 to 9 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is shortened to 7 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is 7 to 8 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is shortened to 8 days and the rapid

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second expansion (for example, an expansion as described in Step D in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is 8 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is 8 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is 9 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is 8 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is 10 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is 7 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is 7 to 10 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is 7 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is 8 to 10 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is 7 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is 9 to 10 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is shortened to 7 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is 7 to 9 days. In some embodiments, the combination of the priming first expansion and rapid second expansion (for example, expansions described as Step B and Step D in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is 14-16 days, as discussed

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in detail below and in the examples and figures. Particularly, it is considered that certain embodiments of the present invention comprise a priming first expansion step in which TILs are activated by exposure to an anti-CD3 antibody, *e.g.*, OKT-3 in the presence of IL-2 or exposure to an antigen in the presence of at least IL-2 and an anti-CD3 antibody *e.g.* OKT-3. In certain embodiments, the TILs which are activated in the priming first expansion step as described above are a first population of TILs *i.e.*, which are a primary cell population.

[00760] The "Step" Designations A, B, C, *etc.*, below are in reference to the non-limiting example in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) and in reference to certain non-limiting embodiments described herein. The ordering of the Steps below and in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) is exemplary and any combination or order of steps, as well as additional steps, repetition of steps, and/or omission of steps is contemplated by the present application and the methods disclosed herein.

A. STEP A: Obtain Patient tumor sample

[00761] In general, TILs are initially obtained from a patient tumor sample ("primary TILs") or from circulating lymphocytes, such as peripherial blood lymphocytes, including perpherial blood lymphocytes having TIL-like characteristics, and are then expanded into a larger population for further manipulation as described herein, optionally cryopreserved, and optionally evaluated for phenotype and metabolic parameters as an indication of TIL health.

[00762] A patient tumor sample may be obtained using methods known in the art, generally via surgical resection, needle biopsy or other means for obtaining a sample that contains a mixture of tumor and TIL cells. In general, the tumor sample may be from any solid tumor, including primary tumors, invasive tumors or metastatic tumors. The tumor sample may also be a liquid tumor, such as a tumor obtained from a hematological malignancy. The solid tumor may be of any cancer type, including, but not limited to, breast, pancreatic, prostate, colorectal, lung, brain, renal, stomach, and skin (including but not limited to squamous cell carcinoma, basal cell carcinoma, and melanoma). In some embodiments, the cancer is selected from cervical cancer, head and neck cancer (including, for example, head and neck squamous cell carcinoma (HNSCC)), glioblastoma (GBM), gastrointestinal cancer, ovarian cancer, sarcoma, pancreatic cancer, bladder cancer, breast cancer, triple negative breast cancer, and non-small cell lung carcinoma. In some embodiments, useful TILs are obtained

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from malignant melanoma tumors, as these have been reported to have particularly high levels of TILs.

[00763] Once obtained, the tumor sample is generally fragmented using sharp dissection into small pieces of between 1 to about 8 mm^3, with from about 2-3 mm^3 being particularly useful. The TILs are cultured from these fragments using enzymatic tumor digests. Such tumor digests may be produced by incubation in enzymatic media (*e.g.*, Roswell Park Memorial Institute (RPMI) 1640 buffer, 2 mM glutamate, 10 mcg/mL gentamicine, 30 units/mL of DNase and 1.0 mg/mL of collagenase) followed by mechanical dissociation (*e.g.*, using a tissue dissociator). Tumor digests may be produced by placing the tumor in enzymatic media and mechanically dissociating the tumor for approximately 1 minute, followed by incubation for 30 minutes at 37 °C in 5% CO_2, followed by repeated cycles of mechanical dissociation and incubation under the foregoing conditions until only small tissue pieces are present. At the end of this process, if the cell suspension contains a large number of red blood cells or dead cells, a density gradient separation using FICOLL branched hydrophilic polysaccharide may be performed to remove these cells. Alternative methods known in the art may be used, such as those described in U.S. Patent Application Publication No. 2012/0244133 A1, the disclosure of which is incorporated by reference herein. Any of the foregoing methods may be used in any of the embodiments described herein for methods of expanding TILs or methods treating a cancer.

[00764] Tumor dissociating enzyme mixtures can include one or more dissociating (digesting) enzymes such as, but not limited to, collagenase (including any blend or type of collagenase), Accutase™, Accumax™, hyaluronidase, neutral protease (dispase), chymotrypsin, chymopapain, trypsin, caseinase, elastase, papain, protease type XIV (pronase), deoxyribonuclease I (DNase), trypsin inhibitor, any other dissociating or proteolytic enzyme, and any combination thereof.

[00765] In some embodiments, the dissociating enzymes are reconstituted from lyophilized enzymes. In some embodiments, lyophilized enzymes are reconstituted in an amount of sterile buffer such as HBSS.

[00766] In some instances, collagenase (such as animal free- type 1 collagenase) is reconstitued in 10 ml of sterile HBSS or another buffer. The lyophilized stock enzyme may be at a concentration of 2892 PZ U/vial. In some embodiments, collagenase is reconstituted in 5 ml to 15 ml buffer. In some embodiment, after reconstitution the collagenase stock

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ranges from about 100 PZ U/ml-about 400 PZ U/ml, *e.g.*, about 100 PZ U/ml-about 400 PZ U/ml, about 100 PZ U/ml-about 350 PZ U/ml, about 100 PZ U/ml-about 300 PZ U/ml, about 150 PZ U/ml-about 400 PZ U/ml, about 100 PZ U/ml, about 150 PZ U/ml, about 200 PZ U/ml, about 210 PZ U/ml, about 220 PZ U/ml, about 230 PZ U/ml, about 240 PZ U/ml, about 250 PZ U/ml, about 260 PZ U/ml, about 270 PZ U/ml, about 280 PZ U/ml, about 289.2 PZ U/ml, about 300 PZ U/ml, about 350 PZ U/ml, or about 400 PZ U/ml.

[00767] In some embodiments, neutral protease is reconstituted in 1-ml of sterile HBSS or another buffer. The lyophilized stock enzyme may be at a concentration of 175 DMC U/vial. The lyophilized stock enzyme may be at a concentration of 175 DMC/mL. In some embodiments, after reconstitution the neutral protease stock ranges from about 100 DMC/ml-about 400 DMC/ml, *e.g.*, about 100 DMC/ml-about 400 DMC/ml, about 100 DMC/ml-about 350 DMC/ml, about 100 DMC/ml-about 300 DMC/ml, about 150 DMC/ml-about 400 DMC/ml, about 100 DMC/ml, about 110 DMC/ml, about 120 DMC/ml, about 130 DMC/ml, about 140 DMC/ml, about 150 DMC/ml, about 160 DMC/ml, about 170 DMC/ml, about 175 DMC/ml, about 180 DMC/ml, about 190 DMC/ml, about 200 DMC/ml, about 250 DMC/ml, about 300 DMC/ml, about 350 DMC/ml, or about 400 DMC/ml.

[00768] In some embodiments, DNAse I is reconstituted in 1-ml of sterile HBSS or another buffer. The lyophilized stock enzyme was at a concentration of 4 KU/vial. In some embodiments, after reconstitution the DNase I stock ranges from about 1 KU/ml-10 KU/ml, *e.g.*, about 1 KU/ml, about 2 KU/ml, about 3 KU/ml, about 4 KU/ml, about 5 KU/ml, about 6 KU/ml, about 7 KU/ml, about 8 KU/ml, about 9 KU/ml, or about 10 KU/ml.

[00769] In some embodiments, the stock of enzymes could change so verify the concentration of the lyophilized stock and amend the final amount of enzyme added to the digest cocktail accordingly.

[00770] In some embodiments, the enzyme mixture includes neutral protease, DNase, and collagenase.

[00771] In some embodiments, the enzyme mixture includes about 10.2-ul of neutral protease (0.36 DMC U/ml), 21.3-ul of collagenase (1.2 PZ/ml) and 250-ul of DNAse I (200 U/ml) in about 4.7-ml of sterile HBSS.

[00772] As indicated above, in some embodiments, the TILs are derived from solid tumors. In some embodiments, the solid tumors are not fragmented. In some embodiments, the solid tumors are not fragmented and are subjected to enzymatic digestion as whole tumors. In some

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embodiments, the tumors are digested in in an enzyme mixture comprising collagenase, DNase, and hyaluronidase. In some embodiments, the tumors are digested in in an enzyme mixture comprising collagenase, DNase, and hyaluronidase for 1-2 hours. In some embodiments, the tumors are digested in in an enzyme mixture comprising collagenase, DNase, and hyaluronidase for 1-2 hours at 37°C, 5% CO_2. In some embodiments, the tumors are digested in in an enzyme mixture comprising collagenase, DNase, and hyaluronidase for 1-2 hours at 37°C, 5% CO_2 with rotation. In some embodiments, the tumors are digested overnight with constant rotation. In some embodiments, the tumors are digested overnight at 37°C, 5% CO_2 with constant rotation. In some embodiments, the whole tumor is combined with the enzymes to form a tumor digest reaction mixture.

[00773] In some embodiments, the tumor is reconstituted with the lyophilized enzymes in a sterile buffer. In some embodiments, the buffer is sterile HBSS.

[00774] In some embodiments, the enxyme mixture comprises collagenase. In some embodiments, the collagenase is collagenase IV. In some embodiments, the working stock for the collagenase is a 100 mg/ml 10X working stock.

[00775] In some embodiments, the enzyme mixture comprises DNAse. In some embodiments, the working stock for the DNAse is a 10,000IU/ml 10X working stock.

[00776] In some embodiments, the enzyme mixture comprises hyaluronidase. In some embodiments, the working stock for the hyaluronidase is a 10-mg/ml 10X working stock.

[00777] In some embodiments, the enzyme mixture comprises 10 mg/ml collagenase, 1000 IU/ml DNAse, and 1 mg/ml hyaluronidase.

[00778] In some embodiments, the enzyme mixture comprises 10 mg/ml collagenase, 500 IU/ml DNAse, and 1 mg/ml hyaluronidase.

[00779] In general, the cell suspension obtained from the tumor is called a "primary cell population" or a "freshly obtained" or a "freshly isolated" cell population. In certain embodiments, the freshly obtained cell population of TILs is exposed to a cell culture medium comprising antigen presenting cells, IL-12 and OKT-3.

[00780] In some embodiments, fragmentation includes physical fragmentation, including, for example, dissection as well as digestion. In some embodiments, the fragmentation is physical fragmentation. In some embodiments, the fragmentation is dissection. In some embodiments, the fragmentation is by digestion. In some embodiments, TILs can be initially

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cultured from enzymatic tumor digests and tumor fragments obtained from patients. In some embodiments, TILs can be initially cultured from enzymatic tumor digests and tumor fragments obtained from patients.

[00781] In some embodiments, where the tumor is a solid tumor, the tumor undergoes physical fragmentation after the tumor sample is obtained in, for example, Step A (as provided in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, the fragmentation occurs before cryopreservation. In some embodiments, the fragmentation occurs after cryopreservation. In some embodiments, the fragmentation occurs after obtaining the tumor and in the absence of any cryopreservation. In some embodiments, the step of fragmentation is an *in vitro* or *ex-vivo* process. In some embodiments, the tumor is fragmented and 10, 20, 30, 40 or more fragments or pieces are placed in each container for the priming first expansion. In some embodiments, the tumor is fragmented and 30 or 40 fragments or pieces are placed in each container for the priming first expansion. In some embodiments, the tumor is fragmented and 40 fragments or pieces are placed in each container for the priming first expansion. In some embodiments, the multiple fragments comprise about 4 to about 50 fragments, wherein each fragment has a volume of about 27 mm^3. In some embodiments, the multiple fragments comprise about 30 to about 60 fragments with a total volume of about 1300 mm^3 to about 1500 mm^3. In some embodiments, the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3. In some embodiments, the multiple fragments comprise about 50 fragments with a total mass of about 1 gram to about 1.5 grams. In some embodiments, the multiple fragments comprise about 4 fragments.

[00782] In some embodiments, the TILs are obtained from tumor fragments. In some embodiments, the tumor fragment is obtained by sharp dissection. In some embodiments, the tumor fragment is between about 1 mm^3 and 10 mm^3. In some embodiments, the tumor fragment is between about 1 mm^3 and 8 mm^3. In some embodiments, the tumor fragment is about 1 mm^3. In some embodiments, the tumor fragment is about 2 mm^3. In some embodiments, the tumor fragment is about 3 mm^3. In some embodiments, the tumor fragment is about 4 mm^3. In some embodiments, the tumor fragment is about 5 mm^3. In some embodiments, the tumor fragment is about 6 mm^3. In some embodiments, the tumor fragment is about 7 mm^3. In some embodiments, the tumor fragment is about 8 mm^3. In some embodiments, the tumor fragment is about 9 mm^3. In some embodiments, the tumor fragment is about 10 mm^3. In some embodiments, the tumor fragments are 1-4 mm x 1-4 mm x 1-4

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mm. In some embodiments, the tumor fragments are 1 mm x 1 mm x 1 mm. In some embodiments, the tumor fragments are 2 mm x 2 mm x 2 mm. In some embodiments, the tumor fragments are 3 mm x 3 mm x 3 mm. In some embodiments, the tumor fragments are 4 mm x 4 mm x 4 mm.

[00783] In some embodiments, the tumors are fragmented in order to minimize the amount of hemorrhagic, necrotic, and/or fatty tissues on each piece. In some embodiments, the tumors are fragmented in order to minimize the amount of hemorrhagic tissue on each piece. In some embodiments, the tumors are fragmented in order to minimize the amount of necrotic tissue on each piece. In some embodiments, the tumors are fragmented in order to minimize the amount of fatty tissue on each piece. In certain embodiments, the step of fragmentation of the tumor is an *in vitro* or *ex-vivo* method.

[00784] In some embodiments, the tumor fragmentation is performed in order to maintain the tumor internal structure. In some embodiments, the tumor fragmentation is performed without preforming a sawing motion with a scalpel. In some embodiments, the TILs are obtained from tumor digests. In some embodiments, tumor digests were generated by incubation in enzyme media, for example but not limited to RPMI 1640, 2 mM GlutaMAX, 10 mg/mL gentamicin, 30 U/mL DNase, and 1.0 mg/mL collagenase, followed by mechanical dissociation (GentleMACS, Miltenyi Biotec, Auburn, CA). After placing the tumor in enzyme media, the tumor can be mechanically dissociated for approximately 1 minute. The solution can then be incubated for 30 minutes at 37 °C in 5% CO_2 and it then mechanically disrupted again for approximately 1 minute. After being incubated again for 30 minutes at 37 °C in 5% CO_2, the tumor can be mechanically disrupted a third time for approximately 1 minute. In some embodiments, after the third mechanical disruption if large pieces of tissue were present, 1 or 2 additional mechanical dissociations were applied to the sample, with or without 30 additional minutes of incubation at 37 °C in 5% CO_2. In some embodiments, at the end of the final incubation if the cell suspension contained a large number of red blood cells or dead cells, a density gradient separation using Ficoll can be performed to remove these cells.

[00785] In some embodiments, the cell suspension prior to the priming first expansion step is called a "primary cell population" or a "freshly obtained" or "freshly isolated" cell population.

[00786] In some embodiments, cells can be optionally frozen after sample isolation (*e.g.*, after obtaining the tumor sample and/or after obtaining the cell suspension from the tumor sample) and stored frozen prior to entry into the expansion described in Step B, which is described in further detail below, as well as exemplified in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G).

1. Core/Small Biopsy Derived TILS

[00787] In some embodiments, TILs are initially obtained from a patient tumor sample ("primary TILs") obtained by a core biopsy or similar procedure and then expanded into a larger population for further manipulation as described herein, optionally cryopreserved, and optionally evaluated for phenotype and metabolic parameters.

[00788] In some emboidments, a patient tumor sample may be obtained using methods known in the art, generally via small biopsy, core biopsy, needle biopsy or other means for obtaining a sample that contains a mixture of tumor and TIL cells. In general, the tumor sample may be from any solid tumor, including primary tumors, invasive tumors or metastatic tumors. The tumor sample may also be a liquid tumor, such as a tumor obtained from a hematological malignancy. In some embodiments, the sample can be from multiple small tumor samples or biopsies. In some embodiments, the sample can comprise multiple tumor samples from a single tumor from the same patient. In some embodiments, the sample can comprise multiple tumor samples from one, two, three, or four tumors from the same patient. In some embodiments, the sample can comprise multiple tumor samples from multiple tumors from the same patient. The solid tumor may be of any cancer type, including, but not limited to, breast, pancreatic, prostate, colorectal, lung, brain, renal, stomach, and skin (including but not limited to squamous cell carcinoma, basal cell carcinoma, and melanoma). In some embodiments, the cancer is selected from cervical cancer, head and neck cancer (including, for example, head and neck squamous cell carcinoma (HNSCC)), glioblastoma (GBM), gastrointestinal cancer, ovarian cancer, sarcoma, pancreatic cancer, bladder cancer, breast cancer, triple negative breast cancer, and non-small cell lung carcinoma (NSCLC). In some embodiments, useful TILs are obtained from malignant melanoma tumors, as these have been reported to have particularly high levels of TILs.

[00789] In general, the cell suspension obtained from the tumor core or fragment is called a "primary cell population" or a "freshly obtained" or a "freshly isolated" cell

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population. In certain embodiments, the freshly obtained cell population of TILs is exposed to a cell culture medium comprising antigen presenting cells, IL-2 and OKT-3.

[00790] In some embodiments, if the tumor is metastatic and the primary lesion has been efficiently treated/removed in the past, removal of one of the metastatic lesions may be needed. In some embodiments, the least invasive approach is to remove a skin lesion, or a lymph node on the neck or axillary area when available. In some embodiments, a skin lesion is removed or small biopsy thereof is removed. In some embodiments, a lymph node or small biopsy thereof is removed. In some embodiments, a lung or liver metastatic lesion, or an intra-abdominal or thoracic lymph node or small biopsy thereof can be employed.

[00791] In some embodiments, the tumor is a melanoma. In some embodiments, the small biopsy for a melanoma comprises a mole or portion thereof.

[00792] In some embodiments, the small biopsy is a punch biopsy. In some embodiments, the punch biopsy is obtained with a circular blade pressed into the skin. In some embodiments, the punch biopsy is obtained with a circular blade pressed into the skin. around a suspicious mole. In some embodiments, the punch biopsy is obtained with a circular blade pressed into the skin, and a round piece of skin is removed. In some embodiments, the small biopsy is a punch biopsy and round portion of the tumor is removed.

[00793] In some embodiments, the small biopsy is an excisional biopsy. In some embodiments, the small biopsy is an excisional biopsy and the entire mole or growth is removed. In some embodiments, the small biopsy is an excisional biopsy and the entire mole or growth is removed along with a small border of normal-appearing skin.

[00794] In some embodiments, the small biopsy is an incisional biopsy. In some embodiments, the small biopsy is an incisional biopsy and only the most irregular part of a mole or growth is taken. In some embodiments, the small biopsy is an incisional biopsy and the incisional biopsy is used when other techniques can't be completed, such as if a suspicious mole is very large.

[00795] In some embodiments, the small biopsy is a lung biopsy. In some embodiments, the small biopsy is obtained by bronchoscopy. Generally, bronchoscopy, the patient is put under anesthesia, and a small tool goes through the nose or mouth, down the throat, and into the bronchial passages, where small tools are used to remove some tissue. In some embodiments, where the tumor or growth cannot be reached via bronchoscopy, a transthoracic needle biopsy can be employed. Generally, for a transthoracic needle biopsy,

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the patient is also under anesthesia and a needle is inserted through the skin directly into the suspicious spot to remove a small sample of tissue. In some embodiments, a transthoracic needle biopsy may require interventional radiology (for example, the use of x-rays or CT scan to guide the needle). In some embodiments, the small biopsy is obtained by needle biopsy. In some embodiments, the small biopsy is obtained endoscopic ultrasound (for example, an endoscope with a light and is placed through the mouth into the esophagus). In some embodiments, the small biopsy is obtained surgically.

[00796] In some embodiments, the small biopsy is a head and neck biopsy. In some embodiments, the small biopsy is an incisional biopsy. In some embodiments, the small biopsy is an incisional biopsy, wherein a small piece of tissue is cut from an abnormal-looking area. In some embodiments, if the abnormal region is easily accessed, the sample may be taken without hospitalization. In some embodiments, if the tumor is deeper inside the mouth or throat, the biopsy may need to be done in an operating room, with general anesthesia. In some embodiments, the small biopsy is an excisional biopsy. In some embodiments, the small biopsy is an excisional biopsy, wherein the whole area is removed. In some embodiments, the small biopsy is a fine needle aspiration (FNA). In some embodiments, the small biopsy is a fine needle aspiration (FNA), wherein a very thin needle attached to a syringe is used to extract (aspirate) cells from a tumor or lump. In some embodiments, the small biopsy is a punch biopsy. In some embodiments, the small biopsy is a punch biopsy, wherein punch forceps are used to remove a piece of the suspicious area.

[00797] In some embodiments, the small biopsy is a cervical biopsy. In some embodiments, the small biopsy is obtained via colposcopy. Generally, colposcopy methods employ the use of a lighted magnifying instrument attached to magnifying binoculars (a colposcope) which is then used to biopsy a small section of the surface of the cervix. In some embodiments, the small biopsy is a conization/cone biopsy. In some embodiments, the small biopsy is a conization/cone biopsy, wherein an outpatient surgery may be needed to remove a larger piece of tissue from the cervix. In some embodiments, the cone biopsy, in addition to helping to confirm a diagnosis, a cone biopsy can serve as an initial treatment.

[00798] The term "solid tumor" refers to an abnormal mass of tissue that usually does not contain cysts or liquid areas. Solid tumors may be benign or malignant. The term "solid tumor cancer refers to malignant, neoplastic, or cancerous solid tumors. Solid tumor cancers include, but are not limited to, sarcomas, carcinomas, and lymphomas, such as cancers of the lung, breast, triple negative breast cancer, prostate, colon, rectum, and bladder. In some

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embodiments, the cancer is selected from cervical cancer, head and neck cancer, glioblastoma, ovarian cancer, sarcoma, pancreatic cancer, bladder cancer, breast cancer, triple negative breast cancer, and non-small cell lung carcinoma. The tissue structure of solid tumors includes interdependent tissue compartments including the parenchyma (cancer cells) and the supporting stromal cells in which the cancer cells are dispersed and which may provide a supporting microenvironment.

[00799] In some embodiments, the sample from the tumor is obtained as a fine needle aspirate (FNA), a core biopsy, a small biopsy (including, for example, a punch biopsy). In some embodiments, sample is placed first into a G-Rex 10. In some embodiments, sample is placed first into a G-Rex 10 when there are 1 or 2 core biopsy and/or small biopsy samples. In some embodiments, sample is placed first into a G-Rex 100 when there are 3, 4, 5, 6, 8, 9, or 10 or more core biopsy and/or small biopsy samples. In some embodiments, sample is placed first into a G-Rex 500 when there are 3, 4, 5, 6, 8, 9, or 10 or more core biopsy and/or small biopsy samples.

[00800] The FNA can be obtained from a tumor selected from the group consisting of lung, melanoma, head and neck, cervical, ovarian, pancreatic, glioblastoma, colorectal, and sarcoma. In some embodiments, the FNA is obtained from a lung tumor, such as a lung tumor from a patient with non-small cell lung cancer (NSCLC). In some cases, the patient with NSCLC has previously undergone a surgical treatment.

[00801] TILs described herein can be obtained from an FNA sample. In some cases, the FNA sample is obtained or isolated from the patient using a fine gauge needle ranging from an 18 gauge needle to a 25 gauge needle. The fine gauge needle can be 18 gauge, 19 gauge, 20 gauge, 21 gauge, 22 gauge, 23 gauge, 24 gauge, or 25 gauge. In some embodiments, the FNA sample from the patient can contain at least 400,000 TILs, *e.g.*, 400,000 TILs, 450,000 TILs, 500,000 TILs, 550,000 TILs, 600,000 TILs, 650,000 TILs, 700,000 TILs, 750,000 TILs, 800,000 TILs, 850,000 TILs, 900,000 TILs, 950,000 TILs, or more.

[00802] In some cases, the TILs described herein are obtained from a core biopsy sample. In some cases, the core biopsy sample is obtained or isolated from the patient using a surgical or medical needle ranging from an 11 gauge needle to a 16 gauge needle. The needle can be 11 gauge, 12 gauge, 13 gauge, 14 gauge, 15 gauge, or 16 gauge. In some embodiments, the core biopsy sample from the patient can contain at least 400,000 TILs, *e.g.*,

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400,000 TILs, 450,000 TILs, 500,000 TILs, 550,000 TILs, 600,000 TILs, 650,000 TILs, 700,000 TILs, 750,000 TILs, 800,000 TILs, 850,000 TILs, 900,000 TILs, 950,000 TILs, or more.

[00803] In general, the harvested cell suspension is called a "primary cell population" or a "freshly harvested" cell population.

[00804] In some embodiments, the TILs are not obtained from tumor digests. In some embodiments, the solid tumor cores are not fragmented.

[00805] In some embodiments, the TILs are obtained from tumor digests. In some embodiments, tumor digests were generated by incubation in enzyme media, for example but not limited to RPMI 1640, 2mM GlutaMAX, 10 mg/mL gentamicin, 30 U/mL DNase, and 1.0 mg/mL collagenase, followed by mechanical dissociation (GentleMACS, Miltenyi Biotec, Auburn, CA). After placing the tumor in enzyme media, the tumor can be mechanically dissociated for approximately 1 minute. The solution can then be incubated for 30 minutes at 37 °C in 5% CO_2 and it then mechanically disrupted again for approximately 1 minute. After being incubated again for 30 minutes at 37 °C in 5% CO_2, the tumor can be mechanically disrupted a third time for approximately 1 minute. In some embodiments, after the third mechanical disruption if large pieces of tissue were present, 1 or 2 additional mechanical dissociations were applied to the sample, with or without 30 additional minutes of incubation at 37 °C in 5% CO_2. In some embodiments, at the end of the final incubation if the cell suspension contained a large number of red blood cells or dead cells, a density gradient separation using Ficoll can be performed to remove these cells.

[00806] In some embodiments, obtaining the first population of TILs comprises a multilesional sampling method.

[00807] Tumor dissociating enzyme mixtures can include one or more dissociating (digesting) enzymes such as, but not limited to, collagenase (including any blend or type of collagenase), Accutase™, Accumax™, hyaluronidase, neutral protease (dispase), chymotrypsin, chymopapain, trypsin, caseinase, elastase, papain, protease type XIV (pronase), deoxyribonuclease I (DNase), trypsin inhibitor, any other dissociating or proteolytic enzyme, and any combination thereof.

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[00808] In some embodiments, the dissociating enzymes are reconstituted from lyophilized enzymes. In some embodiments, lyophilized enzymes are reconstituted in an amount of sterile buffer such as HBSS.

[00809] In some instances, collagenase (such as animal free- type 1 collagenase) is reconstitued in 10 ml of sterile HBSS or another buffer. The lyophilized stock enzyme may be at a concentration of 2892 PZ U/vial. In some embodiments, collagenase is reconstituted in 5 ml to 15 ml buffer. In some embodiment, after reconstitution the collagenase stock ranges from about 100 PZ U/ml-about 400 PZ U/ml, *e.g.*, about 100 PZ U/ml-about 400 PZ U/ml, about 100 PZ U/ml-about 350 PZ U/ml, about 100 PZ U/ml-about 300 PZ U/ml, about 150 PZ U/ml-about 400 PZ U/ml, about 100 PZ U/ml, about 150 PZ U/ml, about 200 PZ U/ml, about 210 PZ U/ml, about 220 PZ U/ml, about 230 PZ U/ml, about 240 PZ U/ml, about 250 PZ U/ml, about 260 PZ U/ml, about 270 PZ U/ml, about 280 PZ U/ml, about 289.2 PZ U/ml, about 300 PZ U/ml, about 350 PZ U/ml, or about 400 PZ U/ml.

[00810] In some embodiments neutral protease is reconstituted in 1-ml of sterile HBSS or another buffer. The lyophilized stock enzyme may be at a concentration of 175 DMC U/vial. The lyophilized stock enzyme may be at a concentration of 175 DMC/mL. In some embodiments, after reconstitution the neutral protease stock ranges from about 100 DMC/ml-about 400 DMC/ml, *e.g.*, about 100 DMC/ml-about 400 DMC/ml, about 100 DMC/ml-about 350 DMC/ml, about 100 DMC/ml-about 300 DMC/ml, about 150 DMC/ml-about 400 DMC/ml, about 100 DMC/ml, about 110 DMC/ml, about 120 DMC/ml, about 130 DMC/ml, about 140 DMC/ml, about 150 DMC/ml, about 160 DMC/ml, about 170 DMC/ml, about 175 DMC/ml, about 180 DMC/ml, about 190 DMC/ml, about 200 DMC/ml, about 250 DMC/ml, about 300 DMC/ml, about 350 DMC/ml, or about 400 DMC/ml.

[00811] In some embodiments, DNAse I is reconstituted in 1-ml of sterile HBSS or another buffer. The lyophilized stock enzyme was at a concentration of 4 KU/vial. In some embodiments, after reconstitution the DNase I stock ranges from about 1 KU/ml-10 KU/ml, *e.g.*, about 1 KU/ml, about 2 KU/ml, about 3 KU/ml, about 4 KU/ml, about 5 KU/ml, about 6 KU/ml, about 7 KU/ml, about 8 KU/ml, about 9 KU/ml, or about 10 KU/ml.

[00812] In some embodiments, the stock of enzymes could change so verify the concentration of the lyophilized stock and amend the final amount of enzyme added to the digest cocktail accordingly.

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[00813] In some embodiments, the enzyme mixture includes neutral protease, collagenase and DNase.

[00814] In some embodiments, the enzyme mixture includes about 10.2-ul of neutral protease (0.36 DMC U/ml), 21.3-ul of collagenase (1.2 PZ/ml) and 250-ul of DNAse I (200 U/ml) in about 4.7-ml of sterile HBSS.

2. Pleural Effusion TILs

[00815] In some embodiments, the sample is a pleural fluid sample. In some embodiments, the source of the TILs for expansion according to the processes described herein is a pleural fluid sample. In some embodiments, the sample is a pleural effusion derived sample. In some embodiments, the source of the TILs for expansion according to the processes described herein is a pleural effusion derived sample. See, for example, methods described in U.S. Patent Publication US 2014/0295426, incorporated herein by reference in its entirety for all purposes.

[00816] In some embodiments, any pleural fluid or pleural effusion suspected of and/or containing TILs can be employed. Such a sample may be derived from a primary or metastatic lung cancer, such as NSCLC or SCLC. In some embodiments, the sample may be secondary metastatic cancer cells which originated from another organ, *e.g.*, breast, ovary, colon or prostate. In some embodiments, the sample for use in the expansion methods described herein is a pleural exudate. In some embodiments, the sample for use in the expansion methods described herein is a pleural transudate. Other biological samples may include other serous fluids containing TILs, including, *e.g.*, ascites fluid from the abdomen or pancreatic cyst fluid. Ascites fluid and pleural fluids involve very similar chemical systems; both the abdomen and lung have mesothelial lines and fluid forms in the pleural space and abdominal spaces in the same matter in malignancies and such fluids in some embodiments contain TILs. In some embodiments, wherein the disclosure exemplifies pleural fluid, the same methods may be performed with similar results using ascites or other cyst fluids containing TILs.

[00817] In some embodiments, the pleural fluid is in unprocessed form, directly as removed from the patient. In some embodiments, the unprocessed pleural fluid is placed in a standard blood collection tube, such as an EDTA or Heparin tube, prior to the contacting step. In some embodiments, the unprocessed pleural fluid is placed in a standard CellSave® tube (Veridex) prior to the contacting step. In some embodiments, the sample is placed in the CellSave tube

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immediately after collection from the patient to avoid a decrease in the number of viable TILs. The number of viable TILs can decrease to a significant extent within 24 hours, if left in the untreated pleural fluid, even at 4°C. In some embodiments, the sample is placed in the appropriate collection tube within 1 hour, 5 hours, 10 hours, 15 hours, or up to 24 hours after removal from the patient. In some embodiments, the sample is placed in the appropriate collection tube within 1 hour, 5 hours, 10 hours, 15 hours, or up to 24 hours after removal from the patient at 4°C.

[00818] In some embodiments, the pleural fluid sample from the chosen subject may be diluted. In one embodiment, the dilution is 1:10 pleural fluid to diluent. In other embodiments, the dilution is 1:9 pleural fluid to diluent. In other embodiments, the dilution is 1:8 pleural fluid to diluent. In other embodiments, the dilution is 1:5 pleural fluid to diluent. In other embodiments, the dilution is 1:2 pleural fluid to diluent. In other embodiments, the dilution is 1:1 pleural fluid to diluent. In some embodiments, diluents include saline, phosphate buffered saline, another buffer or a physiologically acceptable diluent. In some embodiments, the sample is placed in the CellSave tube immediately after collection from the patient and dilution to avoid a decrease in the viable TILs, which may occur to a significant extent within 24-48 hours, if left in the untreated pleural fluid, even at 4°C. In some embodiments, the pleural fluid sample is placed in the appropriate collection tube within 1 hour, 5 hours, 10 hours, 15 hours, 24 hours, 36 hours, up to 48 hours after removal from the patient, and dilution. In some embodiments, the pleural fluid sample is placed in the appropriate collection tube within 1 hour, 5 hours, 10 hours, 15 hours, 24 hours, 36 hours, up to 48 hours after removal from the patient, and dilution at 4°C.

[00819] In some embodiments, pleural fluid samples are concentrated by conventional means prior further processing steps. In some embodiments, this pre-treatment of the pleural fluid is preferable in circumstances in which the pleural fluid must be cryopreserved for shipment to a laboratory performing the method or for later analysis (*e.g.*, later than 24-48 hours post-collection). In some embodiments, the pleural fluid sample is prepared by centrifuging the pleural fluid sample after its withdrawal from the subject and resuspending the centrifugate or pellet in buffer. In some embodiments, the pleural fluid sample is subjected to multiple centrifugations and resuspensions, before it is cryopreserved for transport or later analysis and/or processing.

[00820] In some embodiments, pleural fluid samples are concentrated prior to further processing steps by using a filtration method. In some embodiments, the pleural fluid sample

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used in the contacting step is prepared by filtering the fluid through a filter containing a known and essentially uniform pore size that allows for passage of the pleural fluid through the membrane but retains the tumor cells. In some embodiments, the diameter of the pores in the membrane may be at least 4 μM. In other embodiments the pore diameter may be 5 μM or more, and in other embodiment, any of 6, 7, 8, 9, or 10 μM. After filtration, the cells, including TILs, retained by the membrane may be rinsed off the membrane into a suitable physiologically acceptable buffer. Cells, including TILs, concentrated in this way may then be used in the contacting step of the method.

[00821] In some embodiments, pleural fluid sample (including, for example, the untreated pleural fluid), diluted pleural fluid, or the resuspended cell pellet, is contacted with a lytic reagent that differentially lyses non-nucleated red blood cells present in the sample. In some embodiments, this step is performed prior to further processing steps in circumstances in which the pleural fluid contains substantial numbers of RBCs. Suitable lysing reagents include a single lytic reagent or a lytic reagent and a quench reagent, or a lytic agent, a quench reagent and a fixation reagent. Suitable lytic systems are marketed commercially and include the BD Pharm Lyse™ system (Becton Dickenson). Other lytic systems include the Versalyse™ system, the FACSlyse™ system (Becton Dickenson), the Immunoprep™ system or Erythrolyse II system (Beckman Coulter, Inc.), or an ammonium chloride system. In some embodiments, the lytic reagent can vary with the primary requirements being efficient lysis of the red blood cells, and the conservation of the TILs and phenotypic properties of the TILs in the pleural fluid. In addition to employing a single reagent for lysis, the lytic systems useful in methods described herein can include a second reagent, *e.g.*, one that quenches or retards the effect of the lytic reagent during the remaining steps of the method, *e.g.*, Stabilyse™ reagent (Beckman Coulter, Inc.). A conventional fixation reagent may also be employed depending upon the choice of lytic reagents or the preferred implementation of the method.

[00822] In some embodiments, the pleural fluid sample, unprocessed, diluted or multiply centrifuged or processed as described herein above is cryopreserved at a temperature of about −140°C prior to being further processed and/or expanded as provided herein.

3. Methods of Expanding Peripheral Blood Lymphocytes (PBLs) from Peripheral Blood

[00823] PBL Method 1. In some embodiments of the invention, PBLs are expanded using the processes described herein. In some embodiments of the invention, the method comprises

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obtaining a PBMC sample from whole blood. In some embodiments, the method comprises enriching T-cells by isolating pure T-cells from PBMCs using negative selection of a non-CD19+ fraction. In some embodiments, the method comprises enriching T-cells by isolating pure T-cells from PBMCs using magnetic bead-based negative selection of a non-CD19+ fraction.

[00824] In some embodiments of the invention, PBL Method 1 is performed as follows: On Day 0, a cryopreserved PBMC sample is thawed and PBMCs are counted. T-cells are isolated using a Human Pan T-Cell Isolation Kit and LS columns (Miltenyi Biotec).

[00825] PBL Method 2. In some embodiments of the invention, PBLs are expanded using PBL Method 2, which comprises obtaining a PBMC sample from whole blood. The T-cells from the PBMCs are enriched by incubating the PBMCs for at least three hours at 37°C and then isolating the non-adherent cells.

[00826] In some embodiments of the invention, PBL Method 2 is performed as follows: On Day 0, the cryopreserved PMBC sample is thawed and the PBMC cells are seeded at 6 million cells per well in a 6 well plate in CM-2 media and incubated for 3 hours at 37 degrees Celsius. After 3 hours, the non-adherent cells, which are the PBLs, are removed and counted.

[00827] PBL Method 3. In some embodiments of the invention, PBLs are expanded using PBL Method 3, which comprises obtaining a PBMC sample from peripheral blood. B-cells are isolated using a CD19+ selection and T-cells are selected using negative selection of the non-CD19+ fraction of the PBMC sample.

[00828] In some embodiments of the invention, PBL Method 3 is performed as follows: On Day 0, cryopreserved PBMCs derived from peripheral blood are thawed and counted. CD19+ B-cells are sorted using a CD19 Multisort Kit, Human (Miltenyi Biotec). Of the non-CD19+ cell fraction, T-cells are purified using the Human Pan T-cell Isolation Kit and LS Columns (Miltenyi Biotec).

[00829] In some embodiments, PBMCs are isolated from a whole blood sample. In some embodiments, the PBMC sample is used as the starting material to expand the PBLs. In some embodiments, the sample is cryopreserved prior to the expansion process. In other embodiments, a fresh sample is used as the starting material to expand the PBLs. In some embodiments of the invention, T-cells are isolated from PBMCs using methods known in the art. In some embodiments, the T-cells are isolated using a Human Pan T-cell isolation kit and

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LS columns. In some embodiments of the invention, T-cells are isolated from PBMCs using antibody selection methods known in the art, for example, CD19 negative selection.

[00830] In some embodiments of the invention, the PBMC sample is incubated for a period of time at a desired temperature effective to identify the non-adherent cells. In some embodiments of the invention, the incubation time is about 3 hours. In some embodiments of the invention, the temperature is about 37° Celsius. The non-adherent cells are then expanded using the process described above.

[00831] In some embodiments, the PBMC sample is from a subject or patient who has been optionally pre-treated with a regimen comprising a kinase inhibitor or an ITK inhibitor. In some embodiments, the tumor sample is from a subject or patient who has been pre-treated with a regimen comprising a kinase inhibitor or an ITK inhibitor. In some embodiments, the PBMC sample is from a subject or patient who has been pre-treated with a regimen comprising a kinase inhibitor or an ITK inhibitor, has undergone treatment for at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, or 1 year or more. In other embodiments, the PBMCs are derived from a patient who is currently on an ITK inhibitor regimen, such as ibrutinib.

[00832] In some embodiments, the PBMC sample is from a subject or patient who has been pre-treated with a regimen comprising a kinase inhibitor or an ITK inhibitor and is refractory to treatment with a kinase inhibitor or an ITK inhibitor, such as ibrutinib.

[00833] In some embodiments, the PBMC sample is from a subject or patient who has been pre-treated with a regimen comprising a kinase inhibitor or an ITK inhibitor but is no longer undergoing treatment with a kinase inhibitor or an ITK inhibitor. In some embodiments, the PBMC sample is from a subject or patient who has been pre-treated with a regimen comprising a kinase inhibitor or an ITK inhibitor but is no longer undergoing treatment with a kinase inhibitor or an ITK inhibitor and has not undergone treatment for at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, or at least 1 year or more. In other embodiments, the PBMCs are derived from a patient who has prior exposure to an ITK inhibitor, but has not been treated in at least 3 months, at least 6 months, at least 9 months, or at least 1 year.

[00834] In an embodment of the invention, at Day 0, cells are selected for CD19+ and sorted accordingly. In some embodiments of the invention, the selection is made using antibody

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binding beads. In some embodiments of the invention, pure T-cells are isolated on Day 0 from the PBMCs.

[00835] In some embodiments of the invention, for patients that are not pre-treated with ibrutinib or other ITK inhibitor, 10-15ml of Buffy Coat will yield about 5×10^9 PBMC, which, in turn, will yield about 5.5×10^7 PBLs.

[00836] In some embodiments of the invention, for patients that are pre-treated with ibrutinib or other ITK inhibitor, the expansion process will yield about 20×10^9 PBLs. In some embodiments of the invention, 40.3×10^6 PBMCs will yield about 4.7×10^5 PBLs.

[00837] In any of the foregoing embodiments, PBMCs may be derived from a whole blood sample, by apheresis, from the buffy coat, or from any other method known in the art for obtaining PBMCs.

4. Methods of Expanding Marrow Infiltrating Lymphocytes (MILs) from PBMCs Derived from Bone Marrow

[00838] MIL Method 3. In some embodiments of the invention, the method comprises obtaining PBMCs from the bone marrow. On Day 0, the PBMCs are selected for CD3+/CD33+/CD20+/CD14+ and sorted, and the non-CD3+/CD33+/CD20+/CD14+ cell fraction is sonicated and a portion of the sonicated cell fraction is added back to the selected cell fraction.

[00839] In some embodiments of the invention, MIL Method 3 is performed as follows: On Day 0, a cryopreserved sample of PBMCs is thawed and PBMCs are counted. The cells are stained with CD3, CD33, CD20, and CD14 antibodies and sorted using a S3e cell sorted (Bio-Rad). The cells are sorted into two fractions – an immune cell fraction (or the MIL fraction) (CD3+CD33+CD20+CD14+) and an AML blast cell fraction (non-CD3+CD33+CD20+CD14+).

[00840] In some embodiments of the invention, PBMCs are obtained from bone marrow. In some embodiments, the PBMCs are obtained from the bone marrow through apheresis, aspiration, needle biopsy, or other similar means known in the art. In some embodiments, the PBMCs are fresh. In other embodiments, the PBMCs are cryopreserved.

[00841] In some embodiments of the invention, MILs are expanded from 10-50 ml of bone marrow aspirate. In some embodiments of the invention, 10ml of bone marrow aspirate is obtained from the patient. In other embodiments, 20ml of bone marrow aspirate is obtained

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from the patient. In other embodiments, 30ml of bone marrow aspirate is obtained from the patient. In other embodiments, 40ml of bone marrow aspirate is obtained from the patient. In other embodiments, 50ml of bone marrow aspirate is obtained from the patient.

[00842] In some embodiments of the invention, the number of PBMCs yielded from about 10-50ml of bone marrow aspirate is about 5×10^7 to about 10×10^7 PBMCs. In other embodiments, the number of PMBCs yielded is about 7×10^7 PBMCs.

[00843] In some embodiments of the invention, about 5×10^7 to about 10×10^7 PBMCs, yields about 0.5×10^6 to about 1.5×10^6 MILs. In some embodiments of the invention, about 1×10^6 MILs is yielded.

[00844] In some embodiments of the invention, 12×10^6 PBMC derived from bone marrow aspirate yields approximately 1.4×10^5 MILs.

[00845] In any of the foregoing embodiments, PBMCs may be derived from a whole blood sample, from bone marrow, by apheresis, from the buffy coat, or from any other method known in the art for obtaining PBMCs.

B. STEP B: Priming First Expansion

[00846] In some embodiments, the present methods provide for younger TILs, which may provide additional therapeutic benefits over older TILs (*i.e.*, TILs which have further undergone more rounds of replication prior to administration to a subject/patient). Features of young TILs have been described in the literature, for example Donia, at al., *Scandinavian Journal of Immunology,* 75:157–167 (2012); Dudley et al., *Clin Cancer Res,* 16:6122-6131 (2010); Huang et al., *J Immunother*, 28(3):258–267 (2005); Besser et al., *Clin Cancer Res*, 19(17):OF1-OF9 (2013); Besser et al., *J Immunother* 32:415–423 (2009); Robbins, et al., *J Immunol* 2004; 173:7125-7130; Shen et al., J Immunother, 30:123–129 (2007); Zhou, et al., *J Immunother*, 28:53–62 (2005); and Tran, et al., J Immunother, 31:742–751 (2008), all of which are incorporated herein by reference in their entireties.

[00847] After dissection or digestion of tumor fragments and/or tumor fragments, for example such as described in Step A of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), the resulting cells are cultured in serum containing IL-2, OKT-3, and either feeder cells (*e.g.*, antigen-presenting feeder cells) and/or culture supernatant from a first culture of APCs comprising OKT-3, under conditions that favor the growth of TILs over tumor and other cells. In some embodiments, the IL-2,

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OKT-3, and feeder cells are added at culture initiation along with the tumor digest and/or tumor fragments (*e.g.*, at Day 0). In some embodiments, the tumor digests and/or tumor fragments are incubated in a container with up to 60 fragments per container. In some embodiments, the tumor digests and/or tumor fragments are incubated in a container with up to 80 fragments per container. In some embodiments, the tumor digests and/or tumor fragments are incubated in a container with up to 100 fragments per container In some embodiments, the tumor digests and/or tumor fragments are incubated in a container with up to 60 fragments per container and with 6000 IU/mL of IL-2. In some embodiments, the tumor digests and/or tumor fragments are incubated in a container with up to 80 fragments per container and with 6000 IU/mL of IL-2. In some embodiments, the tumor digests and/or tumor fragments are incubated in a container with up to 100 fragments per container and with 6000 IU/mL of IL-2. In some embodiments, this primary cell population is cultured for a period of days, generally from 1 to 8 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this period is refered to activation 1. In some embodiments, this primary cell population is cultured for a period of days, generally from 1 to 7 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, priming first expansion occurs for a period of 1 to 8 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, priming first expansion occurs for a period of 1 to 7 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, priming first expansion occurs for a period of 1 to 3 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, priming first expansion occurs for a period of 1 to 4 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, priming first expansion occurs for a period of 1 to 5 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, priming first expansion occurs for a period of 1 to 6 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this priming first expansion occurs for a period of 5 to 8 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this priming first expansion occurs for a period of 5 to 7 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this priming first expansion occurs for a period of about 6 to 8 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this priming first expansion occurs for a period of about 6 to 7 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this priming first

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expansion occurs for a period of about 7 to 8 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this priming first expansion occurs for a period of about 7 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this priming first expansion occurs for a period of about 8 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this priming first expansion occurs for a period of about 6 to 11 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this priming first expansion occurs for a period of about 7 to 11 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this priming first expansion occurs for a period of about 8 to 11 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this priming first expansion occurs for a period of about 9 to 11 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this priming first expansion occurs for a period of about 10 to 11 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this priming first expansion occurs for a period of about 9 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this priming first expansion occurs for a period of about 10 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this priming first expansion occurs for a period of about 11 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells.

[00848] In some embodiments, expansion of TILs may be performed using a priming first expansion step (for example such as those described in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include processes referred to as pre-REP or priming REP and which contains feeder cells or feeder cell culture supernatant from Day 0 and/or from culture initiation) as described below and herein, followed by a rapid second expansion (Step D, including processes referred to as rapid expansion protocol (REP) steps) as described below under Step D and herein, followed by optional cryopreservation, and followed by a second Step D (including processes referred to as restimulation REP steps) as described below and herein. The TILs obtained from this process may be optionally characterized for phenotypic characteristics and metabolic parameters as described herein. In some embodiments, the tumor fragment is between about 1 mm^3 and 10 mm^3.

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[00849] In some embodiments, the first expansion culture medium is referred to as "CM", an abbreviation for culture media. In some embodiments, CM for Step B consists of RPMI 1640 with GlutaMAX, supplemented with 10% human AB serum, 25 mM Hepes, and 10 mg/mL gentamicin.

[00850] In some embodiments, there are less than or equal to 240 tumor fragments. In some embodiments, there are less than or equal to 240 tumor fragments placed in less than or equal to 4 containers. In some embodiments, the containers are GREX100 MCS flasks. In some embodiments, less than or equal to 60 tumor fragments are placed in 1 container. In some embodiments, there are less than or equal to 200 tumor fragments. In some embodiments, there are less than or equal to 200 tumor fragments placed in less than or equal to 5 containers. In some embodiments, less than or equal to 50 tumor fragments are placed in 1 container. In some embodiments, each container comprises less than or equal to 500 mL of media per container. In some embodiments, the media comprises IL-2. In some embodiments, the media comprises 6000 IU/mL of IL-2. In some embodiments, the media comprises antigen-presenting feeder cells (also referred to herein as "antigen-presenting cells"). In some embodiments, the media comprises 2.5×10^8 antigen-presenting feeder cells per container. In some embodiments, the media comprises OKT-3. In some embodiments, the media comprises 30 ng/mL of OKT-3 per container. In some embodiments, the container is a GREX100 MCS flask. In some embodiments, the media comprises 6000 IU/mL of IL-2, 30 ng of OKT-3, and 2.5×10^8 antigen-presenting feeder cells. In some embodiments, the media comprises 6000 IU/mL of IL-2, 30 ng/mL of OKT-3, and 2.5×10^8 antigen-presenting feeder cells per container.

[00851] After preparation of the tumor fragments, the resulting cells (*i.e.*, fragments which is a primary cell population) are cultured in media containing IL-2, antigen-presenting feeder cells and OKT-3 under conditions that favor the growth of TILs over tumor and other cells and which allow for TIL priming and accelerated growth from initiation of the culture on Day 0. In some embodiments, the tumor digests and/or tumor fragments are incubated in with 6000 IU/mL of IL-2, as well as antigen-presenting feeder cells and OKT-3. This primary cell population is cultured for a period of days, generally from 1 to 8 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, the growth media during the priming first expansion comprises IL-2 or a variant thereof, as well as antigen-presenting feeder cells and OKT-3. In some embodiments, this primary cell population is cultured for a period of days, generally from 1 to 7 days, resulting in a bulk TIL population,

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generally about 1×10^8 bulk TIL cells. In some embodiments, the growth media during the priming first expansion comprises IL-2 or a variant thereof, as well as antigen-presenting feeder cells and OKT-3. In some embodiments, the IL-2 is recombinant human IL-2 (rhIL-2). In some embodiments the IL-2 stock solution has a specific activity of $20\text{-}30\times10^6$ IU/mg for a 1 mg vial. In some embodiments the IL-2 stock solution has a specific activity of 20×10^6 IU/mg for a 1 mg vial. In some embodiments the IL-2 stock solution has a specific activity of 25×10^6 IU/mg for a 1 mg vial. In some embodiments the IL-2 stock solution has a specific activity of 30×10^6 IU/mg for a 1 mg vial. In some embodiments, the IL- 2 stock solution has a final concentration of $4\text{-}8\times10^6$ IU/mg of IL-2. In some embodiments, the IL- 2 stock solution has a final concentration of $5\text{-}7\times10^6$ IU/mg of IL-2. In some embodiments, the IL- 2 stock solution has a final concentration of 6×10^6 IU/mg of IL-2. In some embodiments, the IL-2 stock solution is prepare as described in Example C. In some embodiments, the priming first expansion culture media comprises about 10,000 IU/mL of IL-2, about 9,000 IU/mL of IL-2, about 8,000 IU/mL of IL-2, about 7,000 IU/mL of IL-2, about 6000 IU/mL of IL-2 or about 5,000 IU/mL of IL-2. In some embodiments, the priming first expansion culture media comprises about 9,000 IU/mL of IL-2 to about 5,000 IU/mL of IL-2. In some embodiments, the priming first expansion culture media comprises about 8,000 IU/mL of IL-2 to about 6,000 IU/mL of IL-2. In some embodiments, the priming first expansion culture media comprises about 7,000 IU/mL of IL-2 to about 6,000 IU/mL of IL-2. In some embodiments, the priming first expansion culture media comprises about 6,000 IU/mL of IL-2. In some embodiments, the cell culture medium further comprises IL-2. In some embodiments, the priming first expansion cell culture medium comprises about 3000 IU/mL of IL-2. In some embodiments, the priming first expansion cell culture medium further comprises IL-2. In some embodiments, the priming first expansion cell culture medium comprises about 3000 IU/mL of IL-2. In some embodiments, the priming first expansion cell culture medium comprises about 1000 IU/mL, about 1500 IU/mL, about 2000 IU/mL, about 2500 IU/mL, about 3000 IU/mL, about 3500 IU/mL, about 4000 IU/mL, about 4500 IU/mL, about 5000 IU/mL, about 5500 IU/mL, about 6000 IU/mL, about 6500 IU/mL, about 7000 IU/mL, about 7500 IU/mL, or about 8000 IU/mL of IL-2. In some embodiments, the priming first expansion cell culture medium comprises between 1000 and 2000 IU/mL, between 2000 and 3000 IU/mL, between 3000 and 4000 IU/mL, between 4000 and 5000 IU/mL, between 5000 and 6000 IU/mL, between 6000 and 7000 IU/mL, between 7000 and 8000 IU/mL, or about 8000 IU/mL of IL-2.

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[00852] In some embodiments, priming first expansion culture media comprises about 500 IU/mL of IL-15, about 400 IU/mL of IL-15, about 300 IU/mL of IL-15, about 200 IU/mL of IL-15, about 180 IU/mL of IL-15, about 160 IU/mL of IL-15, about 140 IU/mL of IL-15, about 120 IU/mL of IL-15, or about 100 IU/mL of IL-15. In some embodiments, the priming first expansion culture media comprises about 500 IU/mL of IL-15 to about 100 IU/mL of IL-15. In some embodiments, the priming first expansion culture media comprises about 400 IU/mL of IL-15 to about 100 IU/mL of IL-15. In some embodiments, the priming first expansion culture media comprises about 300 IU/mL of IL-15 to about 100 IU/mL of IL-15. In some embodiments, the priming first expansion culture media comprises about 200 IU/mL of IL-15. In some embodiments, the priming first expansion cell culture medium comprises about 180 IU/mL of IL-15. In some embodiments, the priming first expansion cell culture medium further comprises IL-15. In some embodiments, the priming first expansion cell culture medium comprises about 180 IU/mL of IL-15.

[00853] In some embodiments, priming first expansion culture media comprises about 20 IU/mL of IL-21, about 15 IU/mL of IL-21, about 12 IU/mL of IL-21, about 10 IU/mL of IL-21, about 5 IU/mL of IL-21, about 4 IU/mL of IL-21, about 3 IU/mL of IL-21, about 2 IU/mL of IL-21, about 1 IU/mL of IL-21, or about 0.5 IU/mL of IL-21. In some embodiments, the priming first expansion culture media comprises about 20 IU/mL of IL-21 to about 0.5 IU/mL of IL-21. In some embodiments, the priming first expansion culture media comprises about 15 IU/mL of IL-21 to about 0.5 IU/mL of IL-21. In some embodiments, the priming first expansion culture media comprises about 12 IU/mL of IL-21 to about 0.5 IU/mL of IL-21. In some embodiments, the priming first expansion culture media comprises about 10 IU/mL of IL-21 to about 0.5 IU/mL of IL-21. In some embodiments, the priming first expansion culture media comprises about 5 IU/mL of IL-21 to about 1 IU/mL of IL-21. In some embodiments, the priming first expansion culture media comprises about 2 IU/mL of IL-21. In some embodiments, the priming first expansion cell culture medium comprises about 1 IU/mL of IL-21. In some embodiments, the priming first expansion cell culture medium comprises about 0.5 IU/mL of IL-21. In some embodiments, the cell culture medium further comprises IL-21. In some embodiments, the priming first expansion cell culture medium comprises about 1 IU/mL of IL-21.

[00854] In some embodiments, the priming first expansion cell culture medium comprises OKT-3 antibody. In some embodiments, the priming first expansion cell culture medium comprises about 30 ng/mL of OKT-3 antibody. In some embodiments, the priming first

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expansion cell culture medium comprises about 0.1 ng/mL, about 0.5 ng/mL, about 1 ng/mL, about 2.5 ng/mL, about 5 ng/mL, about 7.5 ng/mL, about 10 ng/mL, about 15 ng/mL, about 20 ng/mL, about 25 ng/mL, about 30 ng/mL, about 35 ng/mL, about 40 ng/mL, about 50 ng/mL, about 60 ng/mL, about 70 ng/mL, about 80 ng/mL, about 90 ng/mL, about 100 ng/mL, about 200 ng/mL, about 500 ng/mL, and about 1 μg/mL of OKT-3 antibody. In some embodiments, the cell culture medium comprises between 0.1 ng/mL and 1 ng/mL, between 1 ng/mL and 5 ng/mL, between 5 ng/mL and 10 ng/mL, between 10 ng/mL and 20 ng/mL, between 20 ng/mL and 30 ng/mL, between 30 ng/mL and 40 ng/mL, between 40 ng/mL and 50 ng/mL, and between 50 ng/mL and 100 ng/mL of OKT-3 antibody. In some embodiments, the cell culture medium comprises between 15 ng/ml and 30 ng/mL of OKT-3 antibody. In some embodiments, the cell culture medium comprises 30 ng/mL of OKT-3 antibody. In some embodiments, the OKT-3 antibody is muromonab. *See*, Table 1 above.

[00855] In some embodiments, the priming first expansion cell culture medium comprises one or more TNFRSF agonists in a cell culture medium. In some embodiments, the TNFRSF agonist comprises a 4-1BB agonist. In some embodiments, the TNFRSF agonist is a 4-1BB agonist, and the 4-1BB agonist is selected from the group consisting of urelumab, utomilumab, EU-101, a fusion protein, and fragments, derivatives, variants, biosimilars, and combinations thereof. In some embodiments, the TNFRSF agonist is added at a concentration sufficient to achieve a concentration in the cell culture medium of between 0.1 μg/mL and 100 μg/mL. In some embodiments, the TNFRSF agonist is added at a concentration sufficient to achieve a concentration in the cell culture medium of between 20 μg/mL and 40 μg/mL.

[00856] In some embodiments, in addition to one or more TNFRSF agonists, the priming first expansion cell culture medium further comprises IL-2 at an initial concentration of about 3000 IU/mL and OKT-3 antibody at an initial concentration of about 30 ng/mL, and wherein the one or more TNFRSF agonists comprises a 4-1BB agonist. In some embodiments, in addition to one or more TNFRSF agonists, the priming first expansion cell culture medium further comprises IL-2 at an initial concentration of about 6000 IU/mL and OKT-3 antibody at an initial concentration of about 30 ng/mL, and wherein the one or more TNFRSF agonists comprises a 4-1BB agonist.

[00857] In some embodiments, the priming first expansion culture medium is referred to as "CM", an abbreviation for culture media. In some embodiments, it is referred to as CM1 (culture medium 1). In some embodiments, CM consists of RPMI 1640 with GlutaMAX, supplemented with 10% human AB serum, 25 mM Hepes, and 10 mg/mL gentamicin. In

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some embodiments, the CM is the CM1 described in the Examples, *see*, Example A. In some embodiments, the priming first expansion occurs in an initial cell culture medium or a first cell culture medium. In some embodiments, the priming first expansion culture medium or the initial cell culture medium or the first cell culture medium comprises IL-2, OKT-3 and antigen-presenting feeder cells (also referred to herein as feeder cells).

[00858] In some embodiments, the culture medium used in the expansion processes disclosed herein is a serum-free medium or a defined medium. In some embodiments, the serum-free or defined medium comprises a basal cell medium and a serum supplement and/or a serum replacement. In some embodiments, the serum-free or defined medium is used to prevent and/or decrease experimental variation due in part to the lot-to-lot variation of serum-containing media.

[00859] In some embodiments, the serum-free or defined medium comprises a basal cell medium and a serum supplement and/or serum replacement. In some embodiments, 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.

[00860] In some embodiments, the serum supplement or serum replacement includes, but is not limited to one or more of CTS™ OpTmizer T-Cell Expansion Serum Supplement, CTS™ Immune Cell Serum Replacement, 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 one or more trace elements. In some embodiments, the defined 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+}. In some embodiments, the defined medium further comprises L-glutamine, sodium bicarbonate and/or 2-mercaptoethanol.

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[00861] In some embodiments, the CTS™OpTmizer™ T-cell Immune Cell Serum Replacement is used with conventional growth media, including but not limited to CTS™ OpTmizer™ T-cell Expansion Basal Medium, CTS™ OpTmizer™ T-cell Expansion SFM, CTS™ AIM-V Medium, CST™ 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.

[00862] In some embodiments, the total serum replacement concentration (vol%) in the serum-free or defined medium is 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 total serum-free or defined medium. In some embodiments, the total serum replacement concentration is about 3% of the total volume of the serum-free or defined medium. In some embodiments, the total serum replacement concentration is about 5% of the total volume of the serum-free or defined medium. In some embodiments, the total serum replacement concentration is about 10% of the total volume of the serum-free or defined medium.

[00863] In some embodiments, the serum-free or defined medium is CTS™ OpTmizer™ T-cell Expansion SFM (ThermoFisher Scientific). Any formulation of CTS™ OpTmizer™ is useful in the present invention. CTS™ OpTmizer™ T-cell Expansion SFM is a combination of 1L CTS™ OpTmizer™ T-cell Expansion Basal Medium and 26 mL CTS™ OpTmizer™ T-Cell Expansion Supplement, which are mixed together prior to use. In some embodiments, the CTS™ OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific). In some embodiments, the CTS™ OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), along with 2-mercaptoethanol at 55mM. In some embodiments, the CTS™ OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and the final concentration of 2-mercaptoethanol in the media is 55μM.

[00864] In some embodiments, the defined medium is CTS™ OpTmizer™ T-cell Expansion SFM (ThermoFisher Scientific). Any formulation of CTS™ OpTmizer™ is useful in the present invention. CTS™ OpTmizer™ T-cell Expansion SFM is a combination of 1L CTS™ OpTmizer™ T-cell Expansion Basal Medium and 26 mL CTS™ OpTmizer™

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T-Cell Expansion Supplement, which are mixed together prior to use. In some embodiments, the CTS™ OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), along with 2-mercaptoethanol at 55mM. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), 55mM of 2-mercaptoethanol, and 2mM of L-glutamine. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), 55mM of 2-mercaptoethanol, and 2mM of L-glutamine, and further comprises about 1000 IU/mL to about 8000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), 55mM of 2-mercaptoethanol, and 2mM of L-glutamine, and further comprises about 3000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), 55mM of 2-mercaptoethanol, and 2mM of L-glutamine, and further comprises about 6000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and 55mM of 2-mercaptoethanol, and further comprises about 1000 IU/mL to about 8000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and 55mM of 2-mercaptoethanol, and further comprises about 3000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and 55mM of 2-mercaptoethanol, and further comprises about 1000 IU/mL to about 6000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and about 2mM glutamine, and further comprises about 1000 IU/mL to about 8000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and about 2mM glutamine, and further comprises about 3000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and about 2mM glutamine, and further comprises about 6000 IU/mL of IL-2. In some

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embodiments, the CTS™ OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and the final concentration of 2-mercaptoethanol in the media is 55μM.

[00865] In some embodiments, the serum-free medium or defined medium is supplemented with 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. In some embodiments, the serum-free medium or defined medium is supplemented with glutamine (*i.e.*, GlutaMAX®) at a concentration of about 2mM.

[00866] In some embodiments, the serum-free medium or defined medium is supplemented with 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. In some embodiments, the serum-free medium or defined medium is supplemented with 2-mercaptoethanol at a concentration of about 55mM. In some embodiments, the final concentration of 2-mercaptoethanol in the media is 55μM.

[00867] In some embodiments, the defined media described in International PCT Publication No. WO/1998/030679, which is herein incorporated by reference, are useful in the present invention. In that publication, serum-free eukaryotic cell culture media are described. The serum-free, eukaryotic cell culture medium includes a basal cell culture medium supplemented with a serum-free supplement capable of supporting the growth of cells in serum- free culture. The serum-free eukaryotic cell culture medium supplement comprises or is obtained by combining one or more ingredients selected from the group consisting of 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 trace elements, and one or more antibiotics. In some embodiments, the defined medium further comprises L-glutamine, sodium bicarbonate and/or beta-mercaptoethanol. In some embodiments, the defined medium comprises an albumin or an albumin substitute and one or more ingredients selected from group consisting of 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, and one or more trace

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elements. In some embodiments, the defined 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+}. In some embodiments, the basal cell media is selected from the group consisting of 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.

[00868] In some embodiments, the concentration of glycine in the defined medium is in the range of from about 5-200 mg/L, the concentration of L- histidine is about 5-250 mg/L, the concentration of L-isoleucine is about 5-300 mg/L, the concentration of L-methionine is about 5-200 mg/L, the concentration of L-phenylalanine is about 5-400 mg/L, the concentration of L-proline is about 1-1000 mg/L, the concentration of L- hydroxyproline is about 1-45 mg/L, the concentration of L-serine is about 1-250 mg/L, the concentration of L-threonine is about 10-500 mg/L, the concentration of L-tryptophan is about 2-110 mg/L, the concentration of L-tyrosine is about 3-175 mg/L, the concentration of L-valine is about 5-500 mg/L, the concentration of thiamine is about 1-20 mg/L, the concentration of reduced glutathione is about 1-20 mg/L, the concentration of L-ascorbic acid-2-phosphate is about 1-200 mg/L, the concentration of iron saturated transferrin is about 1-50 mg/L, the concentration of insulin is about 1-100 mg/L, the concentration of sodium selenite is about 0.000001-0.0001 mg/L, and the concentration of albumin (*e.g.*, AlbuMAX® I) is about 5000-50,000 mg/L.

[00869] In some embodiments, 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 4. In other embodiments, the non-trace element moiety ingredients in the defined medium are present in the final concentrations listed in the column under the heading "A Preferred Embodiment of the 1X Medium" in Table 4. In other embodiments, the defined medium is a basal cell medium comprising a serum free supplement. In some of these embodiments, the serum free supplement comprises

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non-trace moiety ingredients of the type and in the concentrations listed in the column under the heading "A Preferred Embodiment in Supplement" in Table 4.

TABLE 4: Concentrations of Non-Trace Element Moiety Ingredients

Ingredient	A preferred embodiment in supplement (mg/L) (About)	Concentration range in 1X medium (mg/L) (About)	A preferred embodiment in 1X medium (mg/L) (About)
Glycine	150	5-200	53
L-Histidine	940	5-250	183
L-Isoleucine	3400	5-300	615
L-Methionine	90	5-200	44
L-Phenylalanine	1800	5-400	336
L-Proline	4000	1-1000	600
L-Hydroxyproline	100	1-45	15
L-Serine	800	1-250	162
L-Threonine	2200	10-500	425
L-Tryptophan	440	2-110	82
L-Tyrosine	77	3-175	84
L-Valine	2400	5-500	454
Thiamine	33	1-20	9
Reduced Glutathione	10	1-20	1.5
Ascorbic Acid-2-PO_4 (Mg Salt)	330	1-200	50
Transferrin (iron saturated)	55	1-50	8
Insulin	100	1-100	10
Sodium Selenite	0.07	0.000001-0.0001	0.00001
AlbuMAX®I	83,000	5000-50,000	12,500

[00870] In some embodiments, the osmolarity of the defined medium is between about 260 and 350 mOsmol. In some embodiments, the osmolarity is between about 280 and 310 mOsmol. In some embodiments, the defined medium is supplemented with up to about 3.7 g/L, or about 2.2 g/L sodium bicarbonate. The defined medium can be further supplemented with L-glutamine (final concentration of about 2 mM), one or more antibiotics, non-essential amino acids (NEAA; final concentration of about 100 μM), 2-mercaptoethanol (final concentration of about 100 μM).

[00871] In some embodiments, the defined media described in Smith, *et al.*, "Ex vivo expansion of human T cells for adoptive immunotherapy using the novel Xeno-free CTS Immune Cell Serum Replacement," *Clin Transl Immunology*, 4(1) 2015 (doi: 10.1038/cti.2014.31) are useful in the present invention. Briefly, RPMI or CTS™

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OpTmizer™ was used as the basal cell medium, and supplemented with either 0, 2%, 5%, or 10% CTS™ Immune Cell Serum Replacement.

[00872] In some embodiments, the cell medium in the first and/or second gas permeable container is unfiltered. The use of unfiltered cell medium may simplify the procedures necessary to expand the number of cells. In some embodiments, the cell 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).

[00873] In some embodiments, the priming first expansion (including processes such as for example those described in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include those sometimes referred to as the pre-REP or priming REP) process is 1 to 8 days, as discussed in the examples and figures. In some embodiments, the priming first expansion (including processes such as for example those described in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include those sometimes referred to as the pre-REP or priming REP) process is 2 to 8 days. In some embodiments, the priming first expansion (including processes such as for example those described in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include those sometimes referred to as the pre-REP or priming REP) process is 3 to 8 days. In some embodiments, the priming first expansion (including processes such as for example those described in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include those sometimes referred to as the pre-REP or priming REP) process is 4 to 8 days, as discussed in the examples and figures. In some embodiments, the priming first expansion (including processes such as for example those described in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include those sometimes referred to as the pre-REP or priming REP) process is 1 to 7 days, as discussed in the examples and figures. In some embodiments, the priming first expansion (including processes such as for example those described in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include those sometimes referred to as the pre-REP or priming REP) process is 2 to 8 days. In some embodiments, the priming first expansion (including processes such as for example those described in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can

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include those sometimes referred to as the pre-REP or priming REP) process is 2 to 7 days. In some embodiments, the priming first expansion (including processes such as for example those described in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include those sometimes referred to as the pre-REP or priming REP) process is 3 to 8 days.In some embodiments, the priming first expansion (including processes such as for example those described in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include those sometimes referred to as the pre-REP or priming REP) process is 3 to 7 days. In some embodiments, the priming first expansion (including processes such as for example those described in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include those sometimes referred to as the pre-REP or priming REP) process is 4 to 8 days. In some embodiments, the priming first expansion (including processes such as for example those described in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include those sometimes referred to as the pre-REP or priming REP) process is 4 to 7 days. In some embodiments, the priming first expansion (including processes such as for example those described in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include those sometimes referred to as the pre-REP or priming REP) process is 5 to 8 days. In some embodiments, the priming first expansion (including processes such as for example those described in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include those sometimes referred to as the pre-REP or priming REP) process is 5 to 7 days. In some embodiments, the priming first expansion (including processes such as for example those described in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include those sometimes referred to as the pre-REP or priming REP) process is 6 to 8 days. In some embodiments, the priming first expansion (including processes such as for example those described in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include those sometimes referred to as the pre-REP or priming REP) process is 6 to 7 days. In some embodiments, the priming first expansion (including processes such as for example those provided in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include those sometimes referred to as the pre-REP or priming REP) process is 7 to 8 days. In some embodiments, the priming

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first expansion (including processes such as for example those provided in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include those sometimes referred to as the pre-REP or priming REP) process is 8 days.In some embodiments, the priming first expansion (including processes such as for example those provided in Step B of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), which can include those sometimes referred to as the pre-REP or priming REP) process is 7 days.

[00874] In some embodiments, the priming first TIL expansion can proceed for 1 days to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the priming first TIL expansion can proceed for 1 days to 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the priming first TIL expansion can proceed for 2 days to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the priming first TIL expansion can proceed for 2 days to 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the priming first TIL expansion can proceed for 3 days to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the priming first TIL expansion can proceed for 3 days to 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the priming first TIL expansion can proceed for 4 days to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the priming first TIL expansion can proceed for 4 days to 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the priming first TIL expansion can proceed for 5 days to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the priming first TIL expansion can proceed for 5 days to 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the priming first TIL expansion can proceed for 6 days to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated.In some embodiments, the priming first TIL expansion can proceed for 6 days to 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the priming first TIL expansion can proceed for 7 to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In

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some embodiments, the priming first TIL expansion can proceed for 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated.In some embodiments, the priming first TIL expansion can proceed for 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated.

[00875] In some embodiments, the priming first expansion of the TILs can proceed for 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, or 8 days. In some embodiments, the first TIL expansion can proceed for 1 day to 8 days. In some embodiments, the first TIL expansion can proceed for 1 day to 7 days. In some embodiments, the first TIL expansion can proceed for 2 days to 8 days. In some embodiments, the first TIL expansion can proceed for 2 days to 7 days. In some embodiments, the first TIL expansion can proceed for 3 days to 8 days. In some embodiments, the first TIL expansion can proceed for 3 days to 7 days. In some embodiments, the first TIL expansion can proceed for 4 days to 8 days. In some embodiments, the first TIL expansion can proceed for 4 days to 7 days. In some embodiments, the first TIL expansion can proceed for 5 days to 8 days. In some embodiments, the first TIL expansion can proceed for 5 days to 7 days. In some embodiments, the first TIL expansion can proceed for 6 days to 8 days. In some embodiments, the first TIL expansion can proceed for 6 days to 7 days. In some embodiments, the first TIL expansion can proceed for 7 to 8 days. In some embodiments, the first TIL expansion can proceed for 8 days. In some embodiments, the first TIL expansion can proceed for 7 days.

[00876] In some embodiments, a combination of IL-2, IL-7, IL-15, and/or IL-21 are employed as a combination during the priming first expansion. In some embodiments, IL-2, IL-7, IL-15, and/or IL-21 as well as any combinations thereof can be included during the priming first expansion, including, for example during Step B processes according to Figure 1 (in particular, *e.g.,* Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as well as described herein. In some embodiments, a combination of IL-2, IL-15, and IL-21 are employed as a combination during the priming first expansion. In some embodiments, IL-2, IL-15, and IL-21 as well as any combinations thereof can be included during Step B processes according to Figure 1 (in particular, *e.g.,* Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) and as described herein.

[00877] In some embodiments, the priming first expansion, for example, Step B according to Figure 1 (in particular, *e.g.,* Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), is performed in a closed system bioreactor. In some embodiments, a

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closed system is employed for the TIL expansion, as described herein. In some embodiments, a bioreactor is employed. In some embodiments, a bioreactor is employed as the container. In some embodiments, the bioreactor employed is for example a G-REX-10 or a G-REX-100. In some embodiments, the bioreactor employed is a G-REX-100. In some embodiments, the bioreactor employed is a G-REX-10.

1. Feeder Cells and Antigen Presenting Cells

[00878] In some embodiments, the priming first expansion procedures described herein (for example including expansion such as those described in Step B from Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as well as those referred to as pre-REP or priming REP) does not require feeder cells (also referred to herein as "antigen-presenting cells") at the initiation of the TIL expansion, but rather are added during the priming first expansion. In some embodiments, the priming first expansion procedures described herein (for example including expansion such as those described in Step B from Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as well as those referred to as pre-REP or priming REP) does not require feeder cells (also referred to herein as "antigen-presenting cells") at the initiation of the TIL expansion, but rather are added during the priming first expansion at any time during days 4-8. In some embodiments, the priming first expansion procedures described herein (for example including expansion such as those described in Step B from Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as well as those referred to as pre-REP or priming REP) does not require feeder cells (also referred to herein as "antigen-presenting cells") at the initiation of the TIL expansion, but rather are added during the priming first expansion at any time during days 4-7. In some embodiments, the priming first expansion procedures described herein (for example including expansion such as those described in Step B from Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as well as those referred to as pre-REP or priming REP) does not require feeder cells (also referred to herein as "antigen-presenting cells") at the initiation of the TIL expansion, but rather are added during the priming first expansion at any time during days 5-8. In some embodiments, the priming first expansion procedures described herein (for example including expansion such as those described in Step B from Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as well as those referred to as pre-REP or priming REP) does not require feeder cells (also referred to herein

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as "antigen-presenting cells") at the initiation of the TIL expansion, but rather are added during the priming first expansion at any time during days 5-7. In some embodiments, the priming first expansion procedures described herein (for example including expansion such as those described in Step B from Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as well as those referred to as pre-REP or priming REP) does not require feeder cells (also referred to herein as "antigen-presenting cells") at the initiation of the TIL expansion, but rather are added during the priming first expansion at any time during days 6-8. In some embodiments, the priming first expansion procedures described herein (for example including expansion such as those described in Step B from Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as well as those referred to as pre-REP or priming REP) does not require feeder cells (also referred to herein as "antigen-presenting cells") at the initiation of the TIL expansion, but rather are added during the priming first expansion at any time during days 6-7. In some embodiments, the priming first expansion procedures described herein (for example including expansion such as those described in Step B from Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as well as those referred to as pre-REP or priming REP) does not require feeder cells (also referred to herein as "antigen-presenting cells") at the initiation of the TIL expansion, but rather are added during the priming first expansion at any time during day 7 or 8. In some embodiments, the priming first expansion procedures described herein (for example including expansion such as those described in Step B from Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as well as those referred to as pre-REP or priming REP) does not require feeder cells (also referred to herein as "antigen-presenting cells") at the initiation of the TIL expansion, but rather are added during the priming first expansion at any time during day 7. In some embodiments, the priming first expansion procedures described herein (for example including expansion such as those described in Step B from Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as well as those referred to as pre-REP or priming REP) does not require feeder cells (also referred to herein as "antigen-presenting cells") at the initiation of the TIL expansion, but rather are added during the priming first expansion at any time during day 8.

[00879] In some embodiments, the priming first expansion procedures described herein (for example including expansion such as those described in Step B from Figure 1 (in particular,

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e.g., Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as well as those referred to as pre-REP or priming REP) require feeder cells (also referred to herein as "antigen-presenting cells") at the initiation of the TIL expansion and during the priming first expansion. In many embodiments, the feeder cells are peripheral blood mononuclear cells (PBMCs) obtained from standard whole blood units from allogeneic healthy blood donors. The PBMCs are obtained using standard methods such as Ficoll-Paque gradient separation. In some embodiments, 2.5×10^8 feeder cells are used during the priming first expansion. In some embodiments, 1×10^9 feeder cells are used during the priming first expansion. In some embodiments, 1.25×10^9 feeder cells are used during the priming first expansion. In some embodiments, 2.5×10^8 feeder cells per container are used during the priming first expansion. In some embodiments, 1×10^9 feeder cells per container are used during the priming first expansion. In some embodiments, 1.25×10^9 feeder cells per container are used during the priming first expansion. In some embodiments, 2.5×10^8 feeder cells per GREX-10 are used during the priming first expansion. In some embodiments, 1×10^9 feeder cells per GREX-10 are used during the priming first expansion. In some embodiments, 1.25×10^9 feeder cells per GREX-10 are used during the priming first expansion. In some embodiments, 2.5×10^8 feeder cells per GREX-100 are used during the priming first expansion. In some embodiments, 1×10^9 feeder cells per GREX-100 are used during the priming first expansion. In some embodiments, 1.25×10^9 feeder cells per GREX-100 are used during the priming first expansion.

[00880] In general, the allogenic PBMCs are inactivated, either via irradiation or heat treatment, and used in the REP procedures, as described in the examples, which provides an exemplary protocol for evaluating the replication incompetence of irradiate allogeneic PBMCs.

[00881] In some embodiments, PBMCs are considered replication incompetent and acceptable for use in the TIL expansion procedures described herein if the total number of viable cells on day 14 is less than the initial viable cell number put into culture on day 0 of the priming first expansion.

[00882] In some embodiments, PBMCs are considered replication incompetent and acceptable for use in the TIL expansion procedures described herein if the total number of viable cells, cultured in the presence of OKT3 and IL-2, on day 7 have not increased from the initial viable cell number put into culture on day 0 of the priming first expansion. In some embodiments, the PBMCs are cultured in the presence of 30 ng/ml OKT3 antibody and 3000

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IU/ml IL-2. In some embodiments, the PBMCs are cultured in the presence of 30 ng/ml OKT3 antibody and 6000 IU/ml IL-2.

[00883] In some embodiments, PBMCs are considered replication incompetent and acceptable for use in the TIL expansion procedures described herein if the total number of viable cells, cultured in the presence of OKT3 and IL-2, on day 7 have not increased from the initial viable cell number put into culture on day 0 of the priming first expansion. In some embodiments, the PBMCs are cultured in the presence of 5-60 ng/mL OKT3 antibody and 1000-6000 IU/mL IL-2. In some embodiments, the PBMCs are cultured in the presence of 10-50 ng/mL OKT3 antibody and 2000-5000 IU/mL IL-2. In some embodiments, the PBMCs are cultured in the presence of 20-40 ng/mL OKT3 antibody and 2000-4000 IU/mL IL-2. In some embodiments, the PBMCs are cultured in the presence of 25-35 ng/mL OKT3 antibody and 2500-3500 IU/mL IL-2. In some embodiments, the PBMCs are cultured in the presence of 30 ng/mL OKT3 antibody and 6000 IU/mL IL-2. In some embodiments, the PBMCs are cultured in the presence of 15 ng/mL OKT3 antibody and 3000 IU/ml IL-2. In some embodiments, the PBMCs are cultured in the presence of 15 ng/mL OKT3 antibody and 6000 IU/mL IL-2.

[00884] In some embodiments, the antigen-presenting feeder cells are PBMCs. In some embodiments, the antigen-presenting feeder cells are artificial antigen-presenting feeder cells. In some embodiments, the ratio of TILs to antigen-presenting feeder cells in the second expansion is about 1 to 25, about 1 to 50, about 1 to 100, about 1 to 125, about 1 to 150, about 1 to 175, about 1 to 200, about 1 to 225, about 1 to 250, about 1 to 275, about 1 to 300, about 1 to 325, about 1 to 350, about 1 to 375, about 1 to 400, or about 1 to 500. In some embodiments, the ratio of TILs to antigen-presenting feeder cells in the second expansion is between 1 to 50 and 1 to 300. In some embodiments, the ratio of TILs to antigen-presenting feeder cells in the second expansion is between 1 to 100 and 1 to 200.

[00885] In some embodiments, the priming first expansion procedures described herein require a ratio of about 2.5×10^8 feeder cells to about 100×10^6 TILs. In other embodiments, the priming first expansion procedures described herein require a ratio of about 2.5×10^8 feeder cells to about 50×10^6 TILs. In yet another embodiment, the priming first expansion described herein require about 2.5×10^8 feeder cells to about 25×10^6 TILs. In yet another embodiment, the priming first expansion described herein require about 2.5×10^8 feeder cells. In yet another embodiment, the priming first expansion requires one-fourth, one-third, five-twelfths, or one-half of the number of feeder cells used in the rapid second expansion.

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[00886] In some embodiments, the media in the priming first expansion comprises IL-2. In some embodiments, the media in the priming first expansion comprises 6000 IU/mL of IL-2. In some embodiments, the media in the priming first expansion comprises antigen-presenting feeder cells. In some embodiments, the media in the priming first expansion comprises 2.5×10^8 antigen-presenting feeder cells per container. In some embodiments, the media in the priming first expansion comprises OKT-3. In some embodiments, the media comprises 30 ng of OKT-3 per container. In some embodiments, the container is a GREX100 MCS flask. In some embodiments, the media comprises 6000 IU/mL of IL-2, 30 ng/mL of OKT-3, and 2.5×10^8 antigen-presenting feeder cells. In some embodiments, the media comprises 6000 IU/mL of IL-2, 30 ng/mL of OKT-3, and 2.5×10^8 antigen-presenting feeder cells per container. In some embodiments, the media comprises 500 mL of culture medium and 15 μg of OKT-3 per 2.5×10^8 antigen-presenting feeder cells per container. In some embodiments, the media comprises 500 mL of culture medium and 15 μg of OKT-3 per container. In some embodiments, the container is a GREX100 MCS flask. In some embodiments, the media comprises 500 mL of culture medium and 6000 IU/mL of IL-2, 30 ng/mL ng of OKT-3, and 2.5×10^8 antigen-presenting feeder cells. In some embodiments, the media comprises 500 mL of culture medium and 6000 IU/mL of IL-2, 15 μg of OKT-3, and 2.5×10^8 antigen-presenting feeder cells per container. In some embodiments, the media comprises 500 mL of culture medium and 15 μg of OKT-3 per 2.5×10^8 antigen-presenting feeder cells per container.

[00887] In some embodiments, the priming first expansion procedures described herein require an excess of feeder cells over TILs during the second expansion. In many embodiments, the feeder cells are peripheral blood mononuclear cells (PBMCs) obtained from standard whole blood units from allogeneic healthy blood donors. The PBMCs are obtained using standard methods such as Ficoll-Paque gradient separation. In some embodiments, artificial antigen-presenting (aAPC) cells are used in place of PBMCs.

[00888] In general, the allogenic PBMCs are inactivated, either via irradiation or heat treatment, and used in the TIL expansion procedures described herein, including the exemplary procedures described in the figures and examples.

[00889] In some embodiments, artificial antigen presenting cells are used in the priming first expansion as a replacement for, or in combination with, PBMCs.

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[00890] In some embodiments, the priming first expansion procedures described herein, as well as those referred to as pre-REP or priming REP does not require feeder cells (also referred to herein as "antigen-presenting cells"), but rather require a culture supernatant obtained from a culture of antigen-presenting feeder cells that contains OKT-3. In some embodiments, the culture supernatant is obtained from a culture of PBMCs in a culture medium supplemented with IL-2 and OKT-3. In some embodiments, the culture supernatant is obtained from a culture of PBMCs after about 3 or 4 days of culture in a culture medium supplemented with IL-2 and OKT-3. In some embodiments, the culture supernatant is obtained from a culture of PBMCs cultured in a culture medium supplemented with IL-2 and OKT-3 after the growth rate of the PMBCs in culture begins to decline. In some embodiments, the culture supernatant is obtained from a culture of PBMCs cultured in a culture medium supplemented with IL-2 and OKT-3 after the growth rate of the PMBCs in culture has declined about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more. In some embodiments, the culture supernatant is obtained from a culture of PBMCs cultured in a culture medium supplemented with IL-2 and OKT-3 after the culture medium is exhausted or spent. In some embodiments, the culture supernatant is obtained from a culture of PBMCs cultured in a culture medium supplemented with IL-2 and OKT-3 after the culture medium is at least about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more exhausted or spent.

2. Cytokines

[00891] The expansion methods described herein generally use culture media with high doses of a cytokine, in particular IL-2, as is known in the art.

[00892] Alternatively, using combinations of cytokines for the priming first expansion of TILs is additionally possible, with combinations of two or more of IL-2, IL-15 and IL-21 as is generally outlined in International Publication No. WO 2015/189356 and WO 2015/189357, hereby expressly incorporated by reference in their entirety. Thus, possible combinations include IL-2 and IL-15, IL-2 and IL-21, IL-15 and IL-21, and IL-2, IL-15 and IL-21, with the latter finding particular use in many embodiments. The use of combinations of cytokines specifically favors the generation of lymphocytes, and in particular T-cells as described therein.

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TABLE 4: Amino acid sequences of interleukins.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:3 recombinant human IL-2 (rhIL-2)	MAPTSSSTKK TQLQLEHLLL DLQMILNGIN NYKNPKLTRM LTFKFYMPKK ATELKHLQCL EEELKPLEEV LNLAQSKNFH LRPRDLISNI NVIVLELKGS ETTFMCEYAD ETATIVEFLN RWITFCQSII STLT	60 120 134
SEQ ID NO:4 Aldesleukin	PTSSSTKKTQ LQLEHLLLDL QMILNGINNY KNPKLTRMLT FKFYMPKKAT ELKHLQCLEE ELKPLEEVLN LAQSKNFHLR PRDLISNINV IVLELKGSET TFMCEYADET ATIVEFLNRW ITFSQSIIST LT	60 120 132
SEQ ID NO:5 recombinant human IL-4 (rhIL-4)	MHKCDITLQE IIKTLNSLTE QKTLCTELTV TDIFAASKNT TEKETFCRAA TVLRQFYSHH EKDTRCLGAT AQQFHRHKQL IRFLKRLDRN LWGLAGLNSC PVKEANQSTL ENFLERLKTI MREKYSKCSS	60 120 130
SEQ ID NO:6 recombinant human IL-7 (rhIL-7)	MDCDIEGKDG KQYESVLMVS IDQLLDSMKE IGSNCLNNEF NFFKRHICDA NKEGMFLFRA ARKLRQFLKM NSTGDFDLHL LKVSEGTTIL LNCTGQVKGR KPAALGEAQP TKSLEENKSL KEQKKLNDLC FLKRLLQEIK TCWNKILMGT KEH	60 120 153
SEQ ID NO:7 recombinant human IL-15 (rhIL-15)	MNWVNVISDL KKIEDLIQSM HIDATLYTES DVHPSCKVTA MKCFLLELQV ISLESGDASI HDTVENLIIL ANNSLSSNGN VTESGCKECE ELEEKNIKEF LQSFVHIVQM FINTS	60 115
SEQ ID NO:8 recombinant human IL-21 (rhIL-21)	MQDRHMIRMR QLIDIVDQLK NYVNDLVPEF LPAPEDVETN CEWSAFSCFQ KAQLKSANTG NNERIINVSI KKLKRKPPST NAGRRQKHRL TCPSCDSYEK KPPKEFLERF KSLLQKMIHQ HLSSRTHGSE DS	60 120 132

C. STEP C: Priming First Expansion to Rapid Second Expansion Transition

[00893] In some cases, the bulk TIL population obtained from the priming first expansion (which can include expansions sometimes referred to as pre-REP), including, for example the TIL population obtained from for example, Step B as indicated in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), can be subjected to a rapid second expansion (which can include expansions sometimes referred to as Rapid Expansion Protocol (REP)) and then cryopreserved as discussed below. Similarly, in the case where genetically modified TILs will be used in therapy, the expanded TIL population from the priming first expansion or the expanded TIL population from the rapid second expansion can be subjected to genetic modifications for suitable treatments prior to the expansion step or after the priming first expansion and prior to the rapid second expansion.

[00894] In some embodiments, the TILs obtained from the priming first expansion (for example, from Step B as indicated in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) are stored until phenotyped for selection. In some embodiments, the TILs obtained from the priming first expansion (for example, from Step B as indicated in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) are not stored and proceed directly to

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the rapid second expansion. In some embodiments, the TILs obtained from the priming first expansion are not cryopreserved after the priming first expansion and prior to the rapid second expansion. In some embodiments, the transition from the priming first expansion to the second expansion occurs at about 2 days, 3 days, 4, days, 5 days, 6 days, 7 days, or 8 days from when tumor fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the rapid second expansion occurs at about 3 days to 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the rapid second expansion occurs at about 3 days to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the second expansion occurs at about 4 days to 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the second expansion occurs at about 4 days to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the second expansion occurs at about 5 days to 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the second expansion occurs at about 5 days to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the second expansion occurs at about 6 days to 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the second expansion occurs at about 6 days to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the second expansion occurs at about 7 days to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the second expansion occurs at about 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the second expansion occurs at about 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated.

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[00895] In some embodiments, the transition from the priming first expansion to the rapid second expansion occurs at 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, or 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the rapid second expansion occurs 1 day to 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the rapid second expansion occurs 1 day to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the second expansion occurs 2 days to 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the second expansion occurs 2 days to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the second expansion occurs 3 days to 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the second expansion occurs 3 days to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the rapid second expansion occurs 4 days to 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the rapid second expansion occurs 4 days to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the rapid second expansion occurs 5 days to 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the rapid second expansion occurs 5 days to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the rapid second expansion occurs 6 days to 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. . In some embodiments, the transition from the priming first expansion to the rapid second expansion occurs 6 days to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the rapid second expansion occurs 7 days to 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some

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embodiments, the transition from the priming first expansion to the rapid second expansion occurs 7 days from when fragmentation occurs and/or when the first priming expansion step is initiated. In some embodiments, the transition from the priming first expansion to the rapid second expansion occurs 8 days from when fragmentation occurs and/or when the first priming expansion step is initiated.

[00896] In some embodiments, the TILs are not stored after the primary first expansion and prior to the rapid second expansion, and the TILs proceed directly to the rapid second expansion (for example, in some embodiments, there is no storage during the transition from Step B to Step D as shown in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, the transition occurs in closed system, as described herein. In some embodiments, the TILs from the priming first expansion, the second population of TILs, proceeds directly into the rapid second expansion with no transition period.

[00897] In some embodiments, the transition from the priming first expansion to the rapid second expansion, for example, Step C according to Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), is performed in a closed system bioreactor. In some embodiments, a closed system is employed for the TIL expansion, as described herein. In some embodiments, a single bioreactor is employed. In some embodiments, the single bioreactor employed is for example a GREX-10 or a GREX-100. In some embodiments, the closed system bioreactor is a single bioreactor. In some embodiments, the transition from the priming first expansion to the rapid second expansion involves a scale-up in container size. In some embodiments, the priming first expansion is performed in a smaller container than the rapid second expansion. In some embodiments, the priming first expansion is performed in a GREX-100 and the rapid second expansion is performed in a GREX-500.

D. STEP D: Rapid Second Expansion

[00898] In some embodiments, the TIL cell population is further expanded in number after tumor harvest and fragmentation and the priming first expansion, after Step A and Step B, and the transition referred to as Step C, as indicated in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). This further expansion is referred to herein as the rapid second expansion, which can include expansion processes generally referred to in the art as a rapid expansion process (Rapid Expansion

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Protocol or REP; as well as processes as indicated in Step D of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). The rapid second expansion is generally accomplished using a culture media comprising a number of components, including feeder cells and/or feeder cell culture supernatant, a cytokine source, and an anti-CD3 antibody, in a gas-permeable container. In some embodiments, 1 day, 2 days, 3 days, or 4 days after initiation of the rapid second expansion (*i.e.*, at days 8, 9, 10, or 11 of the overall Gen 3 process), the TILs are transferred to a larger volume container. In some embodiments, this rapid second expansion is referred to as activation II.

[00899] In some embodiments, the rapid second expansion (which can include expansions sometimes referred to as REP; as well as processes as indicated in Step D of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) of TIL can be performed using any TIL flasks or containers known by those of skill in the art. In some embodiments, the second TIL expansion can proceed for 1 day, 2 days, 3 days, 4, days, 5 days, 6 days, 7 days, 8 days, 9 days or 10 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 1 days to about 9 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 1 days to about 10 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 2 days to about 9 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 2 days to about 10 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 3 days to about 9 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 3 days to about 10 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 4 days to about 9 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 4 days to about 10 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 5 days to about 9 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 5 days to about 10 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 6 days to about 9 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 6 days to about 10 days after initiation of the rapid second expansion.

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In some embodiments, the second TIL expansion can proceed for about 7 days to about 9 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 7 days to about 10 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 8 days to about 9 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 8 days to about 10 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 9 days to about 10 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 1 day after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 2 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 3 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 4 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 5 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 6 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 7 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 8 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 9 days after initiation of the rapid second expansion. In some embodiments, the second TIL expansion can proceed for about 10 days after initiation of the rapid second expansion.

[00900] In some embodiments, the rapid second expansion can be performed in a gas permeable container using the methods of the present disclosure (including, for example, expansions referred to as REP; as well as processes as indicated in Step D of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, the TILs are expanded in the rapid second expansion in the presence of IL-2, OKT-3, and feeder cells (also referred herein as "antigen-presenting cells"). In some embodiments, the TILs are expanded in the rapid second expansion in the presence of IL-2, OKT-3, and feeder cells, wherein the feeder cells are added to a final concentration that is twice, 2.4 times, 2.5 times, 3 times, 3.5 times or 4 times the concentration of feeder cells present in the priming first expansion. For example, TILs can be rapidly expanded using non-specific T-cell receptor stimulation in the presence of interleukin-2 (IL-2) or interleukin-

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15 (IL-15). The non-specific T-cell receptor stimulus can include, for example, an anti-CD3 antibody, such as about 30 ng/ml of OKT3, a mouse monoclonal anti-CD3 antibody (commercially available from Ortho-McNeil, Raritan, NJ or Miltenyi Biotech, Auburn, CA) or UHCT-1 (commercially available from BioLegend, San Diego, CA, USA). TILs can be expanded to induce further stimulation of the TILs *in vitro* by including one or more antigens during the second expansion, including antigenic portions thereof, such as epitope(s), of the cancer, which can be optionally expressed from a vector, such as a human leukocyte antigen A2 (HLA-A2) binding peptide, *e.g.*, 0.3 μM MART-1 :26-35 (27 L) or gpl 00:209-217 (210M), optionally in the presence of a T-cell growth factor, such as 300 IU/mL IL-2 or IL-15. Other suitable antigens may include, *e.g.*, NY-ESO-1, TRP-1, TRP-2, tyrosinase cancer antigen, MAGE-A3, SSX-2, and VEGFR2, or antigenic portions thereof. TIL may also be rapidly expanded by re-stimulation with the same antigen(s) of the cancer pulsed onto HLA-A2-expressing antigen-presenting cells. Alternatively, the TILs can be further re-stimulated with, *e.g.*, example, irradiated, autologous lymphocytes or with irradiated HLA-A2+ allogeneic lymphocytes and IL-2. In some embodiments, the re-stimulation occurs as part of the second expansion. In some embodiments, the second expansion occurs in the presence of irradiated, autologous lymphocytes or with irradiated HLA-A2+ allogeneic lymphocytes and IL-2.

[00901] In some embodiments, the cell culture medium further comprises IL-2. In some embodiments, the cell culture medium comprises about 3000 IU/mL of IL-2. In some embodiments, the cell culture medium comprises about 1000 IU/mL, about 1500 IU/mL, about 2000 IU/mL, about 2500 IU/mL, about 3000 IU/mL, about 3500 IU/mL, about 4000 IU/mL, about 4500 IU/mL, about 5000 IU/mL, about 5500 IU/mL, about 6000 IU/mL, about 6500 IU/mL, about 7000 IU/mL, about 7500 IU/mL, or about 8000 IU/mL of IL-2. In some embodiments, the cell culture medium comprises between 1000 and 2000 IU/mL, between 2000 and 3000 IU/mL, between 3000 and 4000 IU/mL, between 4000 and 5000 IU/mL, between 5000 and 6000 IU/mL, between 6000 and 7000 IU/mL, between 7000 and 8000 IU/mL, or between 8000 IU/mL of IL-2.

[00902] In some embodiments, the cell culture medium comprises OKT-3 antibody. In some embodiments, the cell culture medium comprises about 30 ng/mL of OKT-3 antibody. In some embodiments, the cell culture medium comprises about 0.1 ng/mL, about 0.5 ng/mL, about 1 ng/mL, about 2.5 ng/mL, about 5 ng/mL, about 7.5 ng/mL, about 10 ng/mL, about 15 ng/mL, about 20 ng/mL, about 25 ng/mL, about 30 ng/mL, about 35 ng/mL, about 40 ng/mL,

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about 50 ng/mL, about 60 ng/mL, about 70 ng/mL, about 80 ng/mL, about 90 ng/mL, about 100 ng/mL, about 200 ng/mL, about 500 ng/mL, and about 1 μg/mL of OKT-3 antibody. In some embodiments, the cell culture medium comprises between 0.1 ng/mL and 1 ng/mL, between 1 ng/mL and 5 ng/mL, between 5 ng/mL and 10 ng/mL, between 10 ng/mL and 20 ng/mL, between 20 ng/mL and 30 ng/mL, between 30 ng/mL and 40 ng/mL, between 40 ng/mL and 50 ng/mL, and between 50 ng/mL and 100 ng/mL of OKT-3 antibody. In some embodiments, the cell culture medium comprises between 30 ng/ml and 60 ng/mL of OKT-3 antibody. In some embodiments, the cell culture medium comprises about 60 ng/mL OKT-3. In some embodiments, the OKT-3 antibody is muromonab.

[00903] In some embodiments, the media in the rapid second expansion comprises IL-2. In some embodiments, the media comprises 6000 IU/mL of IL-2. In some embodiments, the media in the rapid second expansion comprises antigen-presenting feeder cells. In some embodiments, the media in the rapid second expansion comprises 7.5×10^8 antigen-presenting feeder cells per container. In some embodiments, the media in the rapid second expansion comprises OKT-3. In some embodiments, the in the rapid second expansion media comprises 500 mL of culture medium and 30 μg of OKT-3 per container. In some embodiments, the container is a GREX100 MCS flask. In some embodiments, the in the rapid second expansion media comprises 6000 IU/mL of IL-2, 60 ng/mL of OKT-3, and 7.5×10^8 antigen-presenting feeder cells. In some embodiments, the media comprises 500 mL of culture medium and 6000 IU/mL of IL-2, 30 μg of OKT-3, and 7.5×10^8 antigen-presenting feeder cells per container.

[00904] In some embodiments, the media in the rapid second expansion comprises IL-2. In some embodiments, the media comprises 6000 IU/mL of IL-2. In some embodiments, the media in the rapid second expansion comprises antigen-presenting feeder cells. In some embodiments, the media comprises between 5×10^8 and 7.5×10^8antigen-presenting feeder cells per container. In some embodiments, the media in the rapid second expansion comprises OKT-3. In some embodiments, the media in the rapid second expansion comprises 500 mL of culture medium and 30 μg of OKT-3 per container. In some embodiments, the container is a GREX100 MCS flask. In some embodiments, the media in the rapid second expansion comprises 6000 IU/mL of IL-2, 60 ng/mL of OKT-3, and between 5×10^8 and 7.5×10^8 antigen-presenting feeder cells. In some embodiments, the media in the rapid second expansion comprises 500 mL of culture medium and 6000 IU/mL of IL-2, 30 μg of OKT-3, and between 5×10^8 and 7.5×10^8 antigen-presenting feeder cells per container.

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[00905] In some embodiments, the cell culture medium comprises one or more TNFRSF agonists in a cell culture medium. In some embodiments, the TNFRSF agonist comprises a 4-1BB agonist. In some embodiments, the TNFRSF agonist is a 4-1BB agonist, and the 4-1BB agonist is selected from the group consisting of urelumab, utomilumab, EU-101, a fusion protein, and fragments, derivatives, variants, biosimilars, and combinations thereof. In some embodiments, the TNFRSF agonist is added at a concentration sufficient to achieve a concentration in the cell culture medium of between 0.1 μg/mL and 100 μg/mL. In some embodiments, the TNFRSF agonist is added at a concentration sufficient to achieve a concentration in the cell culture medium of between 20 μg/mL and 40 μg/mL.

[00906] In some embodiments, in addition to one or more TNFRSF agonists, the cell culture medium further comprises IL-2 at an initial concentration of about 3000 IU/mL and OKT-3 antibody at an initial concentration of about 30 ng/mL, and wherein the one or more TNFRSF agonists comprises a 4-1BB agonist.

[00907] In some embodiments, a combination of IL-2, IL-7, IL-15, and/or IL-21 are employed as a combination during the second expansion. In some embodiments, IL-2, IL-7, IL-15, and/or IL-21 as well as any combinations thereof can be included during the second expansion, including, for example during a Step D processes according to Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as well as described herein. In some embodiments, a combination of IL-2, IL-15, and IL-21 are employed as a combination during the second expansion. In some embodiments, IL-2, IL-15, and IL-21 as well as any combinations thereof can be included during Step D processes according to Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) and as described herein.

[00908] In some embodiments, the second expansion can be conducted in a supplemented cell culture medium comprising IL-2, OKT-3, antigen-presenting feeder cells, and optionally a TNFRSF agonist. In some embodiments, the second expansion occurs in a supplemented cell culture medium. In some embodiments, the supplemented cell culture medium comprises IL-2, OKT-3, and antigen-presenting feeder cells. In some embodiments, the second cell culture medium comprises IL-2, OKT-3, and antigen-presenting cells (APCs; also referred to as antigen-presenting feeder cells) and/or culture supernatant from a culture of APCs comprising OKT-3. In some embodiments, the second expansion occurs in a cell culture medium comprising IL-2, OKT-3, and antigen-presenting feeder cells (*i.e.*, antigen presenting cells).

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[00909] In some embodiments, the second expansion culture media comprises about 500 IU/mL of IL-15, about 400 IU/mL of IL-15, about 300 IU/mL of IL-15, about 200 IU/mL of IL-15, about 180 IU/mL of IL-15, about 160 IU/mL of IL-15, about 140 IU/mL of IL-15, about 120 IU/mL of IL-15, or about 100 IU/mL of IL-15. In some embodiments, the second expansion culture media comprises about 500 IU/mL of IL-15 to about 100 IU/mL of IL-15. In some embodiments, the second expansion culture media comprises about 400 IU/mL of IL-15 to about 100 IU/mL of IL-15. In some embodiments, the second expansion culture media comprises about 300 IU/mL of IL-15 to about 100 IU/mL of IL-15. In some embodiments, the second expansion culture media comprises about 200 IU/mL of IL-15. In some embodiments, the cell culture medium comprises about 180 IU/mL of IL-15. In some embodiments, the cell culture medium further comprises IL-15. In some embodiments, the cell culture medium comprises about 180 IU/mL of IL-15.

[00910] In some embodiments, the second expansion culture media comprises about 20 IU/mL of IL-21, about 15 IU/mL of IL-21, about 12 IU/mL of IL-21, about 10 IU/mL of IL-21, about 5 IU/mL of IL-21, about 4 IU/mL of IL-21, about 3 IU/mL of IL-21, about 2 IU/mL of IL-21, about 1 IU/mL of IL-21, or about 0.5 IU/mL of IL-21. In some embodiments, the second expansion culture media comprises about 20 IU/mL of IL-21 to about 0.5 IU/mL of IL-21. In some embodiments, the second expansion culture media comprises about 15 IU/mL of IL-21 to about 0.5 IU/mL of IL-21. In some embodiments, the second expansion culture media comprises about 12 IU/mL of IL-21 to about 0.5 IU/mL of IL-21. In some embodiments, the second expansion culture media comprises about 10 IU/mL of IL-21 to about 0.5 IU/mL of IL-21. In some embodiments, the second expansion culture media comprises about 5 IU/mL of IL-21 to about 1 IU/mL of IL-21. In some embodiments, the second expansion culture media comprises about 2 IU/mL of IL-21. In some embodiments, the cell culture medium comprises about 1 IU/mL of IL-21. In some embodiments, the cell culture medium comprises about 0.5 IU/mL of IL-21. In some embodiments, the cell culture medium further comprises IL-21. In some embodiments, the cell culture medium comprises about 1 IU/mL of IL-21.

[00911] In some embodiments, the antigen-presenting feeder cells (APCs) are PBMCs. In some embodiments, the ratio of TILs to PBMCs and/or antigen-presenting cells in the rapid expansion and/or the second expansion is about 1 to 10, about 1 to 15, about 1 to 20, about 1 to 25, about 1 to 30, about 1 to 35, about 1 to 40, about 1 to 45, about 1 to 50, about 1 to 75, about 1 to 100, about 1 to 125, about 1 to 150, about 1 to 175, about 1 to 200, about 1 to 225,

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about 1 to 250, about 1 to 275, about 1 to 300, about 1 to 325, about 1 to 350, about 1 to 375, about 1 to 400, or about 1 to 500. In some embodiments, the ratio of TILs to PBMCs in the rapid expansion and/or the second expansion is between 1 to 50 and 1 to 300. In some embodiments, the ratio of TILs to PBMCs in the rapid expansion and/or the second expansion is between 1 to 100 and 1 to 200.

[00912] In some embodiments, REP and/or the rapid second expansion is performed in flasks with the bulk TILs being mixed with a 100- or 200-fold excess of inactivated feeder cells, wherein the feeder cell concentration is at least 1.1 times (1.1X), 1.2X, 1.3X, 1.4X, 1.5X, 1.6X, 1.7X, 1.8X, 1.8X, 2X, 2.1X2.2X, 2.3X, 2.4X, 2.5X, 2.6X, 2.7X, 2.8X, 2.9X, 3.0X, 3.1X, 3.2X, 3.3X, 3.4X, 3.5X, 3.6X, 3.7X, 3.8X, 3.9X or 4.0X the feeder cell concentration in the priming first expansion, 30 ng/mL OKT3 anti-CD3 antibody and 6000 IU/mL IL-2 in 150 ml media. Media replacement is done (generally 2/3 media replacement via aspiration of 2/3 of spent media and replacement with an equal volume of fresh media) until the cells are transferred to an alternative growth chamber. Alternative growth chambers include G-REX flasks and gas permeable containers as more fully discussed below.

[00913] In some embodiments, the rapid second expansion (which can include processes referred to as the REP process) is 7 to 9 days, as discussed in the examples and figures. In some embodiments, the second expansion is 7 days. In some embodiments, the second expansion is 8 days. In some embodiments, the second expansion is 9 days.

[00914] In some embodiments, the second expansion (which can include expansions referred to as REP, as well as those referred to in Step D of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) may be performed in 500 mL capacity gas permeable flasks with 100 cm gas-permeable silicon bottoms (G-Rex 100, commercially available from Wilson Wolf Manufacturing Corporation, New Brighton, MN, USA), 5×10^6 or 10×10^6 TIL may be cultured with PBMCs in 400 mL of 50/50 medium, supplemented with 5% human AB serum, 3000 IU per mL of IL-2 and 30 ng per ml of anti-CD3 (OKT3). The G-Rex 100 flasks may be incubated at 37°C in 5% CO_2. On day 5, 250 mL of supernatant may be removed and placed into centrifuge bottles and centrifuged at 1500 rpm (491 × g) for 10 minutes. The TIL pellets may be re-suspended with 150 mL of fresh medium with 5% human AB serum, 6000 IU per mL of IL-2, and added back to the original GREX-100 flasks. When TIL are expanded serially in GREX-100 flasks, on day 10 or 11 the TILs can be moved to a larger flask, such as a GREX-500. The cells may be harvested on day 14 of culture. The cells may be harvested on day 15 of culture. The cells

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may be harvested on day 16 of culture. In some embodiments, media replacement is done until the cells are transferred to an alternative growth chamber. In some embodiments, 2/3 of the media is replaced by aspiration of spent media and replacement with an equal volume of fresh media. In some embodiments, alternative growth chambers include GREX flasks and gas permeable containers as more fully discussed below.

[00915] In some embodiments, on day 10 or 11 of the rapid expansion the culture is scaled out and/or scaled up by transfer of the culture into one or more new culture vessels.

[00916] In some embodiments, on day 10 or 11 of the rapid expansion the culture is scaled out by transfer of the culture to a plurality of new culture vessels equal in the size to the original culture vessel in which the rapid expansion was initiated. In some embodiments, each of the new culture vessels is a G-rex 10M culture vessel and the original culture vessel is a G-rex 10M culture vessel. In some embodiments, each of the new culture vessels is a G-rex 100M culture vessel and the original culture vessel is a G-rex 100M culture vessel.

[00917] In some embodiments, on day 10 or 11 of the rapid expansion the culture is scaled up by transfer of the culture to a new culture vessel greater in size than the original culture vessel in which the rapid expansion was initiated.

[00918] In some embodiments, on day 10 or 11 of the rapid expansion the culture is scaled out and scaled up by transfer of the culture into a plurality of new culture vessels greater in size than the original culture vessel in which the rapid expansion was initiated.

[00919] In some embodiments, on day 10 or 11 of the rapid expansion the culture is scaled out by transfer of the culture into 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 new culture vessels equal in size to the original culture vessel in which the rapid expansion was initiated.

[00920] In some embodiments, the culture in the original culture vessel is evenly distributed into the new culture vessels.

[00921] In some embodiments, on day 10 or 11 of the rapid expansion the culture is scaled out by transfer of the culture into 5 new culture vessels equal in size to the original culture vessel in which the rapid expansion was initiated. In some embodiments, the culture in the original culture vessel is evenly distributed into the 5 new culture vessels.

[00922] In some embodiments, on day 10 or 11 of the rapid expansion the culture is scaled out and/or scaled up by transfer of the culture into one or more new culture vessels containing

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fresh culture medium supplemented with IL-2. In some embodiments, each of the new culture vessels contains fresh culture medium that is the same as or different from the culture medium in the original culture vessel in which the rapid expansion was initiated. In some embodiments, each of the new culture vessels contains fresh culture medium that is different from the culture medium in the original culture vessel. In some embodiments, each of the new culture vessels contains fresh DM2 culture medium and the original culture vessel contains DM1 culture medium.

[00923] In some embodiments, the culture medium used in the expansion processes disclosed herein is a serum-free medium or a defined medium. In some embodiments, the serum-free or defined medium comprises a basal cell medium and a serum supplement and/or a serum replacement. In some embodiments, the serum-free or defined medium is used to prevent and/or decrease experimental variation due in part to the lot-to-lot variation of serum-containing media.

[00924] In some embodiments, the serum-free or defined medium comprises a basal cell medium and a serum supplement and/or serum replacement. In some embodiments, 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.

[00925] In some embodiments, the serum supplement or serum replacement includes, but is not limited to one or more of CTS™ OpTmizer T-Cell Expansion Serum Supplement, CTS™ Immune Cell Serum Replacement, 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 one or more trace elements. In some embodiments, the defined 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+}. In some embodiments, the

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defined medium further comprises L-glutamine, sodium bicarbonate and/or 2-mercaptoethanol.

[00926] In some embodiments, the CTS™OpTmizer™ T-cell Immune Cell Serum Replacement is used with conventional growth media, including but not limited to CTS™ OpTmizer™ T-cell Expansion Basal Medium, CTS™ OpTmizer™ T-cell Expansion SFM, CTS™ AIM-V Medium, CST™ 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.

[00927] In some embodiments, the total serum replacement concentration (vol%) in the serum-free or defined medium is 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 total serum-free or defined medium. In some embodiments, the total serum replacement concentration is about 3% of the total volume of the serum-free or defined medium. In some embodiments, the total serum replacement concentration is about 5% of the total volume of the serum-free or defined medium. In some embodiments, the total serum replacement concentration is about 10% of the total volume of the serum-free or defined medium.

[00928] In some embodiments, the serum-free or defined medium is CTS™ OpTmizer™ T-cell Expansion SFM (ThermoFisher Scientific). Any formulation of CTS™ OpTmizer™ is useful in the present invention. CTS™ OpTmizer™ T-cell Expansion SFM is a combination of 1L CTS™ OpTmizer™ T-cell Expansion Basal Medium and 26 mL CTS™ OpTmizer™ T-Cell Expansion Supplement, which are mixed together prior to use. In some embodiments, the CTS™ OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific). In some embodiments, the CTS™ OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), along with 2-mercaptoethanol at 55mM. In some embodiments, the CTS™ OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and the final concentration of 2-mercaptoethanol in the media is 55μM.

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[00929] In some embodiments, the defined medium is CTS™ OpTmizer™ T-cell Expansion SFM (ThermoFisher Scientific). Any formulation of CTS™ OpTmizer™ is useful in the present invention. CTS™ OpTmizer™ T-cell Expansion SFM is a combination of 1L CTS™ OpTmizer™ T-cell Expansion Basal Medium and 26 mL CTS™ OpTmizer™ T-Cell Expansion Supplement, which are mixed together prior to use. In some embodiments, the CTS™ OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), along with 2-mercaptoethanol at 55mM. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), 55mM of 2-mercaptoethanol, and 2mM of L-glutamine. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), 55mM of 2-mercaptoethanol, and 2mM of L-glutamine, and further comprises about 1000 IU/mL to about 8000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), 55mM of 2-mercaptoethanol, and 2mM of L-glutamine, and further comprises about 3000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), 55mM of 2-mercaptoethanol, and 2mM of L-glutamine, and further comprises about 6000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and 55mM of 2-mercaptoethanol, and further comprises about 1000 IU/mL to about 8000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and 55mM of 2-mercaptoethanol, and further comprises about 3000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and 55mM of 2-mercaptoethanol, and further comprises about 1000 IU/mL to about 6000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and about 2mM glutamine, and further comprises about 1000 IU/mL to about 8000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher

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Scientific) and about 2mM glutamine, and further comprises about 3000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and about 2mM glutamine, and further comprises about 6000 IU/mL of IL-2. In some embodiments, the CTS™ OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and the final concentration of 2-mercaptoethanol in the media is 55μM.

[00930] In some embodiments, the serum-free medium or defined medium is supplemented with 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. In some embodiments, the serum-free medium or defined medium is supplemented with glutamine (*i.e.*, GlutaMAX®) at a concentration of about 2mM.

[00931] In some embodiments, the serum-free medium or defined medium is supplemented with 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. In some embodiments, the serum-free medium or defined medium is supplemented with 2-mercaptoethanol at a concentration of about 55mM.

[00932] In some embodiments, the defined media described in International PCT Publication No. WO/1998/030679, which is herein incorporated by reference, are useful in the present invention. In that publication, serum-free eukaryotic cell culture media are described. The serum-free, eukaryotic cell culture medium includes a basal cell culture medium supplemented with a serum-free supplement capable of supporting the growth of cells in serum- free culture. The serum-free eukaryotic cell culture medium supplement comprises or is obtained by combining one or more ingredients selected from the group consisting of 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 trace elements, and one or more antibiotics. In some embodiments, the defined medium further comprises L-glutamine, sodium bicarbonate and/or beta-mercaptoethanol. In some embodiments, the defined medium comprises an albumin or an albumin substitute and one or

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more ingredients selected from group consisting of 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, and one or more trace elements. In some embodiments, the defined 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^{3m}, Ge^{4+}, Se^{4+}, Br, T, Mn^{2+}, P, Si^{4+}, V^{5+}, Mo^{6+}, Ni^{2+}, Rb^{+}, Sn^{2+} and Zr^{4+}. In some embodiments, the basal cell media is selected from the group consisting of 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.

[00933] In some embodiments, the concentration of glycine in the defined medium is in the range of from about 5-200 mg/L, the concentration of L- histidine is about 5-250 mg/L, the concentration of L-isoleucine is about 5-300 mg/L, the concentration of L-methionine is about 5-200 mg/L, the concentration of L-phenylalanine is about 5-400 mg/L, the concentration of L-proline is about 1-1000 mg/L, the concentration of L- hydroxyproline is about 1-45 mg/L, the concentration of L-serine is about 1-250 mg/L, the concentration of L-threonine is about 10-500 mg/L, the concentration of L-tryptophan is about 2-110 mg/L, the concentration of L-tyrosine is about 3-175 mg/L, the concentration of L-valine is about 5-500 mg/L, the concentration of thiamine is about 1-20 mg/L, the concentration of reduced glutathione is about 1-20 mg/L, the concentration of L-ascorbic acid-2-phosphate is about 1-200 mg/L, the concentration of iron saturated transferrin is about 1-50 mg/L, the concentration of insulin is about 1-100 mg/L, the concentration of sodium selenite is about 0.000001-0.0001 mg/L, and the concentration of albumin (*e.g.*, AlbuMAX® I) is about 5000-50,000 mg/L.

[00934] In some embodiments, 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 4. In other embodiments, the non-trace element moiety ingredients in the defined medium are present in the final concentrations listed in the column under the heading "A Preferred Embodiment of the 1X Medium" in

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Table 4. In other embodiments, the defined medium is a basal cell medium comprising a serum free supplement. In some of these embodiments, the serum free supplement comprises non-trace moiety ingredients of the type and in the concentrations listed in the column under the heading "A Preferred Embodiment in Supplement" in Table 4.

[00935] In some embodiments, the osmolarity of the defined medium is between about 260 and 350 mOsmol. In some embodiments, the osmolarity is between about 280 and 310 mOsmol. In some embodiments, the defined medium is supplemented with up to about 3.7 g/L, or about 2.2 g/L sodium bicarbonate. The defined medium can be further supplemented with L-glutamine (final concentration of about 2 mM), one or more antibiotics, non-essential amino acids (NEAA; final concentration of about 100 μM), 2-mercaptoethanol (final concentration of about 100 μM).

[00936] In some embodiments, the defined media described in Smith, *et al.*, "Ex vivo expansion of human T cells for adoptive immunotherapy using the novel Xeno-free CTS Immune Cell Serum Replacement," *Clin Transl Immunology*, 4(1) 2015 (doi: 10.1038/cti.2014.31) are useful in the present invention. Briefly, RPMI or CTS™ OpTmizer™ was used as the basal cell medium, and supplemented with either 0, 2%, 5%, or 10% CTS™ Immune Cell Serum Replacement.

[00937] In some embodiments, the cell medium in the first and/or second gas permeable container is unfiltered. The use of unfiltered cell medium may simplify the procedures necessary to expand the number of cells. In some embodiments, the cell 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).

[00938] In some embodiments, the rapid second expansion (including expansions referred to as REP) is performed and further comprises a step wherein TILs are selected for superior tumor reactivity. Any selection method known in the art may be used. For example, the methods described in U.S. Patent Application Publication No. 2016/0010058 A1, the disclosures of which are incorporated herein by reference, may be used for selection of TILs for superior tumor reactivity.

[00939] Optionally, a cell viability assay can be performed after the rapid second expansion (including expansions referred to as the REP expansion), using standard assays known in the art. For example, a trypan blue exclusion assay can be done on a sample of the bulk TILs, which selectively labels dead cells and allows a viability assessment. In some embodiments,

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TIL samples can be counted and viability determined using a Cellometer K2 automated cell counter (Nexcelom Bioscience, Lawrence, MA). In some embodiments, viability is determined according to the standard Cellometer K2 Image Cytometer Automatic Cell Counter protocol.

[00940] The diverse antigen receptors of T and B lymphocytes are produced by somatic recombination of a limited, but large number of gene segments. These gene segments: V (variable), D (diversity), J (joining), and C (constant), determine the binding specificity and downstream applications of immunoglobulins and T-cell receptors (TCRs). The present invention provides a method for generating TILs which exhibit and increase the T-cell repertoire diversity. In some embodiments, the TILs obtained by the present method exhibit an increase in the T-cell repertoire diversity. In some embodiments, the TILs obtained in the second expansion exhibit an increase in the T-cell repertoire diversity. In some embodiments, the increase in diversity is an increase in the immunoglobulin diversity and/or the T-cell receptor diversity. In some embodiments, the diversity is in the immunoglobulin is in the immunoglobulin heavy chain. In some embodiments, the diversity is in the immunoglobulin is in the immunoglobulin light chain. In some embodiments, the diversity is in the T-cell receptor. In some embodiments, the diversity is in one of the T-cell receptors selected from the group consisting of alpha, beta, gamma, and delta receptors. In some embodiments, there is an increase in the expression of T-cell receptor (TCR) alpha and/or beta. In some embodiments, there is an increase in the expression of T-cell receptor (TCR) alpha. In some embodiments, there is an increase in the expression of T-cell receptor (TCR) beta. In some embodiments, there is an increase in the expression of TCRab (*i.e.*, TCRα/β).

[00941] In some embodiments, the rapid second expansion culture medium (*e.g.*, sometimes referred to as CM2 or the second cell culture medium), comprises IL-2, OKT-3, as well as the antigen-presenting feeder cells (APCs) and/or culture supernatant from a culture of APCs comprising OKT-3, as discussed in more detail below. In some embodiments, the rapid second expansion culture medium (*e.g.*, sometimes referred to as CM2 or the second cell culture medium), comprises 6000 IU/mL IL-2, 30 ug/flask OKT-3, as well as 7.5×10^8 antigen-presenting feeder cells (APCs), as discussed in more detail below. In some embodiments, the rapid second expansion culture medium (*e.g.*, sometimes referred to as CM2 or the second cell culture medium), comprises IL-2, OKT-3, as well as the antigen-presenting feeder cells (APCs), as discussed in more detail below. In some embodiments, the rapid second expansion culture medium (*e.g.*, sometimes referred to as

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CM2 or the second cell culture medium), comprises 6000 IU/mL IL-2, 30 ug/flask OKT-3, as well as 5×10^8 antigen-presenting feeder cells (APCs), as discussed in more detail below.

[00942] In some embodiments, the rapid second expansion, for example, Step D according to Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), is performed in a closed system bioreactor. In some embodiments, a closed system is employed for the TIL expansion, as described herein. In some embodiments, a bioreactor is employed. In some embodiments, a bioreactor is employed as the container. In some embodiments, the bioreactor employed is for example a G-REX-100 or a G-REX-500. In some embodiments, the bioreactor employed is a G-REX-100. In some embodiments, the bioreactor employed is a G-REX-500.

1. Feeder Cells and Antigen Presenting Cells and Culture Supernatants

[00943] In some embodiments, the rapid second expansion procedures described herein (for example including expansion such as those described in Step D from Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as well as those referred to as REP) require an excess of feeder cells during REP TIL expansion and/or during the rapid second expansion and/or culture supernatant from a culture of feeder cells (for example APCs) comprising OKT-3. In many embodiments, the feeder cells are peripheral blood mononuclear cells (PBMCs) obtained from standard whole blood units from healthy blood donors. The PBMCs are obtained using standard methods such as Ficoll-Paque gradient separation.

[00944] In general, the allogenic PBMCs are inactivated, either via irradiation or heat treatment, and used in the REP procedures, as described in the examples, which provides an exemplary protocol for evaluating the replication incompetence of irradiate allogeneic PBMCs.

[00945] In some embodiments, PBMCs are considered replication incompetent and acceptable for use in the TIL expansion procedures described herein if the total number of viable cells on day 7 or 14 is less than the initial viable cell number put into culture on day 0 of the REP and/or day 0 of the second expansion (*i.e.*, the start day of the second expansion).

[00946] In some embodiments, PBMCs are considered replication incompetent and acceptable for use in the TIL expansion procedures described herein if the total number of viable cells, cultured in the presence of OKT3 and IL-2, on day 7 and day 14 has not

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increased from the initial viable cell number put into culture on day 0 of the REP and/or day 0 of the second expansion (*i.e.*, the start day of the second expansion). In some embodiments, the PBMCs are cultured in the presence of 30 ng/ml OKT3 antibody and 3000 IU/ml IL-2. In some embodiments, the PBMCs are cultured in the presence of 60 ng/ml OKT3 antibody and 6000 IU/ml IL-2. In some embodiments, the PBMCs are cultured in the presence of 60 ng/ml OKT3 antibody and 3000 IU/ml IL-2. In some embodiments, the PBMCs are cultured in the presence of 30 ng/ml OKT3 antibody and 6000 IU/ml IL-2.

[00947] In some embodiments, PBMCs are considered replication incompetent and acceptable for use in the TIL expansion procedures described herein if the total number of viable cells, cultured in the presence of OKT3 and IL-2, on day 7 and day 14 has not increased from the initial viable cell number put into culture on day 0 of the REP and/or day 0 of the second expansion (*i.e.*, the start day of the second expansion). In some embodiments, the PBMCs are cultured in the presence of 30-60 ng/ml OKT3 antibody and 1000-6000 IU/ml IL-2. In some embodiments, the PBMCs are cultured in the presence of 30-60 ng/ml OKT3 antibody and 2000-5000 IU/ml IL-2. In some embodiments, the PBMCs are cultured in the presence of 30-60 ng/ml OKT3 antibody and 2000-4000 IU/ml IL-2. In some embodiments, the PBMCs are cultured in the presence of 30-60 ng/ml OKT3 antibody and 2500-3500 IU/ml IL-2. In some embodiments, the PBMCs are cultured in the presence of 30-60 ng/ml OKT3 antibody and 6000 IU/ml IL-2.

[00948] In some embodiments, the antigen-presenting feeder cells are PBMCs. In some embodiments, the antigen-presenting feeder cells are artificial antigen-presenting feeder cells. In some embodiments, the ratio of TILs to antigen-presenting feeder cells in the second expansion is about 1 to 10, about 1 to 25, about 1 to 50, about 1 to 100, about 1 to 125, about 1 to 150, about 1 to 175, about 1 to 200, about 1 to 225, about 1 to 250, about 1 to 275, about 1 to 300, about 1 to 325, about 1 to 350, about 1 to 375, about 1 to 400, or about 1 to 500. In some embodiments, the ratio of TILs to antigen-presenting feeder cells in the second expansion is between 1 to 50 and 1 to 300. In some embodiments, the ratio of TILs to antigen-presenting feeder cells in the second expansion is between 1 to 100 and 1 to 200.

[00949] In some embodiments, 5×10^8 feeder cells are used during the rapid second expansion process. In some embodiments, 2×10^9 feeder cells are used during the rapid second expansion process. In some embodiments, 2.5×10^9 feeder cells are used during the rapid second expansion process.

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[00950] In some embodiments, the second expansion procedures described herein require a ratio of about 5×10^8 feeder cells to about 100×10^6 TILs. In some embodiments, the second expansion procedures described herein require a ratio of about 7.5×10^8 feeder cells to about 100×10^6 TILs. In other embodiments, the second expansion procedures described herein require a ratio of about 5×10^8 feeder cells to about 50×10^6 TILs. In other embodiments, the second expansion procedures described herein require a ratio of about 7.5×10^8 feeder cells to about 50×10^6 TILs. In yet another embodiment, the second expansion procedures described herein require about 5×10^8 feeder cells to about 25×10^6 TILs. In yet another embodiment, the second expansion procedures described herein require about 7.5×10^8 feeder cells to about 25×10^6 TILs. In yet another embodiment, the rapid second expansion requires twice the number of feeder cells as the rapid second expansion. In yet another embodiment, when the priming first expansion described herein requires about 2.5×10^8 feeder cells, the rapid second expansion requires about 5×10^8 feeder cells. In yet another embodiment, when the priming first expansion described herein requires about 2.5×10^8 feeder cells, the rapid second expansion requires about 7.5×10^8 feeder cells. In yet another embodiment, the rapid second expansion requires two times (2.0X), 2.5X, 3.0X, 3.5X or 4.0X the number of feeder cells as the priming first expansion.

[00951] In some embodiments, the rapid second expansion procedures described herein require an excess of feeder cells during the rapid second expansion. In many embodiments, the feeder cells are peripheral blood mononuclear cells (PBMCs) obtained from standard whole blood units from allogeneic healthy blood donors. The PBMCs are obtained using standard methods such as Ficoll-Paque gradient separation. In some embodiments, artificial antigen-presenting (aAPC) cells are used in place of PBMCs. In some emdboidumetns, culture supernatant from a culture of aAPCs comprising OKT-3 is used. In some embodiments, the PBMCs are added to the rapid second expansion at twice the concentration of PBMCs that were added to the priming first expansion.

[00952] In general, the allogenic PBMCs are inactivated, either via irradiation or heat treatment, and used in the TIL expansion procedures described herein, including the exemplary procedures described in the figures and examples.

[00953] In some embodiments, artificial antigen presenting cells are used in the rapid second expansion as a replacement for, or in combination with, PBMCs.

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[00954] In some embodiments, the rapid second expansion procedures described herein, as well as those referred to as REP processes do not require feeder cells (also referred to herein as "antigen-presenting cells"), but rather require a culture supernatant obtained from a culture of antigen-presenting feeder cells that contains OKT-3. In some embodiments, the culture supernatant is obtained from a culture of PBMCs in a culture medium supplemented with IL-2 and OKT-3. In some embodiments, the culture supernatant is obtained from a culture of PBMCs after about 3 or 4 days of culture in a culture medium supplemented with IL-2 and OKT-3. In some embodiments, the culture supernatant is obtained from a culture of PBMCs cultured in a culture medium supplemented with IL-2 and OKT-3 after the growth rate of the PMBCs in culture begins to decline. In some embodiments, the culture supernatant is obtained from a culture of PBMCs cultured in a culture medium supplemented with IL-2 and OKT-3 after the growth rate of the PMBCs in culture has declined about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more. In some embodiments, the culture supernatant is obtained from a culture of PBMCs cultured in a culture medium supplemented with IL-2 and OKT-3 after the culture medium is exhausted or spent. In some embodiments, the culture supernatant is obtained from a culture of PBMCs cultured in a culture medium supplemented with IL-2 and OKT-3 after the culture medium is at least about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more exhausted or spent.

[00955] In one embodiment, neither the priming first expansion procedures nor the rapid second expansion procedures require feeder cells, but rather a culture supernatant obtained from a culture of feeder cells that contains OKT-3. In one embodiment, neither the priming first expansion procedures nor the rapid second expansion procedures require feeder cells, but rather the priming first expansion requires a first culture supernatant obtained from a first culture of feeder cells contains OKT-3, and the rapid second expansion requires a second culture supernatant obtained from a second culture of feeder cells that contains OKT-3. In other embodiments, the priming first expansion procedures require feeder cells, whereas the rapid second expansion procedures require a culture supernatant obtained from a culture of feeder cells that contains OKT-3. In yet another embodiment, the priming first expansion procedures require a culture supernatant obtained from a culture of feeder cells that contain OKT-3, whereas the rapid second expansion procedures require feeder cells. In yet another embodiment, both the priming first expansion procedures and the rapid second expansion procedures require feeder cells.

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[00956] The rapid second expansion methods described herein generally use culture media with high doses of a cytokine, in particular IL-2, as is known in the art.

[00957] Alternatively, using combinations of cytokines for the rapid second expansion of TILs is additionally possible, with combinations of two or more of IL-2, IL-15 and IL-21 as is generally outlined in WO 2015/189356 and WO 2015/189357, hereby expressly incorporated by reference in their entirety. Thus, possible combinations include IL-2 and IL-15, IL-2 and IL-21, IL-15 and IL-21, and IL-2, IL-15 and IL-21, with the latter finding particular use in many embodiments. The use of combinations of cytokines specifically favors the generation of lymphocytes, and in particular T-cells as described therein.

E. STEP E: Harvest TILS

[00958] After the rapid second expansion step, cells can be harvested. In some embodiments the TILs are harvested after one, two, three, four or more expansion steps, for example as provided in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments the TILs are harvested after two expansion steps, for example as provided in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments the TILs are harvested after two expansion steps, one priming first expansion and one rapid second expansion, for example as provided in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G).

[00959] TILs can be harvested in any appropriate and sterile manner, including, for example by centrifugation. Methods for TIL harvesting are well known in the art and any such known methods can be employed with the present process. In some embodiments, TILS are harvested using an automated system.

[00960] Cell harvesters and/or cell processing systems are commercially available from a variety of sources, including, for example, Fresenius Kabi, Tomtec Life Science, Perkin Elmer, and Inotech Biosystems International, Inc. Any cell based harvester can be employed with the present methods. In some embodiments, the cell harvester and/or cell processing system is a membrane-based cell harvester. In some embodiments, cell harvesting is via a cell processing system, such as the LOVO system (manufactured by Fresenius Kabi). The term "LOVO cell processing system" also refers to any instrument or device manufactured by any vendor that can pump a solution comprising cells through a membrane or filter such as a spinning membrane or spinning filter in a sterile and/or closed system environment, allowing

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for continuous flow and cell processing to remove supernatant or cell culture media without pelletization. In some embodiments, the cell harvester and/or cell processing system can perform cell separation, washing, fluid-exchange, concentration, and/or other cell processing steps in a closed, sterile system.

[00961] In some embodiments, the rapid second expansion, for example, Step D according to Figure 1 (in particular, *e.g.,* Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), is performed in a closed system bioreactor. In some embodiments, a closed system is employed for the TIL expansion, as described herein. In some embodiments, a bioreactor is employed. In some embodiments, a bioreactor is employed as the container. In some embodiments, the bioreactor employed is for example a G-REX-100 or a G-REX-500. In some embodiments, the bioreactor employed is a G-REX-100. In some embodiments, the bioreactor employed is a G-REX-500.

[00962] In some embodiments, Step E according to Figure 1 (in particular, *e.g.,* Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), is performed according to the processes described herein. In some embodiments, the closed system is accessed via syringes under sterile conditions in order to maintain the sterility and closed nature of the system. In some embodiments, a closed system as described herein is employed.

[00963] In some embodiments, TILs are harvested according to the methods described in herein. In some embodiments, TILs between days 14 and 16 are harvested using the methods as described herein. In some embodiments, TILs are harvested at 14 days using the methods as described herein. In some embodiments, TILs are harvested at 15 days using the methods as described herein. In some embodiments, TILs are harvested at 16 days using the methods as described herein.

F. STEP F: Final Formulation/ Transfer to Infusion Bag

[00964] After Steps A through E as provided in an exemplary order in Figure 1 (in particular, *e.g.,* Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) and as outlined in detailed above and herein are complete, cells are transferred to a container for use in administration to a patient. In some embodiments, once a therapeutically sufficient number of TILs are obtained using the expansion methods described above, they are transferred to a container for use in administration to a patient.

[00965] In some embodiments, TILs expanded using the methods of the present disclosure are administered to a patient as a pharmaceutical composition. In some embodiments, the

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pharmaceutical composition is a suspension of TILs in a sterile buffer. TILs expanded as disclosed herein may be administered by any suitable route as known in the art. In some embodiments, the TILs are administered as a single intra-arterial or intravenous infusion, which preferably lasts approximately 30 to 60 minutes. Other suitable routes of administration include intraperitoneal, intrathecal, and intralymphatic.

G. PBMC Feeder Cell Ratios

[00966] In some embodiments, the culture media used in expansion methods described herein (see for example, Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) include an anti-CD3 antibody *e.g.* OKT-3. An anti-CD3 antibody in combination with IL-2 induces T cell activation and cell division in the TIL population. This effect can be seen with full length antibodies as well as Fab and F(ab')2 fragments, with the former being generally preferred; see, *e.g.*, Tsoukas *et al.*, *J. Immunol.* **1985,** *135,* 1719, hereby incorporated by reference in its entirety.

[00967] In some embodiments, the number of PBMC feeder layers is calculated as follows:

A. Volume of a T-cell (10 μm diameter): $V = (4/3)\ \pi r^3 = 523.6\ \mu m^3$

B. Columne of G-Rex 100 (M) with a 40 μm (4 cells) height: $V = (4/3)\ \pi r^3 = 4\times10^{12}\ \mu m^3$

C. Number cell required to fill column B: $4\times10^{12}\ \mu m^3\ /\ 523.6\ \mu m^3 = 7.6\times10^{8}\ \mu m^3\ *\ 0.64 = 4.86\times10^{8}$

D. Number cells that can be optimally activated in 4D space: $4.86\times10^{8} / 24 = 20.25\times10^{6}$

E. Number of feeders and TIL extrapolated to G-Rex 500: TIL: 100×10^{6} and Feeder: 2.5×10^{9}

In this calculation, an approximation of the number of mononuclear cells required to provide an icosahedral geometry for activation of TIL in a cylinder with a 100 cm^2 base is used. The calculation derives the experimental result of ~5×10^{8} for threshold activation of T-cells which closely mirrors NCI experimental data.[(1)] (C) The multiplier (0.64) is the random packing density for equivalent spheres as calculated by Jaeger and Nagel in 1992 [(2)]. (D) The divisor 24 is the number of equivalent spheres that could contact a similar object in 4 dimensional space "the Newton number."[(3)].

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(1) Jin, Jianjian, et.al., Simplified Method of the Growth of Human Tumor Infiltrating Lymphocytes (TIL) in Gas-Permeable Flasks to Numbers Needed for Patient Treatment. J Immunother. 2012 Apr; 35(3): 283–292.

(2) Jaeger HM, Nagel SR. Physics of the granular state. Science. 1992 Mar 20;255(5051):1523-31.

(3) O. R. Musin (2003). "The problem of the twenty-five spheres". Russ. Math. Surv. 58 (4): 794–795.

[00968] In some embodiments, the number of antigen-presenting feeder cells exogenously supplied during the priming first expansion is approximately one-half the number of antigen-presenting feeder cells exogenously supplied during the rapid second expansion. In certain embodiments, the method comprises performing the priming first expansion in a cell culture medium which comprises approximately 50% fewer antigen presenting cells as compared to the cell culture medium of the rapid second expansion.

[00969] In other embodiments, the number of antigen-presenting feeder cells (APCs) exogenously supplied during the rapid second expansion is greater than the number of APCs exogenously supplied during the priming first expansion.

[00970] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 20:1.

[00971] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 10:1.

[00972] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 9:1.

[00973] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 8:1.

[00974] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 7:1.

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[00975] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 6:1.

[00976] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 5:1.

[00977] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 4:1.

[00978] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion) is in a range of from at or about 1.1:1 to at or about 3:1.

[00979] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.9:1.

[00980] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.8:1.

[00981] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.7:1.

[00982] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.6:1.

[00983] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.5:1.

[00984] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.4:1.

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[00985] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.3:1.

[00986] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.2:1.

[00987] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.1:1.

[00988] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 1.1:1 to at or about 2:1.

[00989] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 2:1 to at or about 10:1.

[00990] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 2:1 to at or about 5:1.

[00991] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 2:1 to at or about 4:1.

[00992] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 2:1 to at or about 3:1.

[00993] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 2:1 to at or about 2.9:1.

[00994] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 2:1 to at or about 2.8:1.

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[00995] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 2:1 to at or about 2.7:1.

[00996] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 2:1 to at or about 2.6:1.

[00997] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 2:1 to at or about 2.5:1.

[00998] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 2:1 to at or about 2.4:1.

[00999] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 2:1 to at or about 2.3:1.

[001000] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about about 2:1 to at or about 2.2:1.

[001001] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is in a range of from at or about 2:1 to at or about 2.1:1.

[001002] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is at or about 2:1.

[001003] In other embodiments, the ratio of the number of APCs exogenously supplied during the rapid second expansion to the number of APCs exogenously supplied during the priming first expansion is at or about 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1, 1.6:1, 1.7:1, 1.8:1, 1.9:1, 2:1, 2.1:1, 2.2:1, 2.3:1, 2.4:1, 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, 3.5:1, 3.6:1, 3.7:1, 3.8:1, 3.9:1, 4:1, 4.1:1, 4.2:1, 4.3:1, 4.4:1, 4.5:1, 4.6:1, 4.7:1, 4.8:1, 4.9:1, or 5:1.

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[001004] In other embodiments, the number of APCs exogenously supplied during the priming first expansion is at or about 1×10^8, 1.1×10^8, 1.2×10^8, 1.3×10^8, 1.4×10^8, 1.5×10^8, 1.6×10^8, 1.7×10^8, 1.8×10^8, 1.9×10^8, 2×10^8, 2.1×10^8, 2.2×10^8, 2.3×10^8, 2.4×10^8, 2.5×10^8, 2.6×10^8, 2.7×10^8, 2.8×10^8, 2.9×10^8, 3×10^8, 3.1×10^8, 3.2×10^8, 3.3×10^8, 3.4×10^8 or 3.5×10^8 APCs, and the number of APCs exogenously supplied during the rapid second expansion is at or about 3.5×10^8, 3.6×10^8, 3.7×10^8, 3.8×10^8, 3.9×10^8, 4×10^8, 4.1×10^8, 4.2×10^8, 4.3×10^8, 4.4×10^8, 4.5×10^8, 4.6×10^8, 4.7×10^8, 4.8×10^8, 4.9×10^8, 5×10^8, 5.1×10^8, 5.2×10^8, 5.3×10^8, 5.4×10^8, 5.5×10^8, 5.6×10^8, 5.7×10^8, 5.8×10^8, 5.9×10^8, 6×10^8, 6.1×10^8, 6.2×10^8, 6.3×10^8, 6.4×10^8, 6.5×10^8, 6.6×10^8, 6.7×10^8, 6.8×10^8, 6.9×10^8, 7×10^8, 7.1×10^8, 7.2×10^8, 7.3×10^8, 7.4×10^8, 7.5×10^8, 7.6×10^8, 7.7×10^8, 7.8×10^8, 7.9×10^8, 8×10^8, 8.1×10^8, 8.2×10^8, 8.3×10^8, 8.4×10^8, 8.5×10^8, 8.6×10^8, 8.7×10^8, 8.8×10^8, 8.9×10^8, 9×10^8, 9.1×10^8, 9.2×10^8, 9.3×10^8, 9.4×10^8, 9.5×10^8, 9.6×10^8, 9.7×10^8, 9.8×10^8, 9.9×10^8 or 1×10^9 APCs.

[001005] In other embodiments, the number of APCs exogenously supplied during the priming first expansion is in the range of at or about 1.5×10^8 APCs to at or about 3×10^8 APCs, and the number of APCs exogenously supplied during the rapid second expansion is in the range of at or about 4×10^8 APCs to at or about 7.5×10^8 APCs.

[001006] In other embodiments, the number of APCs exogenously supplied during the priming first expansion is in the range of at or about 2×10^8 APCs to at or about 2.5×10^8 APCs, and the number of APCs exogenously supplied during the rapid second expansion is in the range of at or about 4.5×10^8 APCs to at or about 5.5×10^8 APCs.

[001007] In other embodiments, the number of APCs exogenously supplied during the priming first expansion is at or about 2.5×10^8 APCs, and the number of APCs exogenously supplied during the rapid second expansion is at or about 5×10^8 APCs.

[001008] In some embodiments, the number of APCs (including, for example, PBMCs) added at day 0 of the priming first expansion is approximately one-half of the number of PBMCs added at day 7 of the priming first expansion (*e.g.*, day 7 of the method). In certain embodiments, the method comprises adding antigen presenting cells at day 0 of the priming first expansion to the first population of TILs and adding antigen presenting cells at day 7 to the second population of TILs, wherein the number of antigen presenting cells added at day 0 is approximately 50% of the number of antigen presenting cells added at day 7 of the priming first expansion (*e.g.*, day 7 of the method).

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[001009] In other embodiments, the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion is greater than the number of PBMCs exogenously supplied at day 0 of the priming first expansion.

[001010] In other embodiments, the APCs exogenously supplied in the priming first expansion are seeded in the culture flask at a density in a range of at or about 1.0×10^6 APCs/cm^2 to at or about 4.5×10^6 APCs/cm^2.

[001011] In other embodiments, the APCs exogenously supplied in the priming first expansion are seeded in the culture flask at a density in a range of at or about 1.5×10^6 APCs/cm^2 to at or about 3.5×10^6 APCs/cm^2.

[001012] In other embodiments, the APCs exogenously supplied in the priming first expansion are seeded in the culture flask at a density in a range of at or about 2×10^6 APCs/cm^2 to at or about 3×10^6 APCs/cm^2.

[001013] In other embodiments, the APCs exogenously supplied in the priming first expansion are seeded in the culture flask at a density of at or about 2×10^6 APCs/cm^2.

[001014] In other embodiments, the APCs exogenously supplied in the priming first expansion are seeded in the culture flask at a density of at or about 1.0×10^6, 1.1×10^6, 1.2×10^6, 1.3×10^6, 1.4×10^6, 1.5×10^6, 1.6×10^6, 1.7×10^6, 1.8×10^6, 1.9×10^6, 2×10^6, 2.1×10^6, 2.2×10^6, 2.3×10^6, 2.4×10^6, 2.5×10^6, 2.6×10^6, 2.7×10^6, 2.8×10^6, 2.9×10^6, 3×10^6, 3.1×10^6, 3.2×10^6, 3.3×10^6, 3.4×10^6, 3.5×10^6, 3.6×10^6, 3.7×10^6, 3.8×10^6, 3.9×10^6, 4×10^6, 4.1×10^6, 4.2×10^6, 4.3×10^6, 4.4×10^6 or 4.5×10^6 APCs/cm^2.

[001015] In other embodiments, the APCs exogenously supplied in the rapid second expansion are seeded in the culture flask at a density in a range of at or about 2.5×10^6 APCs/cm^2 to at or about 7.5×10^6 APCs/cm^2.

[001016] In other embodiments, the APCs exogenously supplied in the rapid second expansion are seeded in the culture flask at a density in a range of at or about 3.5×10^6 APCs/cm^2 to about 6.0×10^6 APCs/cm^2.

[001017] In other embodiments, the APCs exogenously supplied in the rapid second expansion are seeded in the culture flask at a density in a range of at or about 4.0×10^6 APCs/cm^2 to about 5.5×10^6 APCs/cm^2.

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[001018] In other embodiments, the APCs exogenously supplied in the rapid second expansion are seeded in the culture flask at a density in a range of at or about 4.0×10^6 APCs/cm^2.

[001019] In other embodiments, the APCs exogenously supplied in the rapid second expansion are seeded in the culture flask at a density of at or about 2.5×10^6 APCs/cm^2, 2.6×10^6 APCs/cm^2, 2.7×10^6 APCs/cm^2, 2.8×10^6, 2.9×10^6, 3×10^6, 3.1×10^6, 3.2×10^6, 3.3×10^6, 3.4×10^6, 3.5×10^6, 3.6×10^6, 3.7×10^6, 3.8×10^6, 3.9×10^6, 4×10^6, 4.1×10^6, 4.2×10^6, 4.3×10^6, 4.4×10^6, 4.5×10^6, 4.6×10^6, 4.7×10^6, 4.8×10^6, 4.9×10^6, 5×10^6, 5.1×10^6, 5.2×10^6, 5.3×10^6, 5.4×10^6, 5.5×10^6, 5.6×10^6, 5.7×10^6, 5.8×10^6, 5.9×10^6, 6×10^6, 6.1×10^6, 6.2×10^6, 6.3×10^6, 6.4×10^6, 6.5×10^6, 6.6×10^6, 6.7×10^6, 6.8×10^6, 6.9×10^6, 7×10^6, 7.1×10^6, 7.2×10^6, 7.3×10^6, 7.4×10^6 or 7.5×10^6 APCs/cm^2.

[001020] In other embodiments, the APCs exogenously supplied in the priming first expansion are seeded in the culture flask at a density of at or about 1.0×10^6, 1.1×10^6, 1.2×10^6, 1.3×10^6, 1.4×10^6, 1.5×10^6, 1.6×10^6, 1.7×10^6, 1.8×10^6, 1.9×10^6, 2×10^6, 2.1×10^6, 2.2×10^6, 2.3×10^6, 2.4×10^6, 2.5×10^6, 2.6×10^6, 2.7×10^6, 2.8×10^6, 2.9×10^6, 3×10^6, 3.1×10^6, 3.2×10^6, 3.3×10^6, 3.4×10^6, 3.5×10^6, 3.6×10^6, 3.7×10^6, 3.8×10^6, 3.9×10^6, 4×10^6, 4.1×10^6, 4.2×10^6, 4.3×10^6, 4.4×10^6 or 4.5×10^6 APCs/cm^2 and the the APCs exogenously supplied in the rapid second expansion are seeded in the culture flask at a density of at or about 2.5×10^6 APCs/cm^2, 2.6×10^6 APCs/cm^2, 2.7×10^6 APCs/cm^2, 2.8×10^6, 2.9×10^6, 3×10^6, 3.1×10^6, 3.2×10^6, 3.3×10^6, 3.4×10^6, 3.5×10^6, 3.6×10^6, 3.7×10^6, 3.8×10^6, 3.9×10^6, 4×10^6, 4.1×10^6, 4.2×10^6, 4.3×10^6, 4.4×10^6, 4.5×10^6, 4.6×10^6, 4.7×10^6, 4.8×10^6, 4.9×10^6, 5×10^6, 5.1×10^6, 5.2×10^6, 5.3×10^6, 5.4×10^6, 5.5×10^6, 5.6×10^6, 5.7×10^6, 5.8×10^6, 5.9×10^6, 6×10^6, 6.1×10^6, 6.2×10^6, 6.3×10^6, 6.4×10^6, 6.5×10^6, 6.6×10^6, 6.7×10^6, 6.8×10^6, 6.9×10^6, 7×10^6, 7.1×10^6, 7.2×10^6, 7.3×10^6, 7.4×10^6 or 7.5×10^6 APCs/cm^2.

[001021] In other embodiments, the APCs exogenously supplied in the priming first expansion are seeded in the culture flask at a density in a range of at or about 1.0×10^6 APCs/cm^2 to at or about 4.5×10^6 APCs/cm^2, and the APCs exogenously supplied in the rapid second expansion are seeded in the culture flask at a density in a range of at or about 2.5×10^6 APCs/cm^2 to at or about 7.5×10^6 APCs/cm^2.

[001022] In other embodiments, the APCs exogenously supplied in the priming first expansion are seeded in the culture flask at a density in a range of at or about 1.5×10^6 APCs/cm^2 to at or about 3.5×10^6 APCs/cm^2, and the APCs exogenously supplied in the rapid

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second expansion are seeded in the culture flask at a density in a range of at or about 3.5×10^6 APCs/cm^2 to at or about 6×10^6 APCs/cm^2.

[001023] In other embodiments, the APCs exogenously supplied in the priming first expansion are seeded in the culture flask at a density in a range of at or about 2×10^6 APCs/cm^2 to at or about 3×10^6 APCs/cm^2, and the APCs exogenously supplied in the rapid second expansion are seeded in the culture flask at a density in a range of at or about 4×10^6 APCs/cm^2 to at or about 5.5×10^6 APCs/cm^2.

[001024] In other embodiments, the APCs exogenously supplied in the priming first expansion are seeded in the culture flask at a density at or about 2×10^6 APCs/cm^2 and the APCs exogenously supplied in the rapid second expansion are seeded in the culture flask at a density of at or about 4×10^6 APCs/cm^2.

[001025] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of PBMCs exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 20:1.

[001026] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of PBMCs exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 10:1.

[001027] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of PBMCs exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 9:1.

[001028] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 8:1.

[001029] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 7:1.

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[001030] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 6:1.

[001031] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 5:1.

[001032] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 4:1.

[001033] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 3:1.

[001034] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.9:1.

[001035] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.8:1.

[001036] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.7:1.

[001037] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.6:1.

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[001038] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.5:1.

[001039] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.4:1.

[001040] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.3:1.

[001041] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.2:1.

[001042] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 2.1:1.

[001043] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 1.1:1 to at or about 2:1.

[001044] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 2:1 to at or about 10:1.

[001045] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 2:1 to at or about 5:1.

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[001046] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 2:1 to at or about 4:1.

[001047] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 2:1 to at or about 3:1.

[001048] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 2:1 to at or about 2.9:1.

[001049] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 2:1 to at or about 2.8:1.

[001050] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 2:1 to at or about 2.7:1.

[001051] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 2:1 to at or about 2.6:1.

[001052] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 2:1 to at or about 2.5:1.

[001053] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 2:1 to at or about 2.4:1.

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[001054] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 2:1 to at or about 2.3:1.

[001055] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about about 2:1 to at or about 2.2:1.

[001056] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in a range of from at or about 2:1 to at or about 2.1:1.

[001057] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is at or about 2:1.

[001058] In other embodiments, the ratio of the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion to the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is at or about 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1, 1.6:1, 1.7:1, 1.8:1, 1.9:1, 2:1, 2.1:1, 2.2:1, 2.3:1, 2.4:1, 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, 3.5:1, 3.6:1, 3.7:1, 3.8:1, 3.9:1, 4:1, 4.1:1, 4.2:1, 4.3:1, 4.4:1, 4.5:1, 4.6:1, 4.7:1, 4.8:1, 4.9:1, or 5:1.

[001059] In other embodiments, the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is at or about 1×10^8, 1.1×10^8, 1.2×10^8, 1.3×10^8, 1.4×10^8, 1.5×10^8, 1.6×10^8, 1.7×10^8, 1.8×10^8, 1.9×10^8, 2×10^8, 2.1×10^8, 2.2×10^8, 2.3×10^8, 2.4×10^8, 2.5×10^8, 2.6×10^8, 2.7×10^8, 2.8×10^8, 2.9×10^8, 3×10^8, 3.1×10^8, 3.2×10^8, 3.3×10^8, 3.4×10^8 or 3.5×10^8 APCs (including, for example, PBMCs), and the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion is at or about 3.5×10^8, 3.6×10^8, 3.7×10^8, 3.8×10^8, 3.9×10^8, 4×10^8, 4.1×10^8, 4.2×10^8, 4.3×10^8, 4.4×10^8, 4.5×10^8, 4.6×10^8, 4.7×10^8, 4.8×10^8, 4.9×10^8, 5×10^8, 5.1×10^8, 5.2×10^8, 5.3×10^8, 5.4×10^8, 5.5×10^8, 5.6×10^8, 5.7×10^8, 5.8×10^8, 5.9×10^8, 6×10^8, 6.1×10^8, 6.2×10^8, 6.3×10^8, 6.4×10^8, 6.5×10^8, 6.6×10^8, 6.7×10^8, 6.8×10^8, 6.9×10^8, 7×10^8, 7.1×10^8,

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7.2×10^8, 7.3×10^8, 7.4×10^8, 7.5×10^8, 7.6×10^8, 7.7×10^8, 7.8×10^8, 7.9×10^8, 8×10^8, 8.1×10^8, 8.2×10^8, 8.3×10^8, 8.4×10^8, 8.5×10^8, 8.6×10^8, 8.7×10^8, 8.8×10^8, 8.9×10^8, 9×10^8, 9.1×10^8, 9.2×10^8, 9.3×10^8, 9.4×10^8, 9.5×10^8, 9.6×10^8, 9.7×10^8, 9.8×10^8, 9.9×10^8 or 1×10^9 APCs (including, for example, PBMCs).

[001060] In other embodiments, the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in the range of at or about 1×10^8 APCs (including, for example, PBMCs) to at or about 3.5×10^8 APCs (including, for example, PBMCs), and the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion is in the range of at or about 3.5×10^8 APCs (including, for example, PBMCs) to at or about 1×10^9 APCs (including, for example, PBMCs).

[001061] In other embodiments, the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in the range of at or about 1.5×10^8 APCs to at or about 3×10^8 APCs (including, for example, PBMCs), and the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion is in the range of at or about 4×10^8 APCs (including, for example, PBMCs) to at or about 7.5×10^8 APCs (including, for example, PBMCs).

[001062] In other embodiments, the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is in the range of at or about 2×10^8 APCs (including, for example, PBMCs) to at or about 2.5×10^8 APCs (including, for example, PBMCs), and the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion is in the range of at or about 4.5×10^8 APCs (including, for example, PBMCs) to at or about 5.5×10^8 APCs (including, for example, PBMCs).

[001063] In other embodiments, the number of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion is at or about 2.5×10^8 APCs (including, for example, PBMCs) and the number of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion is at or about 5×10^8 APCs (including, for example, PBMCs).

[001064] In some embodiments, the number of layers of APCs (including, for example, PBMCs) added at day 0 of the priming first expansion is approximately one-half of the number of layers of APCs (including, for example, PBMCs) added at day 7 of the rapid

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second expansion. In certain embodiments, the method comprises adding antigen presenting cell layers at day 0 of the priming first expansion to the first population of TILs and adding antigen presenting cell layers at day 7 to the second population of TILs, wherein the number of antigen presenting cell layer added at day 0 is approximately 50% of the number of antigen presenting cell layers added at day 7.

[001065] In other embodiments, the number of layers of APCs (including, for example, PBMCs) exogenously supplied at day 7 of the rapid second expansion is greater than the number of layers of APCs (including, for example, PBMCs) exogenously supplied at day 0 of the priming first expansion.

[001066] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of at or about 2 cell layers and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of at or about 4 cell layers.

[001067] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of at or about one cell layer and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of at or about 3 cell layers.

[001068] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of at or about 1.5 cell layers to at or about 2.5 cell layers and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of at or about 3 cell layers.

[001069] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of at or about one cell layer and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of at or about 2 cell layers.

[001070] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of of at or about 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 or 3 cell layers and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of at or about 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6,

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5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9 or 8 cell layers.

[001071] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of at or about 1 cell layer to at or about 2 cell layers and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of at or about 3 cell layers to at or about 10 cell layers.

[001072] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of at or about 2 cell layers to at or about 3 cell layers and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of at or about 4 cell layers to at or about 8 cell layers.

[001073] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of at or about 2 cell layers and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of at or about 4 cell layers to at or about 8 cell layers.

[001074] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of at or about 1, 2 or 3 cell layers and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with an average thickness of at or about 3, 4, 5, 6, 7, 8, 9 or 10 cell layers.

[001075] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is in the range of at or about 1:1.1 to at or about 1:10.

[001076] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first

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number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is in the range of at or about 1:1.1 to at or about 1:8.

[001077] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is in the range of at or about 1:1.1 to at or about 1:7.

[001078] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is in the range of at or about 1:1.1 to at or about 1:6.

[001079] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is in the range of at or about 1:1.1 to at or about 1:5.

[001080] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first

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number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is in the range of at or about 1:1.1 to at or about 1:4.

[001081] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is in the range of at or about 1:1.1 to at or about 1:3.

[001082] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is in the range of at or about 1:1.1 to at or about 1:2.

[001083] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is in the range of at or about 1:1.2 to at or about 1:8.

[001084] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first

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number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is in the range of at or about 1:1.3 to at or about 1:7.

[001085] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is in the range of at or about 1:1.4 to at or about 1:6.

[001086] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is in the range of at or about 1:1.5 to at or about 1:5.

[001087] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is in the range of at or about 1:1.6 to at or about 1:4.

[001088] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first

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number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is in the range of at or about 1:1.7 to at or about 1:3.5.

[001089] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is in the range of at or about 1:1.8 to at or about 1:3.

[001090] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is in the range of at or about 1:1.9 to at or about 1:2.5.

[001091] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is at or about 1: 2.

[001092] In other embodiments, day 0 of the priming first expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a first average thickness equal to a first

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number of layers of APCs (including, for example, PBMCs) and day 7 of the rapid second expansion occurs in the presence of layered APCs (including, for example, PBMCs) with a second average thickness equal to a second number of layers of APCs (including, for example, PBMCs), wherein the ratio of the first number of layers of APCs (including, for example, PBMCs) to the second number of layers of APCs (including, for example, PBMCs) is in at or about 1:1.1, 1:1.2, 1:1.3, 1:1.4, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1:1.9, 1:2, 1:2.1, 1:2.2, 1:2.3, 1:2.4, 1:2.5, 1:2.6, 1:2.7, 1:2.8, 1:2.9, 1:3, 1:3.1, 1:3.2, 1:3.3, 1:3.4, 1:3.5, 1:3.6, 1:3.7, 1:3.8, 1:3.9, 1:4, 1:4.1, 1:4.2, 1:4.3, 1:4.4, 1:4.5, 1:4.6, 1:4.7, 1:4.8, 1:4.9, 1:5, 1:5.1, 1:5.2, 1:5.3, 1:5.4, 1:5.5, 1:5.6, 1:5.7, 1:5.8, 1:5.9, 1:6, 1:6.1, 1:6.2, 1:6.3, 1:6.4, 1:6.5, 1:6.6, 1:6.7, 1:6.8, 1:6.9, 1:7, 1:7.1, 1:7.2, 1:7.3, 1:7.4, 1:7.5, 1:7.6, 1:7.7, 1:7.8, 1:7.9, 1:8, 1:8.1, 1:8.2, 1:8.3, 1:8.4, 1:8.5, 1:8.6, 1:8.7, 1:8.8, 1:8.9, 1:9, 1:9.1, 1:9.2, 1:9.3, 1:9.4, 1:9.5, 1:9.6, 1:9.7, 1:9.8, 1:9.9 or 1:10.

[001093] In some embodiments, the number of APCs in the priming first expansion is in the range of about 1.0×10^6 APCs/cm^2 to about 4.5×10^6 APCs/cm^2, and the number of APCs in the rapid second expansion is in the range of about 2.5×10^6 APCs/cm^2 to about 7.5×10^6 APCs/cm^2.

[001094] In some embodiments, the number of APCs in the priming first expansion is in the range of about 1.5×10^6 APCs/cm^2 to about 3.5×10^6 APCs/cm^2, and the number of APCs in the rapid second expansion is in the range of about 3.5×10^6 APCs/cm^2 to about 6.0×10^6 APCs/cm^2.

[001095] In some embodiments, the number of APCs in the priming first expansion is in the range of about 2.0×10^6 APCs/cm^2 to about 3.0×10^6 APCs/cm^2, and the number of APCs in the rapid second expansion is in the range of about 4.0×10^6 APCs/cm^2 to about 5.5×10^6 APCs/cm^2.

H. Optional Cell Medium Components

1. Anti-CD3 Antibodies

[001096] In some embodiments, the culture media used in expansion methods described herein (see for example, Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) include an anti-CD3 antibody. An anti-CD3 antibody in combination with IL-2 induces T cell activation and cell division in the TIL population. This effect can be seen with full length antibodies as well as Fab and F(ab')2

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fragments, with the former being generally preferred; see, *e.g.*, Tsoukas *et al.*, *J. Immunol.* **1985,** *135,* 1719, hereby incorporated by reference in its entirety.

[001097] As will be appreciated by those in the art, there are a number of suitable anti-human CD3 antibodies that find use in the invention, including anti-human CD3 polyclonal and monoclonal antibodies from various mammals, including, but not limited to, murine, human, primate, rat, and canine antibodies. In particular embodiments, the OKT3 anti-CD3 antibody is used (commercially available from Ortho-McNeil, Raritan, NJ or Miltenyi Biotech, Auburn, CA). *See*, Table 1.

2. 4-1BB (CD137) AGONISTS

[001098] In some embodiments, the cell culture medium of the priming first expansion and/or the rapid second expansion comprises a TNFRSF agonist. In some embodiments, the TNFRSF agonist is a 4-1BB (CD137) agonist. The 4-1BB agonist may be any 4-1BB binding molecule known in the art. The 4-1BB binding molecule may be a monoclonal antibody or fusion protein capable of binding to human or mammalian 4-1BB. The 4-1BB agonists or 4-1BB binding molecules may comprise an immunoglobulin heavy chain of any isotype (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. The 4-1BB agonist or 4-1BB binding molecule may have both a heavy and a light chain. As used herein, the term binding molecule also includes antibodies (including full length antibodies), monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), human, humanized or chimeric antibodies, and antibody fragments, *e.g.*, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, epitope-binding fragments of any of the above, and engineered forms of antibodies, *e.g.*, scFv molecules, that bind to 4-1BB. In some embodiments, the 4-1BB agonist is an antigen binding protein that is a fully human antibody. In some embodiments, the 4-1BB agonist is an antigen binding protein that is a humanized antibody. In some embodiments, 4-1BB agonists for use in the presently disclosed methods and compositions include anti-4-1BB antibodies, human anti-4-1BB antibodies, mouse anti-4-1BB antibodies, mammalian anti-4-1BB antibodies, monoclonal anti-4-1BB antibodies, polyclonal anti-4-1BB antibodies, chimeric anti-4-1BB antibodies, anti-4-1BB adnectins, anti-4-1BB domain antibodies, single chain anti-4-1BB fragments, heavy chain anti-4-1BB fragments, light chain anti-4-1BB fragments, anti-4-1BB fusion proteins, and fragments, derivatives, conjugates, variants, or biosimilars

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thereof. Agonistic anti-4-1BB antibodies are known to induce strong immune responses. Lee, *et al.*, *PLOS One* **2013**, *8*, e69677. In some embodiments, the 4-1BB agonist is an agonistic, anti-4-1BB humanized or fully human monoclonal antibody (*i.e.*, an antibody derived from a single cell line). In some embodiments, the 4-1BB agonist is EU-101 (Eutilex Co. Ltd.), utomilumab, or urelumab, or a fragment, derivative, conjugate, variant, or biosimilar thereof. In some embodiments, the 4-1BB agonist is utomilumab or urelumab, or a fragment, derivative, conjugate, variant, or biosimilar thereof.

[001099] In some embodiments, the 4-1BB agonist or 4-1BB binding molecule may also be a fusion protein. In some embodiments, a multimeric 4-1BB agonist, such as a trimeric or hexameric 4-1BB agonist (with three or six ligand binding domains), may induce superior receptor (4-1BBL) clustering and internal cellular signaling complex formation compared to an agonistic monoclonal antibody, which typically possesses two ligand binding domains. Trimeric (trivalent) or hexameric (or hexavalent) or greater fusion proteins comprising three TNFRSF binding domains and IgG1-Fc and optionally further linking two or more of these fusion proteins are described, *e.g.*, in Gieffers, *et al.*, *Mol. Cancer Therapeutics* **2013,** *12,* 2735-47.

[001100] Agonistic 4-1BB antibodies and fusion proteins are known to induce strong immune responses. In some embodiments, the 4-1BB agonist is a monoclonal antibody or fusion protein that binds specifically to 4-1BB antigen in a manner sufficient to reduce toxicity. In some embodiments, the 4-1BB agonist is an agonistic 4-1BB monoclonal antibody or fusion protein that abrogates antibody-dependent cellular toxicity (ADCC), for example NK cell cytotoxicity. In some embodiments, the 4-1BB agonist is an agonistic 4-1BB monoclonal antibody or fusion protein that abrogates antibody-dependent cell phagocytosis (ADCP). In some embodiments, the 4-1BB agonist is an agonistic 4-1BB monoclonal antibody or fusion protein that abrogates complement-dependent cytotoxicity (CDC). In some embodiments, the 4-1BB agonist is an agonistic 4-1BB monoclonal antibody or fusion protein which abrogates Fc region functionality.

[001101] In some embodiments, the 4-1BB agonists are characterized by binding to human 4-1BB (SEQ ID NO:9) with high affinity and agonistic activity. In some embodiments, the 4-1BB agonist is a binding molecule that binds to human 4-1BB (SEQ ID NO:9). In some embodiments, the 4-1BB agonist is a binding molecule that binds to murine 4-1BB (SEQ ID NO:10). The amino acid sequences of 4-1BB antigen to which a 4-1BB agonist or binding molecule binds are summarized in Table 6.

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TABLE 6: Amino acid sequences of 4-1BB antigens.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:9 human 4-1BB, Tumor necrosis factor receptor superfamily, member 9 (Homo sapiens)	MGNSCYNIVA TLLLVLNFER TRSLQDPCSN CPAGTFCDNN RNQICSPCPP NSFSSAGGQR TCDICRQCKG VFRTRKECSS TSNAECDCTP GFHCLGAGCS MCEQDCKQGQ ELTKKGCKDC CFGTFNDQKR GICRPWTNCS LDGKSVLVNG TKERDVVCGP SPADLSPGAS SVTPPAPARE PGHSPQIISF FLALTSTALL FLLFFLTLRF SVVKRGRKKL LYIFKQPFMR PVQTTQEEDG CSCRFPEEEE GGCEL	60 120 180 240 255
SEQ ID NO:10 murine 4-1BB, Tumor necrosis factor receptor superfamily, member 9 (Mus musculus)	MGNNCYNVVV IVLLLVGCEK VGAVQNSCDN CQPGTFCRKY NPVCKSCPPS TFSSIGGQPN CNICRVCAGY FRFKKFCSST HNAECECIEG FHCLGPQCTR CEKDCRPGQE LTKQGCKTCS LGTFNDQNGT GVCRPWTNCS LDGRSVLKTG TTEKDVVCGP PVVSFSPSTT ISVTPEGGPG GHSLQVLTLF LALTSALLLA LIFITLLFSV LKWIRKKFPH IFKQPFKKTT GAAQEEDACS CRCPQEEEGG GGGYEL	60 120 180 240 256

[001102] In some embodiments, the compositions, processes and methods described include a 4-1BB agonist that binds human or murine 4-1BB with a K_D of about 100 pM or lower, binds human or murine 4-1BB with a K_D of about 90 pM or lower, binds human or murine 4-1BB with a K_D of about 80 pM or lower, binds human or murine 4-1BB with a K_D of about 70 pM or lower, binds human or murine 4-1BB with a K_D of about 60 pM or lower, binds human or murine 4-1BB with a K_D of about 50 pM or lower, binds human or murine 4-1BB with a K_D of about 40 pM or lower, or binds human or murine 4-1BB with a K_D of about 30 pM or lower.

[001103] In some embodiments, the compositions, processes and methods described include a 4-1BB agonist that binds to human or murine 4-1BB with a k_{assoc} of about 7.5×10^5 1/M·s or faster, binds to human or murine 4-1BB with a k_{assoc} of about 7.5×10^5 1/M·s or faster, binds to human or murine 4-1BB with a k_{assoc} of about 8×10^5 1/M·s or faster, binds to human or murine 4-1BB with a k_{assoc} of about 8.5×10^5 1/M·s or faster, binds to human or murine 4-1BB with a k_{assoc} of about 9×10^5 1/M·s or faster, binds to human or murine 4-1BB with a k_{assoc} of about 9.5×10^5 1/M·s or faster, or binds to human or murine 4-1BB with a k_{assoc} of about 1×10^6 1/M·s or faster.

[001104] In some embodiments, the compositions, processes and methods described include a 4-1BB agonist that binds to human or murine 4-1BB with a k_{dissoc} of about 2×10^{-5} 1/s or slower, binds to human or murine 4-1BB with a k_{dissoc} of about 2.1×10^{-5} 1/s or slower , binds to human or murine 4-1BB with a k_{dissoc} of about 2.2×10^{-5} 1/s or slower, binds to human or murine 4-1BB with a k_{dissoc} of about 2.3×10^{-5} 1/s or slower, binds to human or murine 4-1BB with a k_{dissoc} of about 2.4×10^{-5} 1/s or slower, binds to human or murine 4-1BB with a k_{dissoc} of about 2.5×10^{-5} 1/s or slower, binds to human or murine 4-1BB with a k_{dissoc} of about 2.6×10^{-5} 1/s or slower or binds to human or murine 4-1BB with a k_{dissoc} of about $2.7 \times$

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10^{-5} 1/s or slower, binds to human or murine 4-1BB with a k_{dissoc} of about 2.8×10^{-5} 1/s or slower, binds to human or murine 4-1BB with a k_{dissoc} of about 2.9×10^{-5} 1/s or slower, or binds to human or murine 4-1BB with a k_{dissoc} of about 3×10^{-5} 1/s or slower.

[001105] In some embodiments, the compositions, processes and methods described include a 4-1BB agonist that binds to human or murine 4-1BB with an IC_{50} of about 10 nM or lower, binds to human or murine 4-1BB with an IC_{50} of about 9 nM or lower, binds to human or murine 4-1BB with an IC_{50} of about 8 nM or lower, binds to human or murine 4-1BB with an IC_{50} of about 7 nM or lower, binds to human or murine 4-1BB with an IC_{50} of about 6 nM or lower, binds to human or murine 4-1BB with an IC_{50} of about 5 nM or lower, binds to human or murine 4-1BB with an IC_{50} of about 4 nM or lower, binds to human or murine 4-1BB with an IC_{50} of about 3 nM or lower, binds to human or murine 4-1BB with an IC_{50} of about 2 nM or lower, or binds to human or murine 4-1BB with an IC_{50} of about 1 nM or lower.

[001106] In some embodiments, the 4-1BB agonist is utomilumab, also known as PF-05082566 or MOR-7480, or a fragment, derivative, variant, or biosimilar thereof. Utomilumab is available from Pfizer, Inc. Utomilumab is an immunoglobulin G2-lambda, anti-[*Homo sapiens* TNFRSF9 (tumor necrosis factor receptor (TNFR) superfamily member 9, 4-1BB, T cell antigen ILA, CD137)], *Homo sapiens* (fully human) monoclonal antibody. The amino acid sequences of utomilumab are set forth in Table 7. Utomilumab comprises glycosylation sites at Asn59 and Asn292; heavy chain intrachain disulfide bridges at positions 22-96 (V_H-V_L), 143-199 (C_H1-C_L), 256-316 (C_H2) and 362-420 (C_H3); light chain intrachain disulfide bridges at positions 22'-87' (V_H-V_L) and 136'-195' (C_H1-C_L); interchain heavy chain-heavy chain disulfide bridges at IgG2A isoform positions 218-218, 219-219, 222-222, and 225-225, at IgG2A/B isoform positions 218-130, 219-219, 222-222, and 225-225, and at IgG2B isoform positions 219-130 (2), 222-222, and 225-225; and interchain heavy chain-light chain disulfide bridges at IgG2A isoform positions 130-213' (2), IgG2A/B isoform positions 218-213' and 130-213', and at IgG2B isoform positions 218-213' (2). The preparation and properties of utomilumab and its variants and fragments are described in U.S. Patent Nos. 8,821,867; 8,337,850; and 9,468,678, and International Patent Application Publication No. WO 2012/032433 A1, the disclosures of each of which are incorporated by reference herein. Preclinical characteristics of utomilumab are described in Fisher, *et al.*, *Cancer Immunolog. & Immunother.* **2012,** *61,* 1721-33. Current clinical trials of utomilumab in a variety of hematological and solid tumor indications include U.S. National Institutes of

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Health clinicaltrials.gov identifiers NCT02444793, NCT01307267, NCT02315066, and NCT02554812.

[001107] In some embodiments, a 4-1BB agonist comprises a heavy chain given by SEQ ID NO:11 and a light chain given by SEQ ID NO:12. In some embodiments, a 4-1BB agonist comprises heavy and light chains having the sequences shown in SEQ ID NO:11 and SEQ ID NO:12, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In some embodiments, a 4-1BB agonist comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:11 and SEQ ID NO:12, respectively. In some embodiments, a 4-1BB agonist comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:11 and SEQ ID NO:12, respectively. In some embodiments, a 4-1BB agonist comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:11 and SEQ ID NO:12, respectively. In some embodiments, a 4-1BB agonist comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:11 and SEQ ID NO:12, respectively. In some embodiments, a 4-1BB agonist comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:11 and SEQ ID NO:12, respectively.

[001108] In some embodiments, the 4-1BB agonist comprises the heavy and light chain CDRs or variable regions (VRs) of utomilumab. In some embodiments, the 4-1BB agonist heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:13, and the 4-1BB agonist light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:14, and conservative amino acid substitutions thereof. In some embodiments, a 4-1BB agonist comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:13 and SEQ ID NO:14, respectively. In some embodiments, a 4-1BB agonist comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:13 and SEQ ID NO:14, respectively. In some embodiments, a 4-1BB agonist comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:13 and SEQ ID NO:14, respectively. In some embodiments, a 4-1BB agonist comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:13 and SEQ ID NO:14, respectively. In some embodiments, a 4-1BB agonist comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:13 and SEQ ID NO:14, respectively. In some embodiments, a 4-1BB

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agonist comprises an scFv antibody comprising V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:13 and SEQ ID NO:14.

[001109] In some embodiments, a 4-1BB agonist comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:18, SEQ ID NO:19, and SEQ ID NO:20, respectively, and conservative amino acid substitutions thereof.

[001110] In some embodiments, the 4-1BB agonist is a 4-1BB agonist biosimilar monoclonal antibody approved by drug regulatory authorities with reference to utomilumab. In some embodiments, the biosimilar monoclonal antibody comprises an 4-1BB antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is utomilumab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is a 4-1BB agonist antibody authorized or submitted for authorization, wherein the 4-1BB agonist antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is utomilumab. The 4-1BB agonist antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is utomilumab. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is utomilumab.

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TABLE 7. Amino acid sequences for 4-1BB agonist antibodies related to utomilumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:11 heavy chain for utomilumab	EVQLVQSGAE VKKPGESLRI SCKGSGYSFS TYWISWVRQM PGKGLEWMGK IYPGDSYTNY SPSFQGQVTI SADKSISTAY LQWSSLKASD TAMYYCARGY GIFDYWGQGT LVTVSSASTK GPSVFPLAPC SRSTSESTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS NFGTQTYTCN VDHKPSNTKV DKTVERKCCV ECPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVVSV LTVVHQDWLN GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PMLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP G	60 120 180 240 300 360 420 441
SEQ ID NO:12 light chain for utomilumab	SYELTQPPSV SVSPGQTASI TCSGDNIGDQ YAHWYQQKPG QSPVLVIYQD KNRPSGIPER FSGSNSGNTA TLTISGTQAM DEADYYCATY TGFGSLAVFG GGTKLTVLGQ PKAAPSVTLF PPSSEELQAN KATLVCLISD FYPGAVTVAW KADSSPVKAG VETTTPSKQS NNKYAASSYL SLTPEQWKSH RSYSCQVTHE GSTVEKTVAP TECS	60 120 180 214
SEQ ID NO:13 heavy chain variable region for utomilumab	EVQLVQSGAE VKKPGESLRI SCKGSGYSFS TYWISWVRQM PGKGLEWMG KIYPGDSYTN YSPSFQGQVT ISADKSISTA YLQWSSLKAS DTAMYYCARG YGIFDYWGQ GTLVTVSS	60 118
SEQ ID NO:14 light chain variable region for utomilumab	SYELTQPPSV SVSPGQTASI TCSGDNIGDQ YAHWYQQKPG QSPVLVIYQD KNRPSGIPER FSGSNSGNTA TLTISGTQAM DEADYYCATY TGFGSLAVFG GGTKLTVL	60 108
SEQ ID NO:15 heavy chain CDR1 for utomilumab	STYWIS	6
SEQ ID NO:16 heavy chain CDR2 for utomilumab	KIYPGDSYTN YSPSFQG	17
SEQ ID NO:17 heavy chain CDR3 for utomilumab	RGYGIFDY	8
SEQ ID NO:18 light chain CDR1 for utomilumab	SGDNIGDQYA H	11
SEQ ID NO:19 light chain CDR2 for utomilumab	QDKNRPS	7
SEQ ID NO:20 light chain CDR3 for utomilumab	ATYTGFGSLA V	11

[001111] In some embodiments, the 4-1BB agonist is the monoclonal antibody urelumab, also known as BMS-663513 and 20H4.9.h4a, or a fragment, derivative, variant, or biosimilar thereof. Urelumab is available from Bristol-Myers Squibb, Inc., and Creative Biolabs, Inc. Urelumab is an immunoglobulin G4-kappa, anti-[*Homo sapiens* TNFRSF9 (tumor necrosis factor receptor superfamily member 9, 4-1BB, T cell antigen ILA, CD137)], *Homo sapiens* (fully human) monoclonal antibody. The amino acid sequences of urelumab are set forth in Table 8. Urelumab comprises N-glycosylation sites at positions 298 (and 298'') heavy chain intrachain disulfide bridges at positions 22-95 (V_H-V_L), 148-204 (C_H1-C_L), 262-322 (C_H2) and 368-426 (C_H3) (and at positions 22''-95'', 148''-204'', 262''-322'', and 368''-426''); light chain intrachain disulfide bridges at positions 23'-88' (V_H-V_L) and 136'-196' (C_H1-C_L) (and at positions 23'''-88''' and 136'''-196'''); interchain heavy chain-heavy chain disulfide bridges at positions 227-227'' and 230-230''; and interchain heavy chain-light chain disulfide bridges at 135-216' and 135''-216'''. The preparation and properties of urelumab and its variants and fragments are described in U.S. Patent Nos. 7,288,638 and 8,962,804, the

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disclosures of which are incorporated by reference herein. The preclinical and clinical characteristics of urelumab are described in Segal, *et al.*, *Clin. Cancer Res.* **2016,** *available at* http:/dx.doi.org/ 10.1158/1078-0432.CCR-16-1272. Current clinical trials of urelumab in a variety of hematological and solid tumor indications include U.S. National Institutes of Health clinicaltrials.gov identifiers NCT01775631, NCT02110082, NCT02253992, and NCT01471210.

[001112] In some embodiments, a 4-1BB agonist comprises a heavy chain given by SEQ ID NO:21 and a light chain given by SEQ ID NO:22. In some embodiments, a 4-1BB agonist comprises heavy and light chains having the sequences shown in SEQ ID NO:21 and SEQ ID NO:22, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In some embodiments, a 4-1BB agonist comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:21 and SEQ ID NO:22, respectively. In some embodiments, a 4-1BB agonist comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:21 and SEQ ID NO:22, respectively. In some embodiments, a 4-1BB agonist comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:21 and SEQ ID NO:22, respectively. In some embodiments, a 4-1BB agonist comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:21 and SEQ ID NO:22, respectively. In some embodiments, a 4-1BB agonist comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:21 and SEQ ID NO:22, respectively.

[001113] In some embodiments, the 4-1BB agonist comprises the heavy and light chain CDRs or variable regions (VRs) of urelumab. In some embodiments, the 4-1BB agonist heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:23, and the 4-1BB agonist light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:24, and conservative amino acid substitutions thereof. In some embodiments, a 4-1BB agonist comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:23 and SEQ ID NO:24, respectively. In some embodiments, a 4-1BB agonist comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:23 and SEQ ID NO:24, respectively. In some embodiments, a 4-1BB agonist comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:23 and SEQ ID NO:24, respectively. In some embodiments, a 4-1BB agonist comprises V_H and V_L regions that are each at least 96% identical to the sequences

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shown in SEQ ID NO:23 and SEQ ID NO:24, respectively. In some embodiments, a 4-1BB agonist comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:23 and SEQ ID NO:24, respectively. In some embodiments, a 4-1BB agonist comprises an scFv antibody comprising V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:23 and SEQ ID NO:24.

[001114] In some embodiments, a 4-1BB agonist comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, respectively, and conservative amino acid substitutions thereof.

[001115] In some embodiments, the 4-1BB agonist is a 4-1BB agonist biosimilar monoclonal antibody approved by drug regulatory authorities with reference to urelumab. In some embodiments, the biosimilar monoclonal antibody comprises an 4-1BB antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is urelumab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is a 4-1BB agonist antibody authorized or submitted for authorization, wherein the 4-1BB agonist antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is urelumab. The 4-1BB agonist antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is urelumab. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is urelumab.

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TABLE 8: Amino acid sequences for 4-1BB agonist antibodies related to urelumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:21 heavy chain for urelumab	QVQLQQWGAG LLKPSETLSL TCAVYGGSFS GYYWSWIRQS PEKGLEWIGE INHGGYVTYN PSLESRVTIS VDTSKNQFSL KLSSVTAADT AVYYCARDYG PGNYDWYFDL WGRGTLVTVS SASTKGPSVF PLAPCSRSTS ESTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTK TYTCNVDHKP SNTKVDKRVE SKYGPPCPPC PAPEFLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV DGVEVHNAKT KPREEQFNST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP SSIEKTISKA KGQPREPQVY TLPPSQEEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH EALHNHYTQK SLSLSLGK	60 120 180 240 300 360 420 448
SEQ ID NO:22 light chain for urelumab	EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RSNWPPALTF CGGTKVEIKR TVAAPSVFIF PPSDEQLKSG TASVVCLLNN FYPREAKVQW KVDNALQSGN SQESVTEQDS KDSTYSLSST LTLSKADYEK HKVYACEVTH QGLSSPVTKS FNRGEC	60 120 180 216
SEQ ID NO:23 variable heavy chain for urelumab	MKHLWFFLLL VAAPRWVLSQ VQLQQWGAGL LKPSETLSLT CAVYGGSFSG YYWSWIRQSP EKGLEWIGEI NHGGYVTYNP SLESRVTISV DTSKNQFSLK LSSVTAADTA VYYCARDYGP	60 120
SEQ ID NO:24 variable light chain for urelumab	MEAPAQLLFL LLLWLPDTTG EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ	60 110
SEQ ID NO:25 heavy chain CDR1 for urelumab	GYYWS	5
SEQ ID NO:26 heavy chain CDR2 for urelumab	EINHGGYVTY NPSLES	16
SEQ ID NO:27 heavy chain CDR3 for urelumab	DYGPGNYDWY FDL	13
SEQ ID NO:28 light chain CDR1 for urelumab	RASQSVSSYL A	11
SEQ ID NO:29 light chain CDR2 for urelumab	DASNRAT	7
SEQ ID NO:30 light chain CDR3 for urelumab	QQRSDWPPAL T	11

[001116] In some embodiments, the 4-1BB agonist is selected from the group consisting of 1D8, 3Elor, 4B4 (BioLegend 309809), H4-1BB-M127 (BD Pharmingen 552532), BBK2 (Thermo Fisher MS621PABX), 145501 (Leinco Technologies B591), the antibody produced by cell line deposited as ATCC No. HB-11248 and disclosed in U.S. Patent No. 6,974,863, 5F4 (BioLegend 31 1503), C65-485 (BD Pharmingen 559446), antibodies disclosed in U.S. Patent Application Publication No. US 2005/0095244, antibodies disclosed in U.S. Patent No. 7,288,638 (such as 20H4.9-IgGl (BMS-663031)), antibodies disclosed in U.S. Patent No. 6,887,673 (such as 4E9 or BMS-554271), antibodies disclosed in U.S. Patent No. 7,214,493, antibodies disclosed in U.S. Patent No. 6,303,121, antibodies disclosed in U.S. Patent No. 6,569,997, antibodies disclosed in U.S. Patent No. 6,905,685 (such as 4E9 or BMS-554271), antibodies disclosed in U.S. Patent No. 6,362,325 (such as 1D8 or BMS-469492; 3H3 or BMS-469497; or 3El), antibodies disclosed in U.S. Patent No. 6,974,863 (such as 53A2); antibodies disclosed in U.S. Patent No. 6,210,669 (such as 1D8, 3B8, or 3El), antibodies described in U.S. Patent No. 5,928,893, antibodies disclosed in U.S. Patent No. 6,303,121,

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antibodies disclosed in U.S. Patent No. 6,569,997, antibodies disclosed in International Patent Application Publication Nos. WO 2012/177788, WO 2015/119923, and WO 2010/042433, and fragments, derivatives, conjugates, variants, or biosimilars thereof, wherein the disclosure of each of the foregoing patents or patent application publications is incorporated by reference here.

[001117] In some embodiments, the 4-1BB agonist is a 4-1BB agonistic fusion protein described in International Patent Application Publication Nos. WO 2008/025516 A1, WO 2009/007120 A1, WO 2010/003766 A1, WO 2010/010051 A1, and WO 2010/078966 A1; U.S. Patent Application Publication Nos. US 2011/0027218 A1, US 2015/0126709 A1, US 2011/0111494 A1, US 2015/0110734 A1, and US 2015/0126710 A1; and U.S. Patent Nos. 9,359,420, 9,340,599, 8,921,519, and 8,450,460, the disclosures of which are incorporated by reference herein.

[001118] In some embodiments, the 4-1BB agonist is a 4-1BB agonistic fusion protein as depicted in Structure I-A (C-terminal Fc-antibody fragment fusion protein) or Structure I-B (N-terminal Fc-antibody fragment fusion protein), or a fragment, derivative, conjugate, variant, or biosimilar thereof, as provided in Figure 17.

[001119] In structures I-A and I-B (see, Figure 17), the cylinders refer to individual polypeptide binding domains. Structures I-A and I-B comprise three linearly-linked TNFRSF binding domains derived from *e.g.*, 4-1BBL (4-1BB ligand, CD137 ligand (CD137L), or tumor necrosis factor superfamily member 9 (TNFSF9)) or an antibody that binds 4-1BB, which fold to form a trivalent protein, which is then linked to a second triavelent protein through IgG1-Fc (including C_H3 and C_H2 domains) is then used to link two of the trivalent proteins together through disulfide bonds (small elongated ovals), stabilizing the structure and providing an agonists capable of bringing together the intracellular signaling domains of the six receptors and signaling proteins to form a signaling complex. The TNFRSF binding domains denoted as cylinders may be scFv domains comprising, *e.g.*, a V_H and a V_L chain connected by a linker that may comprise hydrophilic residues and Gly and Ser sequences for flexibility, as well as Glu and Lys for solubility. Any scFv domain design may be used, such as those described in de Marco, *Microbial Cell Factories*, **2011,** *10*, 44; Ahmad, *et al.*, *Clin. & Dev. Immunol.* **2012,** 980250; Monnier, *et al.*, *Antibodies*, **2013,** *2*, 193-208; or in references incorporated elsewhere herein. Fusion protein structures of this form are described in U.S. Patent Nos. 9,359,420, 9,340,599, 8,921,519, and 8,450,460, the disclosures of which are incorporated by reference herein.

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[001120] Amino acid sequences for the other polypeptide domains of structure I-A are given in Table 9. The Fc domain preferably comprises a complete constant domain (amino acids 17-230 of SEQ ID NO:31) the complete hinge domain (amino acids 1-16 of SEQ ID NO:31) or a portion of the hinge domain (*e.g.*, amino acids 4-16 of SEQ ID NO:31). Preferred linkers for connecting a C-terminal Fc-antibody may be selected from the embodiments given in SEQ ID NO:32 to SEQ ID NO:41, including linkers suitable for fusion of additional polypeptides.

TABLE 9: Amino acid sequences for TNFRSF agonist fusion proteins, including 4-1BB agonist fusion proteins, with C-terminal Fc-antibody fragment fusion protein design (structure I-A).

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:31 Fc domain	KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK	60 120 180 230
SEQ ID NO:32 linker	GGPGSSKSCD KTHTCPPCPA PE	22
SEQ ID NO:33 linker	GGSGSSKSCD KTHTCPPCPA PE	22
SEQ ID NO:34 linker	CCPCSSSSSS SKSCDKTHTC PPCPAPE	27
SEQ ID NO:35 linker	GGSGSSSSSS SKSCDKTHTC PPCPAPE	27
SEQ ID NO:36 linker	CCPCSSSSSS SSSKSCDKTH TCPPCPAPE	29
SEQ ID NO:37 linker	GGSGSSSSSS SSSKSCDKTH TCPPCPAPE	29
SEQ ID NO:38 linker	GGPGSSCSGS SDKTHTCPPC PAPE	24
SEQ ID NO:39 linker	GGPGSSCSGS DKTHTCPPCP APE	23
SEQ ID NO:40 linker	GGPSSSCSDK THTCPPCPAP E	21
SEQ ID NO:41 linker	GGSSSSSSSS GSDKTHTCPP CPAPE	25

[001121] Amino acid sequences for the other polypeptide domains of structure I-B are given in Table 10. If an Fc antibody fragment is fused to the N-terminus of an TNRFSF agonist fusion protein as in structure I-B, the sequence of the Fc module is preferably that shown in SEQ ID NO:42, and the linker sequences are preferably selected from those embodiments set forth in SED ID NO:43 to SEQ ID NO:45.

TABLE 10: Amino acid sequences for TNFRSF agonist fusion proteins, including 4-1BB agonist fusion proteins, with N-terminal Fc-antibody fragment fusion protein design (structure I-B).

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:42 Fc domain	METDTLLLWV LLLWVPAGNG DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPG	60 120 180 240 246

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SEQ ID NO:43 linker	SGSGSGSGSG S	11
SEQ ID NO:44 linker	SSSSSSGSGS GS	12
SEQ ID NO:45 linker	SSSSSSGSGS GSGSGS	16

[001122] In some embodiments, a 4-1BB agonist fusion protein according to structures I-A or I-B comprises one or more 4-1BB binding domains selected from the group consisting of a variable heavy chain and variable light chain of utomilumab, a variable heavy chain and variable light chain of urelumab, a variable heavy chain and variable light chain of utomilumab, a variable heavy chain and variable light chain selected from the variable heavy chains and variable light chains described in Table 11, any combination of a variable heavy chain and variable light chain of the foregoing, and fragments, derivatives, conjugates, variants, and biosimilars thereof.

[001123] In some embodiments, a 4-1BB agonist fusion protein according to structures I-A or I-B comprises one or more 4-1BB binding domains comprising a 4-1BBL sequence. In some embodiments, a 4-1BB agonist fusion protein according to structures I-A or I-B comprises one or more 4-1BB binding domains comprising a sequence according to SEQ ID NO:46. In some embodiments, a 4-1BB agonist fusion protein according to structures I-A or I-B comprises one or more 4-1BB binding domains comprising a soluble 4-1BBL sequence. In some embodiments, a 4-1BB agonist fusion protein according to structures I-A or I-B comprises one or more 4-1BB binding domains comprising a sequence according to SEQ ID NO:47.

[001124] In some embodiments, a 4-1BB agonist fusion protein according to structures I-A or I-B comprises one or more 4-1BB binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:13 and SEQ ID NO:14, respectively, wherein the V_H and V_L domains are connected by a linker. In some embodiments, a 4-1BB agonist fusion protein according to structures I-A or I-B comprises one or more 4-1BB binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:23 and SEQ ID NO:24, respectively, wherein the V_H and V_L domains are connected by a linker. In some embodiments, a 4-1BB agonist fusion protein according to structures I-A or I-B comprises one or more 4-1BB binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the V_H and V_L sequences given in Table 11, wherein the V_H and V_L domains are connected by a linker.

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TABLE 11: Additional polypeptide domains useful as 4-1BB binding domains in fusion proteins or as scFv 4-1BB agonist antibodies.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:46 4-1BBL	MEYASDASLD PEAPWPPAPR ARACRVLPWA LVACLLLLLL LAAACAVFLA CPWAVSGARA SPGSAASPRL REGPELSPDD PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS PRSE	60 120 180 240 254
SEQ ID NO:47 4-1BBL soluble domain	LRQGMFAQLV AQNVLLIDGP LSWYSDPGLA GVSLTGGLSY KEDTKELVVA KAGVYYVFFQ LELRRVVAGE GSGSVSLALH LQPLRSAAGA AALALTVDLP PASSEARNSA FGFQGRLLHL SAGQRLGVHL HTEARARHAW QLTQGATVLG LFRVTPEIPA GLPSPRSE	60 120 168
SEQ ID NO:48 variable heavy chain for 4B4-1-1 version 1	QVQLQQPGAE LVKPGASVKL SCKASGYTFS SYWMHWVKQR PGQVLEWIGE INPGNGHTNY NEKFKSKATL TVDKSSSTAY MQLSSLTSED SAVYYCARSF TTARGFAYWG QGTLVTVS	60 118
SEQ ID NO:49 variable light chain for 4B4-1-1 version 1	DIVMTQSPAT QSVTPGDRVS LSCRASQTIS DYLHWYQQKS HESPRLLIKY ASQSISGIPS RFSGSGSGSD FTLSINSVEP EDVGVYYCQD GHSFPPTFGG GTKLEIK	60 107
SEQ ID NO:50 variable heavy chain for 4B4-1-1 version 2	QVQLQQPGAE LVKPGASVKL SCKASGYTFS SYWMHWVKQR PGQVLEWIGE INPGNGHTNY NEKFKSKATL TVDKSSSTAY MQLSSLTSED SAVYYCARSF TTARGFAYWG QGTLVTVSA	60 119
SEQ ID NO:51 variable light chain for 4B4-1-1 version 2	DIVMTQSPAT QSVTPGDRVS LSCRASQTIS DYLHWYQQKS HESPRLLIKY ASQSISGIPS RFSGSGSGSD FTLSINSVEP EDVGVYYCQD GHSFPPTFGG GTKLEIKR	60 108
SEQ ID NO:52 variable heavy chain for H39E3-2	MDWTWRILFL VAAATCAHSE VQLVESCCCL VQPCGSLRLS CAASGFTFSD YWMSWVRQAP GKGLEWVADI KNDGSYTNYA PSLTNRFTIS RDNAKNSLYL QMNSLRAEDT AVYYCARELT	60 120
SEQ ID NO:53 variable light chain for H39E3-2	MEAPAQLLFL LLLWLPDTTG DIVMTQSPDS LAVSLGERAT INCKSSQSLL SSGNQKNYL WYQQKPGQPP KLLIYYASTR QSGVPDRFSG SGSGTDFTLT ISSLQAEDVA	60 110

[001125] In some embodiments, the 4-1BB agonist is a 4-1BB agonistic single-chain fusion polypeptide comprising (i) a first soluble 4-1BB binding domain, (ii) a first peptide linker, (iii) a second soluble 4-1BB binding domain, (iv) a second peptide linker, and (v) a third soluble 4-1BB binding domain, further comprising an additional domain at the N-terminal and/or C-terminal end, and wherein the additional domain is a Fab or Fc fragment domain. In some embodiments, the 4-1BB agonist is a 4-1BB agonistic single-chain fusion polypeptide comprising (i) a first soluble 4-1BB binding domain, (ii) a first peptide linker, (iii) a second soluble 4-1BB binding domain, (iv) a second peptide linker, and (v) a third soluble 4-1BB binding domain, further comprising an additional domain at the N-terminal and/or C-terminal end, wherein the additional domain is a Fab or Fc fragment domain, wherein each of the soluble 4-1BB domains lacks a stalk region (which contributes to trimerisation and provides a certain distance to the cell membrane, but is not part of the 4-1BB binding domain) and the first and the second peptide linkers independently have a length of 3-8 amino acids.

[001126] In some embodiments, the 4-1BB agonist is a 4-1BB agonistic single-chain fusion polypeptide comprising (i) a first soluble tumor necrosis factor (TNF) superfamily cytokine domain, (ii) a first peptide linker, (iii) a second soluble TNF superfamily cytokine domain,

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(iv) a second peptide linker, and (v) a third soluble TNF superfamily cytokine domain, wherein each of the soluble TNF superfamily cytokine domains lacks a stalk region and the first and the second peptide linkers independently have a length of 3-8 amino acids, and wherein each TNF superfamily cytokine domain is a 4-1BB binding domain.

[001127] In some embodiments, the 4-1BB agonist is a 4-1BB agonistic scFv antibody comprising any of the foregoing V_H domains linked to any of the foregoing V_L domains.

[001128] In some embodiments, the 4-1BB agonist is BPS Bioscience 4-1BB agonist antibody catalog no. 79097-2, commercially available from BPS Bioscience, San Diego, CA, USA. In some embodiments, the 4-1BB agonist is Creative Biolabs 4-1BB agonist antibody catalog no. MOM-18179, commercially available from Creative Biolabs, Shirley, NY, USA.

3. OX40 (CD134) AGONISTS

[001129] In some embodiments, the TNFRSF agonist is an OX40 (CD134) agonist. The OX40 agonist may be any OX40 binding molecule known in the art. The OX40 binding molecule may be a monoclonal antibody or fusion protein capable of binding to human or mammalian OX40. The OX40 agonists or OX40 binding molecules may comprise an immunoglobulin heavy chain of any isotype (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. The OX40 agonist or OX40 binding molecule may have both a heavy and a light chain. As used herein, the term binding molecule also includes antibodies (including full length antibodies), monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), human, humanized or chimeric antibodies, and antibody fragments, *e.g.*, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, epitope-binding fragments of any of the above, and engineered forms of antibodies, *e.g.*, scFv molecules, that bind to OX40. In some embodiments, the OX40 agonist is an antigen binding protein that is a fully human antibody. In some embodiments, the OX40 agonist is an antigen binding protein that is a humanized antibody. In some embodiments, OX40 agonists for use in the presently disclosed methods and compositions include anti-OX40 antibodies, human anti-OX40 antibodies, mouse anti-OX40 antibodies, mammalian anti-OX40 antibodies, monoclonal anti-OX40 antibodies, polyclonal anti-OX40 antibodies, chimeric anti-OX40 antibodies, anti-OX40 adnectins, anti-OX40 domain antibodies, single chain anti-OX40 fragments, heavy chain anti-OX40 fragments, light chain anti-OX40 fragments, anti-OX40 fusion proteins, and fragments,

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derivatives, conjugates, variants, or biosimilars thereof. In some embodiments, the OX40 agonist is an agonistic, anti-OX40 humanized or fully human monoclonal antibody (*i.e.*, an antibody derived from a single cell line).

[001130] In some embodiments, the OX40 agonist or OX40 binding molecule may also be a fusion protein. OX40 fusion proteins comprising an Fc domain fused to OX40L are described, for example, in Sadun, *et al.*, *J. Immunother.* **2009,** *182,* 1481-89. In some embodiments, a multimeric OX40 agonist, such as a trimeric or hexameric OX40 agonist (with three or six ligand binding domains), may induce superior receptor (OX40L) clustering and internal cellular signaling complex formation compared to an agonistic monoclonal antibody, which typically possesses two ligand binding domains. Trimeric (trivalent) or hexameric (or hexavalent) or greater fusion proteins comprising three TNFRSF binding domains and IgG1-Fc and optionally further linking two or more of these fusion proteins are described, *e.g.*, in Gieffers, *et al.*, *Mol. Cancer Therapeutics* **2013,** *12,* 2735-47.

[001131] Agonistic OX40 antibodies and fusion proteins are known to induce strong immune responses. Curti, *et al.*, *Cancer Res.* **2013**, *73,* 7189-98. In some embodiments, the OX40 agonist is a monoclonal antibody or fusion protein that binds specifically to OX40 antigen in a manner sufficient to reduce toxicity. In some embodiments, the OX40 agonist is an agonistic OX40 monoclonal antibody or fusion protein that abrogates antibody-dependent cellular toxicity (ADCC), for example NK cell cytotoxicity. In some embodiments, the OX40 agonist is an agonistic OX40 monoclonal antibody or fusion protein that abrogates antibody-dependent cell phagocytosis (ADCP). In some embodiments, the OX40 agonist is an agonistic OX40 monoclonal antibody or fusion protein that abrogates complement-dependent cytotoxicity (CDC). In some embodiments, the OX40 agonist is an agonistic OX40 monoclonal antibody or fusion protein which abrogates Fc region functionality.

[001132] In some embodiments, the OX40 agonists are characterized by binding to human OX40 (SEQ ID NO:54) with high affinity and agonistic activity. In some embodiments, the OX40 agonist is a binding molecule that binds to human OX40 (SEQ ID NO:54). In some embodiments, the OX40 agonist is a binding molecule that binds to murine OX40 (SEQ ID NO:55). The amino acid sequences of OX40 antigen to which an OX40 agonist or binding molecule binds are summarized in Table 12.

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TABLE 12: Amino acid sequences of OX40 antigens.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:54 human OX40 (Homo sapiens)	MCVGARRLGR GPCAALLLLG LGLSTVTGLH CVGDTYPSND RCCHECRPGN GMVSRCSRSQ NTVCRPCGPG FYNDVVSSKP CKPCTWCNLR SGSERKQLCT ATQDTVCRCR AGTQPLDSYK PGVDCAPCPP GHFSPGDNQA CKPWTNCTLA GKHTLQPASN SSDAICEDRD PPATQPQETQ GPPARPITVQ PTEAWPRTSQ GPSTRPVEVP GGRAVAAILG LGLVLGLLGP LAILLALYLL RRDQRLPPDA HKPPGGGSFR TPIQEEQADA HSTLAKI	60 120 180 240 277
SEQ ID NO:55 murine OX40 (Mus musculus)	MYVWVQQPTA LLLLGLTLGV TARRLNCVKH TYPSGHKCCR ECQPGHGMVS RCDHTRDTLC HPCETGFYNE AVNYDTCKQC TQCNHRSGSE LKQNCTPTQD TVCRCRPGTQ PRQDSGYKLG VDCVPCPPGH FSPGNNQACK PWTNCTLSGK QTRHPASDSL DAVCEDRSLL ATLLWETQRP TFRPTTVQST TVWPRTSELP SPPTLVTPEG PAFAVLLGLG LGLLAPLTVL LALYLLRKAW RLPNTPKPCW GNSFRTPIQE EHTDAHFTLA KI	60 120 180 240 272

[001133] In some embodiments, the compositions, processes and methods described include a OX40 agonist that binds human or murine OX40 with a K_D of about 100 pM or lower, binds human or murine OX40 with a K_D of about 90 pM or lower, binds human or murine OX40 with a K_D of about 80 pM or lower, binds human or murine OX40 with a K_D of about 70 pM or lower, binds human or murine OX40 with a K_D of about 60 pM or lower, binds human or murine OX40 with a K_D of about 50 pM or lower, binds human or murine OX40 with a K_D of about 40 pM or lower, or binds human or murine OX40 with a K_D of about 30 pM or lower.

[001134] In some embodiments, the compositions, processes and methods described include a OX40 agonist that binds to human or murine OX40 with a k_{assoc} of about 7.5×10^5 1/M·s or faster, binds to human or murine OX40 with a k_{assoc} of about 7.5×10^5 1/M·s or faster, binds to human or murine OX40 with a k_{assoc} of about 8×10^5 1/M·s or faster, binds to human or murine OX40 with a k_{assoc} of about 8.5×10^5 1/M·s or faster, binds to human or murine OX40 with a k_{assoc} of about 9×10^5 1/M·s or faster, binds to human or murine OX40 with a k_{assoc} of about 9.5×10^5 1/M·s or faster, or binds to human or murine OX40 with a k_{assoc} of about 1×10^6 1/M·s or faster.

[001135] In some embodiments, the compositions, processes and methods described include a OX40 agonist that binds to human or murine OX40 with a k_{dissoc} of about 2×10^{-5} 1/s or slower, binds to human or murine OX40 with a k_{dissoc} of about 2.1×10^{-5} 1/s or slower , binds to human or murine OX40 with a k_{dissoc} of about 2.2×10^{-5} 1/s or slower, binds to human or murine OX40 with a k_{dissoc} of about 2.3×10^{-5} 1/s or slower, binds to human or murine OX40 with a k_{dissoc} of about 2.4×10^{-5} 1/s or slower, binds to human or murine OX40 with a k_{dissoc} of about 2.5×10^{-5} 1/s or slower, binds to human or murine OX40 with a k_{dissoc} of about 2.6×10^{-5} 1/s or slower or binds to human or murine OX40 with a k_{dissoc} of about 2.7×10^{-5} 1/s or slower, binds to human or murine OX40 with a k_{dissoc} of about 2.8×10^{-5} 1/s or slower, binds to human or murine OX40 with a k_{dissoc} of about 2.9×10^{-5} 1/s or slower, or binds to human or murine OX40 with a k_{dissoc} of about 3×10^{-5} 1/s or slower.

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[001136] In some embodiments, the compositions, processes and methods described include OX40 agonist that binds to human or murine OX40 with an IC_{50} of about 10 nM or lower, binds to human or murine OX40 with an IC_{50} of about 9 nM or lower, binds to human or murine OX40 with an IC_{50} of about 8 nM or lower, binds to human or murine OX40 with an IC_{50} of about 7 nM or lower, binds to human or murine OX40 with an IC_{50} of about 6 nM or lower, binds to human or murine OX40 with an IC_{50} of about 5 nM or lower, binds to human or murine OX40 with an IC_{50} of about 4 nM or lower, binds to human or murine OX40 with an IC_{50} of about 3 nM or lower, binds to human or murine OX40 with an IC_{50} of about 2 nM or lower, or binds to human or murine OX40 with an IC_{50} of about 1 nM or lower.

[001137] In some embodiments, the OX40 agonist is tavolixizumab, also known as MEDI0562 or MEDI-0562. Tavolixizumab is available from the MedImmune subsidiary of AstraZeneca, Inc. Tavolixizumab is immunoglobulin G1-kappa, anti-[*Homo sapiens* TNFRSF4 (tumor necrosis factor receptor (TNFR) superfamily member 4, OX40, CD134)], humanized and chimeric monoclonal antibody. The amino acid sequences of tavolixizumab are set forth in Table 13. Tavolixizumab comprises N-glycosylation sites at positions 301 and 301'', with fucosylated complex bi-antennary CHO-type glycans; heavy chain intrachain disulfide bridges at positions 22-95 (V_H-V_L), 148-204 (C_H1-C_L), 265-325 (C_H2) and 371-429 (C_H3) (and at positions 22''-95'', 148''-204'', 265''-325'', and 371''-429''); light chain intrachain disulfide bridges at positions 23'-88' (V_H-V_L) and 134'-194' (C_H1-C_L) (and at positions 23'''-88''' and 134'''-194'''); interchain heavy chain-heavy chain disulfide bridges at positions 230-230'' and 233-233''; and interchain heavy chain-light chain disulfide bridges at 224-214' and 224''-214'''. Current clinical trials of tavolixizumab in a variety of solid tumor indications include U.S. National Institutes of Health clinicaltrials.gov identifiers NCT02318394 and NCT02705482.

[001138] In some embodiments, a OX40 agonist comprises a heavy chain given by SEQ ID NO:56 and a light chain given by SEQ ID NO:57. In some embodiments, a OX40 agonist comprises heavy and light chains having the sequences shown in SEQ ID NO:56 and SEQ ID NO:57, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In some embodiments, a OX40 agonist comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:56 and SEQ ID NO:57, respectively. In some embodiments, a OX40 agonist comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:56 and SEQ ID NO:57, respectively. In some embodiments, a OX40 agonist

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comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:56 and SEQ ID NO:57, respectively. In some embodiments, a OX40 agonist comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:56 and SEQ ID NO:57, respectively. In some embodiments, a OX40 agonist comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:56 and SEQ ID NO:57, respectively.

[001139] In some embodiments, the OX40 agonist comprises the heavy and light chain CDRs or variable regions (VRs) of tavolixizumab. In some embodiments, the OX40 agonist heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:58, and the OX40 agonist light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:59, and conservative amino acid substitutions thereof. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:58 and SEQ ID NO:59, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:58 and SEQ ID NO:59, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:58 and SEQ ID NO:59, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:58 and SEQ ID NO:59, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:58 and SEQ ID NO:59, respectively. In some embodiments, an OX40 agonist comprises an scFv antibody comprising V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:58 and SEQ ID NO:59.

[001140] In some embodiments, a OX40 agonist comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, respectively, and conservative amino acid substitutions thereof.

[001141] In some embodiments, the OX40 agonist is a OX40 agonist biosimilar monoclonal antibody approved by drug regulatory authorities with reference to tavolixizumab. In some embodiments, the biosimilar monoclonal antibody comprises an OX40 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or

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reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is tavolixizumab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is a OX40 agonist antibody authorized or submitted for authorization, wherein the OX40 agonist antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is tavolixizumab. The OX40 agonist antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is tavolixizumab. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is tavolixizumab.

TABLE 13: Amino acid sequences for OX40 agonist antibodies related to tavolixizumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:56 heavy chain for tavolixizumab	QVQLQESGPG LVKPSQTLSL TCAVYGGSFS SGYWNWIRKH PGKGLEYIGY ISYNGITYHN PSLKSRITIN RDTSKNQYSL QLNSVTPEDT AVYYCARYKY DYDGGHAMDY WGQGTLVTVS SASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKRVE PKSCDKTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRE EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K	60 120 180 240 300 360 420 451
SEQ ID NO:57 light chain for tavolixizumab	DIQMTQSPSS LSASVGDRVT ITCRASQDIS NYLNWYQQKP GKAPKLLIYY TSKLHSGVPS RFSGSGSGTD YTLTISSLQP EDFATYYCQQ GSALPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC	60 120 180 214
SEQ ID NO:58 heavy chain variable region for tavolixizumab	QVQLQESGPG LVKPSQTLSL TCAVYGGSFS SGYWNWIRKH PGKGLEYIGY ISYNGITYHN PSLKSRITIN RDTSKNQYSL QLNSVTPEDT AVYYCARYKY DYDGGHAMDY WGQGTLVT	60 118
SEQ ID NO:59 light chain variable region for tavolixizumab	DIQMTQSPSS LSASVGDRVT ITCRASQDIS NYLNWYQQKP GKAPKLLIYY TSKLHSGVPS RFSGSGSGTD YTLTISSLQP EDFATYYCQQ GSALPWTFGQ GTKVEIKR	60 108
SEQ ID NO:60 heavy chain CDR1 for tavolixizumab	GSFSSGYWN	9

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SEQ ID NO:61 heavy chain CDR2 for tavolixizumab	YIGYISYNGI TYH	13
SEQ ID NO:62 heavy chain CDR3 for tavolixizumab	RYKYDYDGGH AMDY	14
SEQ ID NO:63 light chain CDR1 for tavolixizumab	QDISNYLN	8
SEQ ID NO:64 light chain CDR2 for tavolixizumab	LLIYYTSKLH S	11
SEQ ID NO:65 light chain CDR3 for tavolixizumab	QQGSALPW	8

[001142] In some embodiments, the OX40 agonist is 11D4, which is a fully human antibody available from Pfizer, Inc. The preparation and properties of 11D4 are described in U.S. Patent Nos. 7,960,515; 8,236,930; and 9,028,824, the disclosures of which are incorporated by reference herein. The amino acid sequences of 11D4 are set forth in Table 14.

[001143] In some embodiments, a OX40 agonist comprises a heavy chain given by SEQ ID NO:66 and a light chain given by SEQ ID NO:67. In some embodiments, a OX40 agonist comprises heavy and light chains having the sequences shown in SEQ ID NO:66 and SEQ ID NO:67, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In some embodiments, a OX40 agonist comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:66 and SEQ ID NO:67, respectively. In some embodiments, a OX40 agonist comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:66 and SEQ ID NO:67, respectively. In some embodiments, a OX40 agonist comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:66 and SEQ ID NO:67, respectively. In some embodiments, a OX40 agonist comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:66 and SEQ ID NO:67, respectively. In some embodiments, a OX40 agonist comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:66 and SEQ ID NO:67, respectively.

[001144] In some embodiments, the OX40 agonist comprises the heavy and light chain CDRs or variable regions (VRs) of 11D4. In some embodiments, the OX40 agonist heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:68, and the OX40 agonist light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:69, and conservative amino acid substitutions thereof. In some embodiments, a OX40 agonist

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comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:68 and SEQ ID NO:69, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:68 and SEQ ID NO:69, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:68 and SEQ ID NO:69, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:68 and SEQ ID NO:69, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:68 and SEQ ID NO:69, respectively.

[001145] In some embodiments, a OX40 agonist comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:70, SEQ ID NO:71, and SEQ ID NO:72, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:73, SEQ ID NO:74, and SEQ ID NO:75, respectively, and conservative amino acid substitutions thereof.

[001146] In some embodiments, the OX40 agonist is a OX40 agonist biosimilar monoclonal antibody approved by drug regulatory authorities with reference to 11D4. In some embodiments, the biosimilar monoclonal antibody comprises an OX40 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is 11D4. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is a OX40 agonist antibody authorized or submitted for authorization, wherein the OX40 agonist antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is 11D4. The OX40 agonist antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological

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product, wherein the reference medicinal product or reference biological product is 11D4. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is 11D4.

TABLE 14: Amino acid sequences for OX40 agonist antibodies related to 11D4.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:66 heavy chain for 11D4	EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYSMNWVRQA PGKGLEWVSY ISSSSSTIDY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARES GWYLFDYWGQ GTLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSNFGTQTYT CNVDHKPSNT KVDKTVERKC CVECPPCPAP PVAGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV QFNWYVDGVE VHNAKTKPRE EQFNSTFRVV SVLTVVHQDW LNGKEYKCKV SNKGLPAPIE KTISKTKGQP REPQVYTLPP SREEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPMLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK	60 120 180 240 300 360 420 444
SEQ ID NO:67 light chain for 11D4	DIQMTQSPSS LSASVGDRVT ITCRASQGIS SWLAWYQQKP EKAPKSLIYA ASSLQSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNSYPPTFGG GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC	60 120 180 214
SEQ ID NO:68 heavy chain variable region for 11D4	EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYSMNWVRQA PGKGLEWVSY ISSSSSTIDY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARES GWYLFDYWGQ GTLVTVSS	60 118
SEQ ID NO:69 light chain variable region for 11D4	DIQMTQSPSS LSASVGDRVT ITCRASQGIS SWLAWYQQKP EKAPKSLIYA ASSLQSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNSYPPTFGG GTKVEIK	60 107
SEQ ID NO:70 heavy chain CDR1 for 11D4	SYSMN	5
SEQ ID NO:71 heavy chain CDR2 for 11D4	YISSSSSTID YADSVKG	17
SEQ ID NO:72 heavy chain CDR3 for 11D4	ESGWYLFDY	9
SEQ ID NO:73 light chain CDR1 for 11D4	RASQGISSWL A	11
SEQ ID NO:74 light chain CDR2 for 11D4	AASSLQS	7
SEQ ID NO:75 light chain CDR3 for 11D4	QQYNSYPPT	9

[001147] In some embodiments, the OX40 agonist is 18D8, which is a fully human antibody available from Pfizer, Inc. The preparation and properties of 18D8 are described in U.S. Patent Nos. 7,960,515; 8,236,930; and 9,028,824, the disclosures of which are incorporated by reference herein. The amino acid sequences of 18D8 are set forth in Table 15.

[001148] In some embodiments, a OX40 agonist comprises a heavy chain given by SEQ ID NO:76 and a light chain given by SEQ ID NO:77. In some embodiments, a OX40 agonist comprises heavy and light chains having the sequences shown in SEQ ID NO:76 and SEQ ID NO:77, respectively, or antigen binding fragments, Fab fragments, single-chain variable

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fragments (scFv), variants, or conjugates thereof. In some embodiments, a OX40 agonist comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:76 and SEQ ID NO:77, respectively. In some embodiments, a OX40 agonist comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:76 and SEQ ID NO:77, respectively. In some embodiments, a OX40 agonist comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:76 and SEQ ID NO:77, respectively. In some embodiments, a OX40 agonist comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:76 and SEQ ID NO:77, respectively. In some embodiments, a OX40 agonist comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:76 and SEQ ID NO:77, respectively.

[001149] In some embodiments, the OX40 agonist comprises the heavy and light chain CDRs or variable regions (VRs) of 18D8. In some embodiments, the OX40 agonist heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:78, and the OX40 agonist light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:79, and conservative amino acid substitutions thereof. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:78 and SEQ ID NO:79, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:78 and SEQ ID NO:79, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:78 and SEQ ID NO:79, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:78 and SEQ ID NO:79, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:78 and SEQ ID NO:79, respectively.

[001150] In some embodiments, a OX40 agonist comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:80, SEQ ID NO:81, and SEQ ID NO:82, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:83, SEQ ID NO:84, and SEQ ID NO:85, respectively, and conservative amino acid substitutions thereof.

[001151] In some embodiments, the OX40 agonist is a OX40 agonist biosimilar monoclonal antibody approved by drug regulatory authorities with reference to 18D8. In some

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embodiments, the biosimilar monoclonal antibody comprises an OX40 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is 18D8. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is a OX40 agonist antibody authorized or submitted for authorization, wherein the OX40 agonist antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is 18D8. The OX40 agonist antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is 18D8. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is 18D8.

TABLE 15: Amino acid sequences for OX40 agonist antibodies related to 18D8.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:76 heavy chain for 18D8	EVQLVESGGG LVQPGRSLRL SCAASGFTFD DYAMHWVRQA PGKGLEWVSG ISWNSGSIGY ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TALYYCAKDQ STADYYFYYG MDVWGQGTTV TVSSASTKGP SVFPLAPCSR STSESTAALG CLVKDYFPEP VTVSWNSGAL TSGVHTFPAV LQSSGLYSLS SVVTVPSSNF GTQTYTCNVD HKPSNTKVDK TVERKCCVEC PPCPAPPVAG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVQFNW YVDGVEVHNA KTKPREEQFN STFRVVSVLT VVHQDWLNGK EYKCKVSNKG LPAPIEKTIS KTKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPM LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK	60 120 180 240 300 360 420 450
SEQ ID NO:77 light chain for 18D8	EIVVTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RSNWPTFGQG TKVEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC	60 120 180 213
SEQ ID NO:78 heavy chain variable region for 18D8	EVQLVESGGG LVQPGRSLRL SCAASGFTFD DYAMHWVRQA PGKGLEWVSG ISWNSGSIGY ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TALYYCAKDQ STADYYFYYG MDVWGQGTTV TVSS	60 120 124
SEQ ID NO:79 light chain variable region for 18D8	EIVVTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RSNWPTFGQG TKVEIK	60 106

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SEQ ID NO:80 heavy chain CDR1 for 18D8	DYAMH	5
SEQ ID NO:81 heavy chain CDR2 for 18D8	GISWNSGSIG YADSVKG	17
SEQ ID NO:82 heavy chain CDR3 for 18D8	DQSTADYYFY YGMDV	15
SEQ ID NO:83 light chain CDR1 for 18D8	RASQSVSSYL A	11
SEQ ID NO:84 light chain CDR2 for 18D8	DASNRAT	7
SEQ ID NO:85 light chain CDR3 for 18D8	QQRSNWPT	8

[001152] In some embodiments, the OX40 agonist is Hu119-122, which is a humanized antibody available from GlaxoSmithKline plc. The preparation and properties of Hu119-122 are described in U.S. Patent Nos. 9,006,399 and 9,163,085, and in International Patent Publication No. WO 2012/027328, the disclosures of which are incorporated by reference herein. The amino acid sequences of Hu119-122 are set forth in Table 16.

[001153] In some embodiments, the OX40 agonist comprises the heavy and light chain CDRs or variable regions (VRs) of Hu119-122. In some embodiments, the OX40 agonist heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:86, and the OX40 agonist light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:87, and conservative amino acid substitutions thereof. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:86 and SEQ ID NO:87, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:86 and SEQ ID NO:87, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:86 and SEQ ID NO:87, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:86 and SEQ ID NO:87, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:86 and SEQ ID NO:87, respectively.

[001154] In some embodiments, a OX40 agonist comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:88, SEQ ID NO:89, and SEQ ID NO:90, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:91, SEQ ID NO:92, and SEQ ID NO:93, respectively, and conservative amino acid substitutions thereof.

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[001155] In some embodiments, the OX40 agonist is a OX40 agonist biosimilar monoclonal antibody approved by drug regulatory authorities with reference to Hu119-122. In some embodiments, the biosimilar monoclonal antibody comprises an OX40 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is Hu119-122. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is a OX40 agonist antibody authorized or submitted for authorization, wherein the OX40 agonist antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is Hu119-122. The OX40 agonist antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is Hu119-122. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is Hu119-122.

TABLE 16: Amino acid sequences for OX40 agonist antibodies related to Hu119-122.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:86 heavy chain variable region for Hu119-122	EVQLVESGGG LVQPGGSLRL SCAASEYEFP SHDMSWVRQA PGKGIELVAA INSDGGSTYY PDTMERRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARHY DDYYAWFAYW GQGTMVTVSS	60 120
SEQ ID NO:87 light chain variable region for Hu119-122	EIVLTQSPAT LSLSPGERAT LSCRASKSVS TSGYSYMHWY QQKPGQAPRL LIYLASNLES GVPARFSGSG SGTDFTLTIS SLEPEDFAVY YCQHSRELPL TFGGGTKVEI K	60 111
SEQ ID NO:88 heavy chain CDR1 for Hu119-122	SHDMS	5
SEQ ID NO:89 heavy chain CDR2 for Hu119-122	AINSDGGSTY YPDTMER	17

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SEQ ID NO:90 heavy chain CDR3 for Hu119-122	HYDDYYAWFA Y	11
SEQ ID NO:91 light chain CDR1 for Hu119-122	RASKSVSTSG YSYMH	15
SEQ ID NO:92 light chain CDR2 for Hu119-122	LASNLES	7
SEQ ID NO:93 light chain CDR3 for Hu119-122	QHSRELPLT	9

[001156] In some embodiments, the OX40 agonist is Hu106-222, which is a humanized antibody available from GlaxoSmithKline plc. The preparation and properties of Hu106-222 are described in U.S. Patent Nos. 9,006,399 and 9,163,085, and in International Patent Publication No. WO 2012/027328, the disclosures of which are incorporated by reference herein. The amino acid sequences of Hu106-222 are set forth in Table 17.

[001157] In some embodiments, the OX40 agonist comprises the heavy and light chain CDRs or variable regions (VRs) of Hu106-222. In some embodiments, the OX40 agonist heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:94, and the OX40 agonist light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:95, and conservative amino acid substitutions thereof. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:94 and SEQ ID NO:95, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:94 and SEQ ID NO:95, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:94 and SEQ ID NO:95, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:94 and SEQ ID NO:95, respectively. In some embodiments, a OX40 agonist comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:94 and SEQ ID NO:95, respectively.

[001158] In some embodiments, a OX40 agonist comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:96, SEQ ID NO:97, and SEQ ID NO:98, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:99, SEQ ID NO:100, and SEQ ID NO:101, respectively, and conservative amino acid substitutions thereof.

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[001159] In some embodiments, the OX40 agonist is a OX40 agonist biosimilar monoclonal antibody approved by drug regulatory authorities with reference to Hu106-222. In some embodiments, the biosimilar monoclonal antibody comprises an OX40 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is Hu106-222. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is a OX40 agonist antibody authorized or submitted for authorization, wherein the OX40 agonist antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is Hu106-222. The OX40 agonist antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is Hu106-222. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is Hu106-222.

TABLE 17: Amino acid sequences for OX40 agonist antibodies related to Hu106-222.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:94 heavy chain variable region for Hu106-222	QVQLVQSGSE LKKPGASVKV SCKASGYTFT DYSMHWVRQA PGQGLKWMGW INTETGEPTY ADDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCANPY YDYVSYYAMD YWGQGTTVTV SS	60 120 122
SEQ ID NO:95 light chain variable region for Hu106-222	DIQMTQSPSS LSASVGDRVT ITCKASQDVS TAVAWYQQKP GKAPKLLIYS ASYLYTGVPS RFSGSGSGTD FTFTISSLQP EDIATYYCQQ HYSTPRTFGQ GTKLEIK	60 107
SEQ ID NO:96 heavy chain CDR1 for Hu106-222	DYSMH	5
SEQ ID NO:97 heavy chain CDR2 for Hu106-222	WINTETGEPT YADDFKG	17

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SEQ ID NO:98 heavy chain CDR3 for Hu106-222	PYYDYVSYYA MDY	13
SEQ ID NO:99 light chain CDR1 for Hu106-222	KASQDVSTAV A	11
SEQ ID NO:100 light chain CDR2 for Hu106-222	SASYLYT	7
SEQ ID NO:101 light chain CDR3 for Hu106-222	QQHYSTPRT	9

[001160] In some embodiments, the OX40 agonist antibody is MEDI6469 (also referred to as 9B12). MEDI6469 is a murine monoclonal antibody. Weinberg, *et al.*, *J. Immunother.* **2006**, *29*, 575-585. In some embodiments the OX40 agonist is an antibody produced by the 9B12 hybridoma, deposited with Biovest Inc. (Malvern, MA, USA), as described in Weinberg, *et al.*, *J. Immunother.* **2006**, *29*, 575-585, the disclosure of which is hereby incorporated by reference in its entirety. In some embodiments, the antibody comprises the CDR sequences of MEDI6469. In some embodiments, the antibody comprises a heavy chain variable region sequence and/or a light chain variable region sequence of MEDI6469.

[001161] In some embodiments, the OX40 agonist is L106 BD (Pharmingen Product #340420). In some embodiments, the OX40 agonist comprises the CDRs of antibody L106 (BD Pharmingen Product #340420). In some embodiments, the OX40 agonist comprises a heavy chain variable region sequence and/or a light chain variable region sequence of antibody L106 (BD Pharmingen Product #340420). In some embodiments, the OX40 agonist is ACT35 (Santa Cruz Biotechnology, Catalog #20073). In some embodiments, the OX40 agonist comprises the CDRs of antibody ACT35 (Santa Cruz Biotechnology, Catalog #20073). In some embodiments, the OX40 agonist comprises a heavy chain variable region sequence and/or a light chain variable region sequence of antibody ACT35 (Santa Cruz Biotechnology, Catalog #20073). In some embodiments, the OX40 agonist is the murine monoclonal antibody anti-mCD134/mOX40 (clone OX86), commercially available from InVivoMAb, BioXcell Inc, West Lebanon, NH.

[001162] In some embodiments, the OX40 agonist is selected from the OX40 agonists described in International Patent Application Publication Nos. WO 95/12673, WO 95/21925, WO 2006/121810, WO 2012/027328, WO 2013/028231, WO 2013/038191, and WO 2014/148895; European Patent Application EP 0672141; U.S. Patent Application Publication Nos. US 2010/136030, US 2014/377284, US 2015/190506, and US 2015/132288 (including clones 20E5 and 12H3); and U.S. Patent Nos. 7,504,101, 7,550,140, 7,622,444, 7,696,175,

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7,960,515, 7,961,515, 8,133,983, 9,006,399, and 9,163,085, the disclosure of each of which is incorporated herein by reference in its entirety.

[001163] In some embodiments, the OX40 agonist is an OX40 agonistic fusion protein as depicted in Structure I-A (C-terminal Fc-antibody fragment fusion protein) or Structure I-B (N-terminal Fc-antibody fragment fusion protein), or a fragment, derivative, conjugate, variant, or biosimilar thereof. The properties of structures I-A and I-B are described above and in U.S. Patent Nos. 9,359,420, 9,340,599, 8,921,519, and 8,450,460, the disclosures of which are incorporated by reference herein. Amino acid sequences for the polypeptide domains of structure I-A are given in Table 9. The Fc domain preferably comprises a complete constant domain (amino acids 17-230 of SEQ ID NO:31) the complete hinge domain (amino acids 1-16 of SEQ ID NO:31) or a portion of the hinge domain (*e.g.*, amino acids 4-16 of SEQ ID NO:31). Preferred linkers for connecting a C-terminal Fc-antibody may be selected from the embodiments given in SEQ ID NO:32 to SEQ ID NO:41, including linkers suitable for fusion of additional polypeptides. Likewise, amino acid sequences for the polypeptide domains of structure I-B are given in Table 10. If an Fc antibody fragment is fused to the N-terminus of an TNRFSF fusion protein as in structure I-B, the sequence of the Fc module is preferably that shown in SEQ ID NO:42, and the linker sequences are preferably selected from those embodiments set forth in SED ID NO:43 to SEQ ID NO:45.

[001164] In some embodiments, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains selected from the group consisting of a variable heavy chain and variable light chain of tavolixizumab, a variable heavy chain and variable light chain of 11D4, a variable heavy chain and variable light chain of 18D8, a variable heavy chain and variable light chain of Hu119-122, a variable heavy chain and variable light chain of Hu106-222, a variable heavy chain and variable light chain selected from the variable heavy chains and variable light chains described in Table 18, any combination of a variable heavy chain and variable light chain of the foregoing, and fragments, derivatives, conjugates, variants, and biosimilars thereof.

[001165] In some embodiments, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains comprising an OX40L sequence. In some embodiments, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains comprising a sequence according to SEQ ID NO:102. In some embodiments, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains comprising a soluble OX40L sequence.

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In some embodiments, a OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains comprising a sequence according to SEQ ID NO:103. In some embodiments, a OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains comprising a sequence according to SEQ ID NO:104.

[001166] In some embodiments, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:58 and SEQ ID NO:59, respectively, wherein the V_H and V_L domains are connected by a linker. In some embodiments, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:68 and SEQ ID NO:69, respectively, wherein the V_H and V_L domains are connected by a linker. In some embodiments, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:78 and SEQ ID NO:79, respectively, wherein the V_H and V_L domains are connected by a linker. In some embodiments, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:86 and SEQ ID NO:87, respectively, wherein the V_H and V_L domains are connected by a linker. In some embodiments, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:94 and SEQ ID NO:95, respectively, wherein the V_H and V_L domains are connected by a linker. In some embodiments, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the V_H and V_L sequences given in Table 18, wherein the V_H and V_L domains are connected by a linker.

TABLE 18: Additional polypeptide domains useful as OX40 binding domains in fusion proteins (*e.g.*, structures I-A and I-B) or as scFv OX40 agonist antibodies.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:102 OX40L	MERVQPLEEN VGNAARPRFE RNKLLLVASV IQGLGLLLCF TYICLHFSAL QVSHRYPRIQ SIKVQFTEYK KEKGFILTSQ KEDEIMKVQN NSVIINCDGF YLISLKGYFS QEVNISLHYQ	60 120

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	KDEEPLFQLK KVRSVNSLMV ASLTYKDKVY LNVTTDNTSL DDFHVNGGEL ILIHQNPGEF CVL	180 183
SEQ ID NO:103 OX40L soluble domain	SHRYPRIQSI KVQFTEYKKE KGFILTSQKE DEIMKVQNNS VIINCDGFYL ISLKGYFSQE VNISLHYQKD EEPLFQLKKV RSVNSLMVAS LTYKDKVYLN VTTDNTSLDD FHVNGGELIL IHQNPGEFCV L	60 120 131
SEQ ID NO:104 OX40L soluble domain (alternative)	YPRIQSIKVQ FTEYKKEKGF ILTSQKEDEI MKVQNNSVII NCDGFYLISL KGYFSQEVNI SLHYQKDEEP LFQLKKVRSV NSLMVASLTY KDKVYLNVTT DNTSLDDFHV NGGELILIHQ NPGEFCVL	60 120 128
SEQ ID NO:105 variable heavy chain for 008	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYTMNWVRQA PGKGLEWVSA ISGSGGSTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKDR YSQVHYALDY WGQGTLVTVS	60 120
SEQ ID NO:106 variable light chain for 008	DIVMTQSPDS LPVTPGEPAS ISCRSSQSLL HSNGYNYLDW YLQKAGQSPQ LLIYLGSNRA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCQQYYNHP TTFGQGTK	60 108
SEQ ID NO:107 variable heavy chain for 011	EVQLVESGGG VVQPGRSLRL SCAASGFTFS DYTMNWVRQA PGKGLEWVSS ISGGSTYYAD SRKGRFTISR DNSKNTLYLQ MNNLRAEDTA VYYCARDRYF RQQNAFDYWG QGTLVTVSSA	60 120
SEQ ID NO:108 variable light chain for 011	DIVMTQSPDS LPVTPGEPAS ISCRSSQSLL HSNGYNYLDW YLQKAGQSPQ LLIYLGSNRA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCQQYYNHP TTFGQGTK	60 108
SEQ ID NO:109 variable heavy chain for 021	EVQLVESGGG LVQPRGSLRL SCAASGFTFS SYAMNWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKDR YITLPNALDY WGQGTLVTVS	60 120
SEQ ID NO:110 variable light chain for 021	DIQMTQSPVS LPVTPGEPAS ISCRSSQSLL HSNGYNYLDW YLQKPGQSPQ LLIYLGSNRA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCQQYKSNP PTFGQGTK	60 108
SEQ ID NO:111 variable heavy chain for 023	EVQLVESGGG LVHPGGSLRL SCAGSGFTFS SYAMHWVRQA PGKGLEWVSA IGTGGGTYYA DSVMGRFTIS RDNSKNTLYL QMNSLRAEDT AVYYCARYDN VMGLYWFDYW GQGTLVTVSS	60 120
SEQ ID NO:112 variable light chain for 023	EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RSNWPPAFGG GTKVEIKR	60 108
SEQ ID NO:113 heavy chain variable region	EVQLQQSGPE LVKPGASVKM SCKASGYTFT SYVMHWVKQK PGQGLEWIGY INPYNDGTKY NEKFKGKATL TSDKSSSTAY MELSSLTSED SAVYYCANYY GSSLSMDYWG QGTSVTVSS	60 119
SEQ ID NO:114 light chain variable region	DIQMTQTTSS LSASLGDRVT ISCRASQDIS NYLNWYQQKP DGTVKLLIYY TSRLHSGVPS RFSGSGSGTD YSLTISNLEQ EDIATYFCQQ GNTLPWTFGG GTKLEIKR	60 108
SEQ ID NO:115 heavy chain variable region	EVQLQQSGPE LVKPGASVKI SCKTSGYTFK DYTMHWVKQS HGKSLEWIGG IYPNNGGSTY NQNFKDKATL TVDKSSSTAY MEFRSLTSED SAVYYCARMG YHGPHLDFDV WGAGTTVTVS P	60 120 121
SEQ ID NO:116 light chain variable region	DIVMTQSHKF MSTSLGDRVS ITCKASQDVG AAVAWYQQKP GQSPKLLIYW ASTRHTGVPD RFTGGGSGTD FTLTISNVQS EDLTDYFCQQ YINYPLTFGG GTKLEIKR	60 108
SEQ ID NO:117 heavy chain variable region of humanized antibody	QIQLVQSGPE LKKPGETVKI SCKASGYTFT DYSMHWVKQA PGKGLKWMGW INTETGEPTY ADDFKGRFAF SLETSASTAY LQINNLKNED TATYFCANPY YDYVSYYAMD YWGHGTSVTV SS	60 120 122
SEQ ID NO:118 heavy chain variable region of humanized antibody	QVQLVQSGSE LKKPGASVKV SCKASGYTFT DYSMHWVRQA PGQGLKWMGW INTETGEPTY ADDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCANPY YDYVSYYAMD YWGQGTTVTV SS	60 120 122
SEQ ID NO:119 light chain variable region of humanized antibody	DIVMTQSHKF MSTSVRDRVS ITCKASQDVS TAVAWYQQKP GQSPKLLIYS ASYLYTGVPD RFTGSGSGTD FTFTISSVQA EDLAVYYCQQ HYSTPRTFGG GTKLEIK	60 107
SEQ ID NO:120 light chain variable region of humanized antibody	DIVMTQSHKF MSTSVRDRVS ITCKASQDVS TAVAWYQQKP GQSPKLLIYS ASYLYTGVPD RFTGSGSGTD FTFTISSVQA EDLAVYYCQQ HYSTPRTFGG GTKLEIK	60 107
SEQ ID NO:121 heavy chain variable region of humanized antibody	EVQLVESGGG LVQPGESLKL SCESNEYEFP SHDMSWVRKT PEKRLELVAA INSDGGSTYY PDTMERRFII SRDNTKKTLY LQMSSLRSED TALYYCARHY DDYYAWFAYW GQGTLVTVSA	60 120
SEQ ID NO:122 heavy chain variable region of humanized antibody	EVQLVESGGG LVQPGGSLRL SCAASEYEFP SHDMSWVRQA PGKGLELVAA INSDGGSTYY PDTMERRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARHY DDYYAWFAYW GQGTMVTVSS	60 120

SEQ ID NO:123 light chain variable region of humanized antibody	DIVLTQSPAS LAVSLGQRAT ISCRASKSVS TSGYSYMHWY QQKPGQPPKL LIYLASNLES GVPARFSGSG SGTDFTLNIH PVEEEDAATY YCQHSRELPL TFGAGTKLEL K	60 111
SEQ ID NO:124 light chain variable region of humanized antibody	EIVLTQSPAT LSLSPGERAT LSCRASKSVS TSGYSYMHWY QQKPGQAPRL LIYLASNLES GVPARFSGSG SGTDFTLTIS SLEPEDFAVY YCQHSRELPL TFGGGTKVEI K	60 111
SEQ ID NO:125 heavy chain variable region	MYLGLNYVFI VFLLNGVQSE VKLEESGGGL VQPGGSMKLS CAASGFTFSD AWMDWVRQSP EKGLEWVAEI RSKANNHATY YAESVNGRFT ISRDDSKSSV YLQMNSLRAE DTGIYYCTWG EVFYFDYWGQ GTTLTVSS	60 120 138
SEQ ID NO:126 light chain variable region	MRPSIQFLGL LLFWLHGAQC DIQMTQSPSS LSASLGGKVT ITCKSSQDIN KYIAWYQHKP GKGPRLLIHY TSTLQPGIPS RFSGSGSGRD YSFSISNLEP EDIATYYCLQ YDNLLTFGAG TKLELK	60 120 126

[001167] In some embodiments, the OX40 agonist is a OX40 agonistic single-chain fusion polypeptide comprising (i) a first soluble OX40 binding domain, (ii) a first peptide linker, (iii) a second soluble OX40 binding domain, (iv) a second peptide linker, and (v) a third soluble OX40 binding domain, further comprising an additional domain at the N-terminal and/or C-terminal end, and wherein the additional domain is a Fab or Fc fragment domain. In some embodiments, the OX40 agonist is a OX40 agonistic single-chain fusion polypeptide comprising (i) a first soluble OX40 binding domain, (ii) a first peptide linker, (iii) a second soluble OX40 binding domain, (iv) a second peptide linker, and (v) a third soluble OX40 binding domain, further comprising an additional domain at the N-terminal and/or C-terminal end, wherein the additional domain is a Fab or Fc fragment domain wherein each of the soluble OX40 binding domains lacks a stalk region (which contributes to trimerisation and provides a certain distance to the cell membrane, but is not part of the OX40 binding domain) and the first and the second peptide linkers independently have a length of 3-8 amino acids.

[001168] In some embodiments, the OX40 agonist is an OX40 agonistic single-chain fusion polypeptide comprising (i) a first soluble tumor necrosis factor (TNF) superfamily cytokine domain, (ii) a first peptide linker, (iii) a second soluble TNF superfamily cytokine domain, (iv) a second peptide linker, and (v) a third soluble TNF superfamily cytokine domain, wherein each of the soluble TNF superfamily cytokine domains lacks a stalk region and the first and the second peptide linkers independently have a length of 3-8 amino acids, and wherein the TNF superfamily cytokine domain is an OX40 binding domain.

[001169] In some embodiments, the OX40 agonist is MEDI6383. MEDI6383 is an OX40 agonistic fusion protein and can be prepared as described in U.S. Patent No. 6,312,700, the disclosure of which is incorporated by reference herein.

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[001170] In some embodiments, the OX40 agonist is an OX40 agonistic scFv antibody comprising any of the foregoing V_H domains linked to any of the foregoing V_L domains.

[001171] In some embodiments, the OX40 agonist is Creative Biolabs OX40 agonist monoclonal antibody MOM-18455, commercially available from Creative Biolabs, Inc., Shirley, NY, USA.

[001172] In some embodiments, the OX40 agonist is OX40 agonistic antibody clone Ber-ACT35 commercially available from BioLegend, Inc., San Diego, CA, USA.

I. Optional Cell Viability Analyses

[001173] Optionally, a cell viability assay can be performed after the priming first expansion (sometimes referred to as the initial bulk expansion), using standard assays known in the art. Thus, in certain embodiments, the method comprises performing a cell viability assay subsequent to the priming first expansion. For example, a trypan blue exclusion assay can be done on a sample of the bulk TILs, which selectively labels dead cells and allows a viability assessment. Other assays for use in testing viability can include but are not limited to the Alamar blue assay; and the MTT assay.

1. Cell Counts, Viability, Flow Cytometry

[001174] In some embodiments, cell counts and/or viability are measured. The expression of markers such as but not limited CD3, CD4, CD8, and CD56, as well as any other disclosed or described herein, can be measured by flow cytometry with antibodies, for example but not limited to those commercially available from BD Bio-sciences (BD Biosciences, San Jose, CA) using a FACSCanto™ flow cytometer (BD Biosciences). The cells can be counted manually using a disposable c-chip hemocytometer (VWR, Batavia, IL) and viability can be assessed using any method known in the art, including but not limited to trypan blue staining. The cell viability can also be assayed based on USSN 15/863,634, incorporated by reference herein in its entirety. Cell viability can also be assayed based on U.S. Patent Publication No. 2018/0280436 or International Patent Publication No. WO/2018/081473, both of which are incorporate herein in their entireties for all purposes.

[001175] In some cases, the bulk TIL population can be cryopreserved immediately, using the protocols discussed below. Alternatively, the bulk TIL population can be subjected to REP and then cryopreserved as discussed below. Similarly, in the case where genetically modified

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TILs will be used in therapy, the bulk or REP TIL populations can be subjected to genetic modifications for suitable treatments.

2. Cell Cultures

[001176] In some embodiments, a method for expanding TILs, including those discussed above as well as exemplified in Figure 1, in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G, may include using about 5,000 mL to about 25,000 mL of cell medium, about 5,000 mL to about 10,000 mL of cell medium, or about 5,800 mL to about 8,700 mL of cell medium. In some embodiments, the media is a serum free medium. In some embodiments, the media in the priming first expansion is serum free. In some embodiments, the media in the second expansion is serum free. In some embodiments, the media in the priming first expansion and the second expansion (also referred to as rapid second expansion) are both serum free. In some embodiments, expanding the number of TILs uses no more than one type of cell culture medium. Any suitable cell culture medium may be used, *e.g.*, AIM-V cell medium (L-glutamine, 50 μM streptomycin sulfate, and 10 μM gentamicin sulfate) cell culture medium (Invitrogen, Carlsbad CA). In this regard, the inventive methods advantageously reduce the amount of medium and the number of types of medium required to expand the number of TIL. In some embodiments, expanding the number of TIL may comprise feeding the cells no more frequently than every third or fourth day. Expanding the number of cells in a gas permeable container simplifies the procedures necessary to expand the number of cells by reducing the feeding frequency necessary to expand the cells.

[001177] In some embodiments, the cell culture medium in the first and/or second gas permeable container is unfiltered. The use of unfiltered cell medium may simplify the procedures necessary to expand the number of cells. In some embodiments, the cell medium in the first and/or second gas permeable container lacks beta-mercaptoethanol (BME).

[001178] In some embodiments, the duration of the method comprising obtaining a tumor tissue sample from the mammal; culturing the tumor tissue sample in a first gas permeable container containing cell medium including IL-2, 1X antigen-presenting feeder cells, and OKT-3 for a duration of about 1 to 8 days, *e.g.*, about 8 days as a priming first expansion; transferring the TILs to a second gas permeable container and expanding the number of TILs in the second gas permeable container containing cell medium including IL-2, 2X antigen-

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presenting feeder cells, and OKT-3 for a duration of about 7 to 9 days, *e.g.*, about 7 days, about 8 days, or about 9 days.

[001179] In some embodiments, the duration of the method comprising obtaining a tumor tissue sample from the mammal; culturing the tumor tissue sample in a first gas permeable container containing cell medium including IL-2, 1X antigen-presenting feeder cells, and OKT-3 for a duration of about 1 to 7 days, *e.g.*, about 7 days as a priming first expansion; transferring the TILs to a second gas permeable container and expanding the number of TILs in the second gas permeable container containing cell medium including IL-2, 2X antigen-presenting feeder cells, and OKT-3 for a duration of about 7 to 9 days, *e.g.*, about 7 days, about 8 days, or about 9 days.

[001180] In some embodiments, the duration of the method comprising obtaining a tumor tissue sample from the mammal; culturing the tumor tissue sample in a first gas permeable container containing cell medium including IL-2, 1X antigen-presenting feeder cells, and OKT-3 for a duration of about 1 to 7 days, *e.g.*, about 7 days as a priming first expansion; transferring the TILs to a second gas permeable container and expanding the number of TILs in the second gas permeable container containing cell medium including IL-2, 2X antigen-presenting feeder cells, and OKT-3 for a duration of about 7 to 10 days, *e.g.*, about 7 days, about 8 days, about 9 days or about 10 days.

[001181] In some embodiments, TILs are expanded in gas-permeable containers. Gas-permeable containers have been used to expand TILs using PBMCs using methods, compositions, and devices known in the art, including those described in U.S. Patent Application Publication No. 2005/0106717 A1, the disclosures of which are incorporated herein by reference. In some embodiments, TILs are expanded in gas-permeable bags. In some embodiments, TILs are expanded using a cell expansion system that expands TILs in gas permeable bags, such as the Xuri Cell Expansion System W25 (GE Healthcare). In some embodiments, TILs are expanded using a cell expansion system that expands TILs in gas permeable bags, such as the WAVE Bioreactor System, also known as the Xuri Cell Expansion System W5 (GE Healthcare). In some embodiments, the cell expansion system includes a gas permeable cell bag with a volume selected from the group consisting of about 100 mL, about 200 mL, about 300 mL, about 400 mL, about 500 mL, about 600 mL, about 700 mL, about 800 mL, about 900 mL, about 1 L, about 2 L, about 3 L, about 4 L, about 5 L, about 6 L, about 7 L, about 8 L, about 9 L, and about 10 L.

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[001182] In some embodiments, TILs can be expanded in G-Rex flasks (commercially available from Wilson Wolf Manufacturing). Such embodiments allow for cell populations to expand from about 5×10^5 cells/cm^2 to between 10×10^6 and 30×10^6 cells/cm^2. In some embodiments this is without feeding. In some embodiments, this is without feeding so long as medium resides at a height of about 10 cm in the G-Rex flask. In some embodiments this is without feeding but with the addition of one or more cytokines. In some embodiments, the cytokine can be added as a bolus without any need to mix the cytokine with the medium. Such containers, devices, and methods are known in the art and have been used to expand TILs, and include those described in U.S. Patent Application Publication No. US 2014/0377739A1, International Publication No. WO 2014/210036 A1, U.S. Patent Application Publication No. us 2013/0115617 A1, International Publication No. WO 2013/188427 A1, U.S. Patent Application Publication No. US 2011/0136228 A1, U.S. Patent No. US 8,809,050 B2, International publication No. WO 2011/072088 A2, U.S. Patent Application Publication No. US 2016/0208216 A1, U.S. Patent Application Publication No. US 2012/0244133 A1, International Publication No. WO 2012/129201 A1, U.S. Patent Application Publication No. US 2013/0102075 A1, U.S. Patent No. US 8,956,860 B2, International Publication No. WO 2013/173835 A1, U.S. Patent Application Publication No. US 2015/0175966 A1, the disclosures of which are incorporated herein by reference. Such processes are also described in Jin *et al.*, *J. Immunotherapy*, **2012**, 35:283-292.

J. Optional Genetic Engineering of TILs

[001183] In some embodiments, the expanded TILs of the present invention are further manipulated before, during, or after an expansion step, including during closed, sterile manufacturing processes, each as provided herein, in order to alter protein expression in a transient manner. In some embodiments, the transiently altered protein expression is due to transient gene editing. In some embodiments, the expanded TILs of the present invention are treated with transcription factors (TFs) and/or other molecules capable of transiently altering protein expression in the TILs. In some embodiments, the TFs and/or other molecules that are capable of transiently altering protein expression provide for altered expression of tumor antigens and/or an alteration in the number of tumor antigen-specific T cells in a population of TILs.

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[001184] In certain embodiments, the method comprises genetically editing a population of TILs. In certain embodiments, the method comprises genetically editing the first population of TILs, the second population of TILs and/or the third population of TILs.

[001185] In some embodiments, the present invention includes genetic editing through nucleotide insertion, such as through ribonucleic acid (RNA) insertion, including insertion of messenger RNA (mRNA) or small (or short) interfering RNA (siRNA), into a population of TILs for promotion of the expression of one or more proteins or inhibition of the expression of one or more proteins, as well as simultaneous combinations of both promotion of one set of proteins with inhibition of another set of proteins.

[001186] In some embodiments, the expanded TILs of the present invention undergo transient alteration of protein expression. In some embodiments, the transient alteration of protein expression occurs in the bulk TIL population prior to first expansion, including, for example in the TIL population obtained from for example, Step A as indicated in Figure 1 (particularly Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, the transient alteration of protein expression occurs during the first expansion, including, for example in the TIL population expanded in for example, Step B as indicated in Figure 1 (for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, the transient alteration of protein expression occurs after the first expansion, including, for example in the TIL population in transition between the first and second expansion (*e.g.* the second population of TILs as described herein), the TIL population obtained from for example, Step B and included in Step C as indicated in Figure 1. In some embodiments, the transient alteration of protein expression occurs in the bulk TIL population prior to second expansion, including, for example in the TIL population obtained from for example, Step C and prior to its expansion in Step D as indicated in Figure 1. In some embodiments, the transient alteration of protein expression occurs during the second expansion, including, for example in the TIL population expanded in for example, Step D as indicated in Figure 1 (*e.g.* the third population of TILs). In some embodiments, the transient alteration of protein expression occurs after the second expansion, including, for example in the TIL population obtained from the expansion in for example, Step D as indicated in Figure 1.

[001187] In some embodiments, a method of transiently altering protein expression in a population of TILs includes the step of electroporation. Electroporation methods are known in the art and are described, *e.g.*, in Tsong, *Biophys. J.* **1991,** *60,* 297-306, and U.S. Patent

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Application Publication No. 2014/0227237 A1, the disclosures of each of which are incorporated by reference herein. In some embodiments, a method of transiently altering protein expression in population of TILs includes the step of calcium phosphate transfection. Calcium phosphate transfection methods (calcium phosphate DNA precipitation, cell surface coating, and endocytosis) are known in the art and are described in Graham and van der Eb, *Virology* **1973,** *52,* 456-467; Wigler, *et al.*, *Proc. Natl. Acad. Sci.* **1979,** *76,* 1373-1376; and Chen and Okayarea, *Mol. Cell. Biol.* **1987,** *7,* 2745-2752; and in U.S. Patent No. 5,593,875, the disclosures of each of which are incorporated by reference herein. In some embodiments, a method of transiently altering protein expression in a population of TILs includes the step of liposomal transfection. Liposomal transfection methods, such as methods that employ a 1:1 (w/w) liposome formulation of the cationic lipid *N*-[1-(2,3-dioleyloxy)propyl]-*n,n,n*-trimethylammonium chloride (DOTMA) and dioleoyl phophotidylethanolamine (DOPE) in filtered water, are known in the art and are described in Rose, *et al.*, *Biotechniques* **1991,** *10,* 520-525 and Felgner, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1987,** *84,* 7413-7417 and in U.S. Patent Nos. 5,279,833; 5,908,635; 6,056,938; 6,110,490; 6,534,484; and 7,687,070, the disclosures of each of which are incorporated by reference herein. In some embodiments, a method of transiently altering protein expression in a population of TILs includes the step of transfection using methods described in U.S. Patent Nos. 5,766,902; 6,025,337; 6,410,517; 6,475,994; and 7,189,705; the disclosures of each of which are incorporated by reference herein.

[001188] In some embodiments, the TILs of the present invention are further modified to transiently or permanently suppress the expression of one or more genes using the methods described in International Patent Application Nos. WO 2019/136456 A1 or WO 2019/210131 A1, the disclosures of each of which are incorporated by reference herein, including methods described therein to genetically edit TILs to knockout specific target genes such as the genes that code for PD-1 and CTLA-4.

[001189] In some embodiments, transient alteration of protein expression results in an increase in Stem Memory T cells (TSCMs). TSCMs are early progenitors of antigen-experienced central memory T cells. TSCMs generally display the long-term survival, self-renewal, and multipotency abilities that define stem cells, and are generally desirable for the generation of effective TIL products. TSCM have shown enhanced anti-tumor activity compared with other T cell subsets in mouse models of adoptive cell transfer (Gattinoni *et al.* Nat Med 2009, 2011; Gattinoni, Nature Rev. Cancer, 2012; Cieri *et al.* Blood 2013). In some

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embodiments, transient alteration of protein expression results in a TIL population with a composition comprising a high proportion of TSCM. In some embodiments, transient alteration of protein expression results in an at least 5%, at least 10%, at least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% increase in TSCM percentage. In some embodiments, transient alteration of protein expression results in an at least a 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, or 10-fold increase in TSCMs in the TIL population. In some embodiments, transient alteration of protein expression results in a TIL population with at least at least 5%, at least 10%, at least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% TSCMs. In some embodiments, transient alteration of protein expression results in a therapeutic TIL population with at least at least 5%, at least 10%, at least 10%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% TSCMs.

[001190] In some embodiments, transient alteration of protein expression results in rejuvenation of antigen-experienced T-cells. In some embodiments, rejuvenation includes, for example, increased proliferation, increased T-cell activation, and/or increased antigen recognition.

[001191] In some embodiments, transient alteration of protein expression alters the expression in a large fraction of the T-cells in order to preserve the tumor-derived TCR repertoire. In some embodiments, transient alteration of protein expression does not alter the tumor-derived TCR repertoire. In some embodiments, transient alteration of protein expression maintains the tumor-derived TCR repertoire.

[001192] In some embodiments, transient alteration of protein results in altered expression of a particular gene. In some embodiments, the transient alteration of protein expression targets a gene including but not limited to PD-1 (also referred to as PDCD1 or CC279), TGFBR2, CCR4/5, CBLB (CBL-B), CISH, CCRs (chimeric co-stimulatory receptors), IL-2, IL-12, IL-15, IL-21, NOTCH 1/2 ICD, TIM3, LAG3, TIGIT, TGFβ, CCR2, CCR4, CCR5, CXCR1, CXCR2, CSCR3, CCL2 (MCP-1), CCL3 (MIP-1α), CCL4 (MIP1-β), CCL5 (RANTES), CXCL1/CXCL8, CCL22, CCL17, CXCL1/CXCL8, VHL, CD44, PIK3CD, SOCS1, thymocyte selection associated high mobility group (HMG) box (TOX), ankyrin repeat

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domain 11 (ANKRD11), BCL6 co-repressor (BCOR) and/or cAMP protein kinase A (PKA). In some embodiments, the transient alteration of protein expression targets a gene selected from the group consisting of PD-1, TGFBR2, CCR4/5, CBLB (CBL-B), CISH, CCRs (chimeric co-stimulatory receptors), IL-2, IL-12, IL-15, IL-21, NOTCH 1/2 ICD, TIM3, LAG3, TIGIT, TGFβ, CCR2, CCR4, CCR5, CXCR1, CXCR2, CSCR3, CCL2 (MCP-1), CCL3 (MIP-1α), CCL4 (MIP1-β), CCL5 (RANTES), CXCL1/CXCL8, CCL22, CCL17, CXCL1/CXCL8, VHL, CD44, PIK3CD, SOCS1, thymocyte selection associated high mobility group (HMG) box (TOX), ankyrin repeat domain 11 (ANKRD11), BCL6 co-repressor (BCOR) and/or cAMP protein kinase A (PKA). In some embodiments, the transient alteration of protein expression targets PD-1. In some embodiments, the transient alteration of protein expression targets TGFBR2. In some embodiments, the transient alteration of protein expression targets CCR4/5. In some embodiments, the transient alteration of protein expression targets CBLB. In some embodiments, the transient alteration of protein expression targets CISH. In some embodiments, the transient alteration of protein expression targets CCRs (chimeric co-stimulatory receptors). In some embodiments, the transient alteration of protein expression targets IL-2. In some embodiments, the transient alteration of protein expression targets IL-12. In some embodiments, the transient alteration of protein expression targets IL-15. In some embodiments, the transient alteration of protein expression targets IL-21. In some embodiments, the transient alteration of protein expression targets NOTCH 1/2 ICD. In some embodiments, the transient alteration of protein expression targets TIM3. In some embodiments, the transient alteration of protein expression targets LAG3. In some embodiments, the transient alteration of protein expression targets TIGIT. In some embodiments, the transient alteration of protein expression targets TGFβ. In some embodiments, the transient alteration of protein expression targets CCR1. In some embodiments, the transient alteration of protein expression targets CCR2. In some embodiments, the transient alteration of protein expression targets CCR4. In some embodiments, the transient alteration of protein expression targets CCR5. In some embodiments, the transient alteration of protein expression targets CXCR1. In some embodiments, the transient alteration of protein expression targets CXCR2. In some embodiments, the transient alteration of protein expression targets CSCR3. In some embodiments, the transient alteration of protein expression targets CCL2 (MCP-1). In some embodiments, the transient alteration of protein expression targets CCL3 (MIP-1α). In some embodiments, the transient alteration of protein expression targets CCL4 (MIP1-β). In some embodiments, the transient alteration of protein expression targets CCL5 (RANTES). In

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some embodiments, the transient alteration of protein expression targets CXCL1. In some embodiments, the transient alteration of protein expression targets CXCL8. In some embodiments, the transient alteration of protein expression targets CCL22. In some embodiments, the transient alteration of protein expression targets CCL17. In some embodiments, the transient alteration of protein expression targets VHL. In some embodiments, the transient alteration of protein expression targets CD44. In some embodiments, the transient alteration of protein expression targets PIK3CD. In some embodiments, the transient alteration of protein expression targets SOCS1. In some embodiments, the transient alteration of protein expression targets thymocyte selection associated high mobility group (HMG) box (TOX). In some embodiments, the transient alteration of protein expression targets ankyrin repeat domain 11 (ANKRD11). In some embodiments, the transient alteration of protein expression targets BCL6 co-repressor (BCOR). In some embodiments, the transient alteration of protein expression targets cAMP protein kinase A (PKA).

[001193] In some embodiments, the transient alteration of protein expression results in increased and/or overexpression of a chemokine receptor. In some embodiments, the chemokine receptor that is overexpressed by transient protein expression includes a receptor with a ligand that includes but is not limited to CCL2 (MCP-1), CCL3 (MIP-1α), CCL4 (MIP1-β), CCL5 (RANTES), CXCL1, CXCL8, CCL22, and/or CCL17.

[001194] In some embodiments, the transient alteration of protein expression results in a decrease and/or reduced expression of PD-1, CTLA-4, TIM-3, LAG-3, TIGIT, TGFβR2, and/or TGFβ (including resulting in, for example, TGFβ pathway blockade). In some embodiments, the transient alteration of protein expression results in a decrease and/or reduced expression of CBLB (CBL-B). In some embodiments, the transient alteration of protein expression results in a decrease and/or reduced expression of CISH.

[001195] In some embodiments, the transient alteration of protein expression results in increased and/or overexpression of chemokine receptors in order to, for example, improve TIL trafficking or movement to the tumor site. In some embodiments, the transient alteration of protein expression results in increased and/or overexpression of a CCR (chimeric co-stimulatory receptor). In some embodiments, the transient alteration of protein expression results in increased and/or overexpression of a chemokine receptor selected from the group consisting of CCR1, CCR2, CCR4, CCR5, CXCR1, CXCR2, and/or CSCR3.

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[001196] In some embodiments, the transient alteration of protein expression results in increased and/or overexpression of an interleukin. In some embodiments, the transient alteration of protein expression results in increased and/or overexpression of an interleukin selected from the group consisting of IL-2, IL-12, IL-15, and/or IL-21.

[001197] In some embodiments, the transient alteration of protein expression results in increased and/or overexpression of NOTCH 1/2 ICD. In some embodiments, the transient alteration of protein expression results in increased and/or overexpression of VHL. In some embodiments, the transient alteration of protein expression results in increased and/or overexpression of CD44. In some embodiments, the transient alteration of protein expression results in increased and/or overexpression of PIK3CD. In some embodiments, the transient alteration of protein expression results in increased and/or overexpression of SOCS1,

[001198] In some embodiments, the transient alteration of protein expression results in decreased and/or reduced expression of cAMP protein kinase A (PKA).

[001199] In some embodiments, the transient alteration of protein expression results in decreased and/or reduced expression of a molecule selected from the group consisting of PD-1, LAG3, TIM3, CTLA-4, TIGIT, CISH, TGFβR2, PKA, CBLB, BAFF (BR3), and combinations thereof. In some embodiments, the transient alteration of protein expression results in decreased and/or reduced expression of two molecules selected from the group consisting of PD-1, LAG3, TIM3, CTLA-4, TIGIT, CISH, TGFβR2, PKA, CBLB, BAFF (BR3), and combinations thereof. In some embodiments, the transient alteration of protein expression results in decreased and/or reduced expression of PD-1 and one molecule selected from the group consisting of LAG3, TIM3, CTLA-4, TIGIT, CISH, TGFβR2, PKA, CBLB, BAFF (BR3), and combinations thereof. In some embodiments, the transient alteration of protein expression results in decreased and/or reduced expression of PD-1, LAG-3, CISH, CBLB, TIM3, and combinations thereof. In some embodiments, the transient alteration of protein expression results in decreased and/or reduced expression of PD-1 and one of LAG3, CISH, CBLB, TIM3, and combinations thereof. In some embodiments, the transient alteration of protein expression results in decreased and/or reduced expression of PD-1 and LAG3. In some embodiments, the transient alteration of protein expression results in decreased and/or reduced expression of PD-1 and CISH. In some embodiments, the transient alteration of protein expression results in decreased and/or reduced expression of PD-1 and CBLB. In some embodiments, the transient alteration of protein expression results in decreased and/or reduced expression of LAG3 and CISH. In some embodiments, the

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transient alteration of protein expression results in decreased and/or reduced expression of LAG3 and CBLB. In some embodiments, the transient alteration of protein expression results in decreased and/or reduced expression of CISH and CBLB. In some embodiments, the transient alteration of protein expression results in decreased and/or reduced expression of TIM3 and PD-1. In some embodiments, the transient alteration of protein expression results in decreased and/or reduced expression of TIM3 and LAG3. In some embodiments, the transient alteration of protein expression results in decreased and/or reduced expression of TIM3 and CISH. In some embodiments, the transient alteration of protein expression results in decreased and/or reduced expression of TIM3 and CBLB.

[001200] In some embodiments, an adhesion molecule selected from the group consisting of CCR2, CCR4, CCR5, CXCR2, CXCR3, CX3CR1, and combinations thereof, is inserted by a gammaretroviral or lentiviral method into the first population of TILs, second population of TILs, or harvested population of TILs (*e.g.*, the expression of the adhesion molecule is increased).

[001201] In some embodiments, the transient alteration of protein expression results in decreased and/or reduced expression of a molecule selected from the group consisting of PD-1, LAG3, TIM3, CTLA-4, TIGIT, CISH, TGFβR2, PKA, CBLB, BAFF (BR3), and combinations thereof, and increased and/or enhanced expression of CCR2, CCR4, CCR5, CXCR2, CXCR3, CX3CR1, and combinations thereof. In some embodiments, the transient alteration of protein expression results in decreased and/or reduced expression of a molecule selected from the group consisting of PD-1, LAG3, TIM3, CISH, CBLB, and combinations thereof, and increased and/or enhanced expression of CCR2, CCR4, CCR5, CXCR2, CXCR3, CX3CR1, and combinations thereof.

[001202] In some embodiments, there is a reduction in expression of about 5%, about 10%, about 10%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%. In some embodiments, there is a reduction in expression of at least about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%. In some embodiments, there is a reduction in expression of at least about 75%, about 80%, about 85%, about 90%, or about 95%. In some embodiments, there is a reduction in expression of at least about 80%, about 85%, about 90%, or about 95%. In some embodiments, there is a reduction in expression of at least about 85%, about 90%, or about 95%. In some embodiments, there is a reduction in expression of at least about 80%. In

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some embodiments, there is a reduction in expression of at least about 85%, In some embodiments, there is a reduction in expression of at least about 90%. In some embodiments, there is a reduction in expression of at least about 95%. In some embodiments, there is a reduction in expression of at least about 99%.

[001203] In some embodiments, there is an increase in expression of about 5%, about 10%, about 10%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%. In some embodiments, there is an increase in expression of at least about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%. In some embodiments, there is an increase in expression of at least about 75%, about 80%, about 85%, about 90%, or about 95%. In some embodiments, there is an increase in expression of at least about 80%, about 85%, about 90%, or about 95%. In some embodiments, there is an increase in expression of at least about 85%, about 90%, or about 95%. In some embodiments, there is an increase in expression of at least about 80%. In some embodiments, there is an increase in expression of at least about 85%, In some embodiments, there is an increase in expression of at least about 90%. In some embodiments, there is an increase in expression of at least about 95%. In some embodiments, there is an increase in expression of at least about 99%.

[001204] In some embodiments, transient alteration of protein expression is induced by treatment of the TILs with transcription factors (TFs) and/or other molecules capable of transiently altering protein expression in the TILs. In some embodiments, the SQZ vector-free microfluidic platform is employed for intracellular delivery of the transcription factors (TFs) and/or other molecules capable of transiently altering protein expression. Such methods demonstrating the ability to deliver proteins, including transcription factors, to a variety of primary human cells, including T cells, have been described in U.S. Patent Application Publication Nos. US 2019/0093073 A1, US 2018/0201889 A1, and US 2019/0017072 A1, the disclosures of each of which are incorporated by reference herein. Such methods can be employed with the present invention in order to expose a population of TILs to transcription factors (TFs) and/or other molecules capable of inducing transient protein expression, wherein said TFs and/or other molecules capable of inducing transient protein expression provide for increased expression of tumor antigens and/or an increase in the number of tumor antigen-specific T cells in the population of TILs, thus resulting in reprogramming of the TIL population and an increase in therapeutic efficacy of the

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reprogrammed TIL population as compared to a non-reprogrammed TIL population. In some embodiments, the reprogramming results in an increased subpopulation of effector T cells and/or central memory T cells relative to the starting or prior population (*i.e.*, prior to reprogramming) population of TILs, as described herein.

[001205] In some embodiments, the transcription factor (TF) includes but is not limited to TCF-1, NOTCH 1/2 ICD, and/or MYB. In some embodiments, the transcription factor (TF) is TCF-1. In some embodiments, the transcription factor (TF) is NOTCH 1/2 ICD. In some embodiments, the transcription factor (TF) is MYB. In some embodiments, the transcription factor (TF) is administered with induced pluripotent stem cell culture (iPSC), such as the commercially available KNOCKOUT Serum Replacement (Gibco/ThermoFisher), to induce additional TIL reprogramming. In some embodiments, the transcription factor (TF) is administered with an iPSC cocktail to induce additional TIL reprogramming. In some embodiments, the transcription factor (TF) is administered without an iPSC cocktail. In some embodiments, reprogramming results in an increase in the percentage of TSCMs. In some embodiments, reprogramming results in an increase in the percentage of TSCMs by about 5%, about 10%, about 10%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% TSCMs.

[001206] In some embodiments, a method of transient altering protein expression, as described above, may be combined with a method of genetically modifying a population of TILs includes the step of stable incorporation of genes for production of one or more proteins. In certain embodiments, the method comprises a step of genetically modifying a population of TILs. In certain embodiments, the method comprises genetically modifying the first population of TILs, the second population of TILs and/or the third population of TILs. In some embodiments, a method of genetically modifying a population of TILs includes the step of retroviral transduction. In some embodiments, a method of genetically modifying a population of TILs includes the step of lentiviral transduction. Lentiviral transduction systems are known in the art and are described, *e.g.*, in Levine, *et al.*, *Proc. Nat'l Acad. Sci.* **2006,** *103,* 17372-77; Zufferey, *et al., Nat. Biotechnol.* **1997,** *15,* 871-75; Dull, *et al., J. Virology* **1998,** *72,* 8463-71, and U.S. Patent No. 6,627,442, the disclosures of each of which are incorporated by reference herein. In some embodiments, a method of genetically modifying a population of TILs includes the step of gamma-retroviral transduction. Gamma-retroviral transduction systems are known in the art and are described, *e.g.*, Cepko and Pear,

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Cur. Prot. Mol. Biol. **1996,** 9.9.1-9.9.16, the disclosure of which is incorporated by reference herein. In some embodiments, a method of genetically modifying a population of TILs includes the step of transposon-mediated gene transfer. Transposon-mediated gene transfer systems are known in the art and include systems wherein the transposase is provided as DNA expression vector or as an expressible RNA or a protein such that long-term expression of the transposase does not occur in the transgenic cells, for example, a transposase provided as an mRNA (*e.g.*, an mRNA comprising a cap and poly-A tail). Suitable transposon-mediated gene transfer systems, including the salmonid-type Tel-like transposase (SB or Sleeping Beauty transposase), such as SB10, SB11, and SB100x, and engineered enzymes with increased enzymatic activity, are described in, *e.g.*, Hackett, *et al.*, *Mol. Therapy* **2010,** *18,* 674-83 and U.S. Patent No. 6,489,458, the disclosures of each of which are incorporated by reference herein.

[001207] In some embodiments, transient alteration of protein expression in TILs is induced by small interfering RNA (siRNA), sometimes known as short interfering RNA or silencing RNA, which is a double stranded RNA molecule, generally 19-25 base pairs in length. siRNA is used in RNA interference (RNAi), where it interferes with expression of specific genes with complementary nucleotide sequences. siRNA may be used to transiently knockdown genes in TILs also modified to CCRs according to the present invention.

[001208] In some embodiments, transient alteration of protein expression is induced by self-delivering RNA interference (sdRNA), which is a chemically-synthesized asymmetric siRNA duplex with a high percentage of 2'-OH substitutions (typically fluorine or -OCH_3) which comprises a 20-nucleotide antisense (guide) strand and a 13 to 15 base sense (passenger) strand conjugated to cholesterol at its 3' end using a tetraethylenglycol (TEG) linker. Small interfering RNA (siRNA), sometimes known as short interfering RNA or silencing RNA, is a double stranded RNA molecule, generally 19-25 base pairs in length. siRNA is used in RNA interference (RNAi), where it interferes with expression of specific genes with complementary nucleotide sequences. sdRNA are covalently and hydrophobically modified RNAi compounds that do not require a delivery vehicle to enter cells. sdRNAs are generally asymmetric chemically modified nucleic acid molecules with minimal double stranded regions. sdRNA molecules typically contain single stranded regions and double stranded regions, and can contain a variety of chemical modifications within both the single stranded and double stranded regions of the molecule. Additionally, the sdRNA molecules can be attached to a hydrophobic conjugate such as a conventional and advanced sterol-type

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molecule, as described herein. sdRNAs and associated methods for making such sdRNAs have also been described extensively in, for example, U.S. Patent Application Publication Nos. US 2016/0304873 A1, US 2019/0211337 A1, US 2009/0131360 A1, and US 2019/0048341 A1, and U.S. Patent Nos. 10,633,654 and 10,913,948B2, the disclosures of each of which are incorporated by reference herein. To optimize sdRNA structure, chemistry, targeting position, sequence preferences, and the like, an algorithm has been developed and utilized for sdRNA potency prediction. Based on these analyses, functional sdRNA sequences have been generally defined as having over 70% reduction in expression at 1 μM concentration, with a probability over 40%.

[001209] Double stranded DNA (dsRNA) can be generally used to define any molecule comprising a pair of complementary strands of RNA, generally a sense (passenger) and antisense (guide) strands, and may include single-stranded overhang regions. The term dsRNA, contrasted with siRNA, generally refers to a precursor molecule that includes the sequence of an siRNA molecule which is released from the larger dsRNA molecule by the action of cleavage enzyme systems, including Dicer.

[001210] In some embodiments, the method comprises transient alteration of protein expression in a population of TILs, comprising the use of siRNA or sdRNA. Methods of using sdRNA have been described in Khvorova and Watts, *Nat. Biotechnol.* **2017,** *35,* 238–248; Byrne, *et al., J. Ocul. Pharmacol. Ther.* **2013,** *29,* 855-864; and Ligtenberg, *et al., Mol. Therapy,* **2018,** in press, the disclosures of which are incorporated by reference herein. In an embodiment, delivery of siRNA is accomplished using electroporation or cell membrane disruption (such as the squeeze or SQZ method). In some embodiments, delivery of sdRNA to a TIL population is accomplished without use of electroporation, SQZ, or other methods, instead using a 1 to 3 day period in which a TIL population is exposed to sdRNA at a concentration of 1 μM/10,000 TILs in medium. In certain embodiments, the method comprises delivery of siRNA or sdRNA to a TILs population comprising exposing the TILs population to siRNA or sdRNA at a concentration of 1 μM/10,000 TILs in medium for a period of between 1 to 3 days. In some embodiments, delivery of siRNA or sdRNA to a TIL population is accomplished using a 1 to 3 day period in which a TIL population is exposed to siRNA or sdRNA at a concentration of 10 μM/10,000 TILs in medium. In some embodiments, delivery of siRNA or sdRNA to a TIL population is accomplished using a 1 to 3 day period in which a TIL population is exposed to siRNA or sdRNA at a concentration of 50 μM/10,000 TILs in medium. In some embodiments, delivery of siRNA or sdRNA to a

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TIL population is accomplished using a 1 to 3 day period in which a TIL population is exposed to sdRNA at a concentration of between 0.1 μM/10,000 TILs and 50 μM/10,000 TILs in medium. In some embodiments, delivery of siRNA or sdRNA to a TIL population is accomplished using a 1 to 3 day period in which a TIL population is exposed to siRNA or sdRNA at a concentration of between 0.1 μM/10,000 TILs and 50 μM/10,000 TILs in medium, wherein the exposure to siRNA or sdRNA is performed two, three, four, or five times by addition of fresh siRNA or sdRNA to the media. Other suitable processes are described, for example, in U.S. Patent Application Publication No. US 2011/0039914 A1, US 2013/0131141 A1, and US 2013/0131142 A1, and U.S. Patent No. 9,080,171, the disclosures of which are incorporated by reference herein.

[001211] In some embodiments, siRNA or sdRNA is inserted into a population of TILs during manufacturing. In some embodiments, the siRNA or sdRNA encodes RNA that interferes with NOTCH 1/2 ICD, PD-1, CTLA-4 TIM-3, LAG-3, TIGIT, TGFβ, TGFBR2, cAMP protein kinase A (PKA), BAFF BR3, CISH, and/or CBLB. In some embodiments, the reduction in expression is determined based on a percentage of gene silencing, for example, as assessed by flow cytometry and/or qPCR. In some embodiments, there is a reduction in expression of about 5%, about 10%, about 10%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%. In some embodiments, there is a reduction in expression of at least about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%. In some embodiments, there is a reduction in expression of at least about 75%, about 80%, about 85%, about 90%, or about 95%. In some embodiments, there is a reduction in expression of at least about 80%, about 85%, about 90%, or about 95%. In some embodiments, there is a reduction in expression of at least about 85%, about 90%, or about 95%. In some embodiments, there is a reduction in expression of at least about 80%. In some embodiments, there is a reduction in expression of at least about 85%, In some embodiments, there is a reduction in expression of at least about 90%. In some embodiments, there is a reduction in expression of at least about 95%. In some embodiments, there is a reduction in expression of at least about 99%.

[001212] The self-deliverable RNAi technology based on the chemical modification of siRNAs or sdRNAs can be employed with the methods of the present invention to successfully deliver the siRNA or sdRNAs to the TILs as described herein. The combination of backbone modifications with asymmetric siRNA or sdRNA structure and a hydrophobic

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ligand (*see*, for eample, Ligtenberg, *et al.*, *Mol. Therapy,* **2018** and US20160304873) allow sdRNAs or sd RNAs to penetrate cultured mammalian cells without additional formulations and methods by simple addition to the culture media, capitalizing on the nuclease stability of siRNA or sdRNAs. This stability allows the support of constant levels of RNAi-mediated reduction of target gene activity simply by maintaining the active concentration of siRNA or sdRNA in the media. While not being bound by theory, the backbone stabilization of siRNA or sdRNA provides for extended reduction in gene expression effects which can last for months in non-dividing cells.

[001213] In some embodiments, over 95% transfection efficiency of TILs and a reduction in expression of the target by various specific siRNA or sdRNA occurs. In some embodiments, siRNA or sdRNAs containing several unmodified ribose residues were replaced with fully modified sequences to increase potency and/or the longevity of RNAi effect. In some embodiments, a reduction in expression effect is maintained for 12 hours, 24 hours, 36 hours, 48 hours, 5 days, 6 days, 7 dyas, or 8 days or more. In some embodiments, the reduction in expression effect decreases at 10 days or more post siRNA or sdRNA treatment of the TILs. In some embodiments, more than 70% reduction in expression of the target expression is maintained. In some embodiments, more than 70% reduction in expression of the target expression is maintained TILs. In some embodiments, a reduction in expression in the PD-1/PD-L1 pathway allows for the TILs to exhibit a more potent in vivo effect, which is in some embodiments, due to the avoidance of the suppressive effects of the PD-1/PD-L1 pathway. In some embodiments, a reduction in expression of PD-1 by siRNA or sdRNA results in an increase TIL proliferation.

[001214] In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a 70% reduction in expression of the target gene. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a 75% reduction in expression of the target gene. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit an 80% reduction in expression of the target gene. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit an 85% reduction in expression of the target gene. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a 90% reduction in expression of the target gene. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a 95% reduction in expression of the target gene. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a 99% reduction in expression of the target gene. In some embodiments, the siRNA or sdRNA

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sequences used in the invention exhibit a reduction in expression of the target gene when delivered at a concentration of about 0.25 μM to about 4 μM. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a reduction in expression of the target gene when delivered at a concentration of about 0.25 μM. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a reduction in expression of the target gene when delivered at a concentration of about 0.5 μM. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a reduction in expression of the target gene when delivered at a concentration of about 0.75 μM. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a reduction in expression of the target gene when delivered at a concentration of about 1.0 μM. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a reduction in expression of the target gene when delivered at a concentration of about 1.25 μM. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a reduction in expression of the target gene when delivered at a concentration of about 1.5 μM. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a reduction in expression of the target gene when delivered at a concentration of about 1.75 μM. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a reduction in expression of the target gene when delivered at a concentration of about 2.0 μM. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a reduction in expression of the target gene when delivered at a concentration of about 2.25 μM. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a reduction in expression of the target gene when delivered at a concentration of about 2.5 μM. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a reduction in expression of the target gene when delivered at a concentration of about 2.75 μM. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a reduction in expression of the target gene when delivered at a concentration of about 3.0 μM. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a reduction in expression of the target gene when delivered at a concentration of about 3.25 μM. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a reduction in expression of the target gene when delivered at a concentration of about 3.5 μM. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a reduction in expression of the target gene when delivered at a concentration of about 3.75 μM. In some embodiments, the siRNA or sdRNA sequences used in the invention exhibit a reduction in expression of the target gene when delivered at a concentration of about 4.0 μM.

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[001215] In some emodiments, the siRNA or sdRNA oligonucleotide agents comprise one or more modification to increase stability and/or effectiveness of the therapeutic agent, and to effect efficient delivery of the oligonucleotide to the cells or tissue to be treated. Such modifications can include a 2'-O-methyl modification, a 2'-O-Fluro modification, a diphosphorothioate modification, 2' F modified nucleotide, a2'-O-methyl modified and/or a 2'deoxy nucleotide. In some embodiments, the oligonucleotide is modified to include one or more hydrophobic modifications including, for example, sterol, cholesterol, vitamin D, naphtyl, isobutyl, benzyl, indol, tryptophane, and/or phenyl. In an additional particular embodiment, chemically modified nucleotides are combination of phosphorothioates, 2'-O-methyl, 2'deoxy, hydrophobic modifications and phosphorothioates. In some embodiments, the sugars can be modified and modified sugars can include but are not limited to D-ribose, 2'-O-alkyl (including 2'-O-methyl and 2'-0-ethyl), *i.e.*, 2'-alkoxy, 2'-amino, 2'-S-alkyl, 2'-halo (including 2'-fluoro), T- methoxyethoxy, 2'-allyloxy (-$OCH_2CH{=}CH_2$), 2'-propargyl, 2'-propyl, ethynyl, ethenyl, propenyl, and cyano and the like. In one embodiment, the sugar moiety can be a hexose and incorporated into an oligonucleotide as described in Augustyns, et al., Nucl. Acids. Res. 18:4711 (1992), the disclosure of which is incorporated by reference herein.

[001216] In some embodiments, the double-stranded siRNA or sdRNA oligonucleotide of the invention is double-stranded over its entire length, *i.e.*, with no overhanging single-stranded sequence at either end of the molecule, *i.e.*, is blunt-ended. In some embodiments, the individual nucleic acid molecules can be of different lengths. In other words, a double-stranded oligonucleotide of the invention is not double-stranded over its entire length. For instance, when two separate nucleic acid molecules are used, one of the molecules, *e.g.*, the first molecule comprising an antisense sequence, can be longer than the second molecule hybridizing thereto (leaving a portion of the molecule single-stranded). In some embodiments, when a single nucleic acid molecule is used a portion of the molecule at either end can remain single-stranded.

[001217] In some embodiments, a double-stranded siRNA or sdRNA oligonucleotide of the invention contains mismatches and/or loops or bulges, but is double-stranded over at least about 70% of the length of the oligonucleotide. In some embodiments, a double-stranded siRNA or sdRNA oligonucleotide of the invention is double-stranded over at least about 80% of the length of the oligonucleotide. In other embodiments, a double-stranded oligonucleotide of the invention is double-stranded over at least about 90%-95% of the length of the

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oligonucleotide. In some embodiments, a double-stranded siRNA or sdRNA oligonucleotide of the invention is double-stranded over at least about 96%-98% of the length of the oligonucleotide. In some embodiments, the double-stranded siRNA or sdRNA oligonucleotide of the invention contains at least or up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 mismatches.

[001218] In some embodiments, the siRNA or sdRNA oligonucleotide can be substantially protected from nucleases *e.g.*, by modifying the 3' or 5' linkages (*e.g.*, U.S. Pat. No. 5,849,902 and WO 98/13526). For example, oligonucleotides can be made resistant by the inclusion of a "blocking group." The term "blocking group" as used herein refers to substituents (*e.g.*, other than OH groups) that can be attached to oligonucleotides or nucleomonomers, either as protecting groups or coupling groups for synthesis (*e.g.*, FITC, propyl (CH_2-CH_2-CH_3), glycol (-0-CH_2-CH_2-O-) phosphate ($PO_3^{2"}$), hydrogen phosphonate, or phosphoramidite). "Blocking groups" can also include "end blocking groups" or "exonuclease blocking groups" which protect the 5' and 3' termini of the oligonucleotide, including modified nucleotides and non-nucleotide exonuclease resistant structures.

[001219] In some embodiments, at least a portion of the contiguous polynucleotides within the siRNA or sdRNA are linked by a substitute linkage, *e.g.*, a phosphorothioate linkage.

[001220] In some embodiments, chemical modification can lead to at least a 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 enhancements in cellular uptake of an siRNA or sdRNA. In some embodiments, at least one of the C or U residues includes a hydrophobic modification. In some embodiments, a plurality of Cs and Us contain a hydrophobic modification. In some embodiments, at least 10%, 15%, 20%, 30%, 40%, 50%, 55%, 60% 65%, 70%, 75%, 80%, 85%, 90% or at least 95% of the Cs and Us can contain a hydrophobic modification. In some embodiments, all of the Cs and Us contain a hydrophobic modification.

[001221] In some embodiments, the siRNA or sdRNA molecules exhibit enhanced endosomal release through the incorporation of protonatable amines. In some embodiments, protonatable amines are incorporated in the sense strand (in the part of the molecule which is discarded after RISC loading). In some embodiments, the siRNA or sdRNA compounds of the invention comprise an asymmetric compound comprising a duplex region (required for

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efficient RISC entry of 10-15 bases long) and single stranded region of 4-12 nucleotides long; with a 13 nucleotide duplex. In some embodiments, a 6 nucleotide single stranded region is employed. In some embodiments, the single stranded region of the siRNA or sdRNA comprises 2-12 phosphorothioate intemucleotide linkages (referred to as phosphorothioate modifications). In some embodiments, 6-8 phosphorothioate intemucleotide linkages are employed. In some embodiments, the siRNA or sdRNA compounds of the invention also include a unique chemical modification pattern, which provides stability and is compatible with RISC entry. The guide strand, for example, may also be modified by any chemical modification which confirms stability without interfering with RISC entry. In some embodiments, the chemical modification pattern in the guide strand includes the majority of C and U nucleotides being 2' F modified and the 5 ' end being phosphorylated.

[001222] In some embodiments, at least 30% of the nucleotides in the siRNA or sdRNA are modified. In some embodiments, at least 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% of the nucleotides in the siRNA or sdRNA are modified. In some embodiments, 100% of the nucleotides in the siRNA or sdRNA are modified.

[001223] In some embodiments, the siRNA or sdRNA molecules have minimal double stranded regions. In some embodiments the region of the molecule that is double stranded ranges from 8-15 nucleotides long. In some embodiments, the region of the molecule that is double stranded is 8, 9, 10, 11, 12, 13, 14 or 15 nucleotides long. In some embodiments the double stranded region is 13 nucleotides long. There can be 100% complementarity between the guide and passenger strands, or there may be one or more mismatches between the guide and passenger strands. In some embodiments, on one end of the double stranded molecule, the molecule is either blunt-ended or has a one-nucleotide overhang. The single stranded region of the molecule is in some embodiments between 4-12 nucleotides long. In some embodiments, the single stranded region can be 4, 5, 6, 7, 8, 9, 10, 11 or 12 nucleotides long. In some embodiments, the single stranded region can also be less than 4 or greater than 12 nucleotides long. In certain embodiments, the single stranded region is 6 or 7 nucleotides long.

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[001224] In some embodiments, the siRNA or sdRNA molecules have increased stability. In some instances, a chemically modified siRNA or sdRNA molecule has a half-life in media that is longer than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more than 24 hours, including any intermediate values. In some embodiments, the sd-rxRNA has a half-life in media that is longer than 12 hours.

[001225] In some embodiments, the siRNA or sdRNA is optimized for increased potency and/or reduced toxicity. In some embodiments, nucleotide length of the guide and/or passenger strand, and/or the number of phosphorothioate modifications in the guide and/or passenger strand, can in some aspects influence potency of the RNA molecule, while replacing 2'-fluoro (2'F) modifications with 2'-0-methyl (2'OMe) modifications can in some aspects influence toxicity of the molecule. In some embodiments, reduction in 2'F content of a molecule is predicted to reduce toxicity of the molecule. In some embodiments, the number of phosphorothioate modifications in an RNA molecule can influence the uptake of the molecule into a cell, for example the efficiency of passive uptake of the molecule into a cell. In some embodiments, the sdRNA has no 2'F modification and yet are characterized by equal efficacy in cellular uptake and tissue penetration.

[001226] In some embodiments, a guide strand is approximately 18-19 nucleotides in length and has approximately 2-14 phosphate modifications. For example, a guide strand can contain 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or more than 14 nucleotides that are phosphate-modified. The guide strand may contain one or more modifications that confer increased stability without interfering with RISC entry. The phosphate modified nucleotides, such as phosphorothioate modified nucleotides, can be at the 3' end, 5' end or spread throughout the guide strand. In some embodiments, the 3' terminal 10 nucleotides of the guide strand contain 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 phosphorothioate modified nucleotides. The guide strand can also contain 2'F and/or 2'OMe modifications, which can be located throughout the molecule. In some embodiments, the nucleotide in position one of the guide strand (the nucleotide in the most 5' position of the guide strand) is 2'OMe modified and/or phosphorylated. C and U nucleotides within the guide strand can be 2'F modified. For example, C and U nucleotides in positions 2-10 of a 19 nt guide strand (or corresponding positions in a guide strand of a different length) can be 2'F modified. C and U nucleotides within the guide strand can also be 2'OMe modified. For example, C and U nucleotides in positions 11-18 of a 19 nt guide strand (or corresponding positions in a guide strand of a different length) can be 2'OMe modified. In some embodiments, the nucleotide at the most 3' end of the guide strand is unmodified. In

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certain embodiments, the majority of Cs and Us within the guide strand are 2'F modified and the 5' end of the guide strand is phosphorylated. In other embodiments, position 1 and the Cs or Us in positions 11-18 are 2'OMe modified and the 5' end of the guide strand is phosphorylated. In other embodiments, position 1 and the Cs or Us in positions 11-18 are 2'OMe modified, the 5' end of the guide strand is phosphorylated, and the Cs or Us in position 2-10 are 2'F modified.

[001227] The self-deliverable RNAi technology provides a method of directly transfecting cells with the RNAi agent (whether siRNA, sdRNA, or other RNAi agents), without the need for additional formulations or techniques. The ability to transfect hard-to-transfect cell lines, high *in vivo* activity, and simplicity of use, are characteristics of the compositions and methods that present significant functional advantages over traditional siRNA-based techniques, and as such, the sdRNA methods are employed in several embodiments related to the methods of reduction in expression of the target gene in the TILs of the present invention. The sdRNAi methods allows direct delivery of chemically synthesized compounds to a wide range of primary cells and tissues, both *ex-vivo* and *in vivo*. The sdRNAs described in some embodiments of the invention herein are commercially available from Advirna LLC, Worcester, MA, USA.

[001228] The siRNA and sdRNA are formed as hydrophobically-modified siRNA-antisense oligonucleotide hybrid structures, and are disclosed, for example in Byrne et al., December 2013, J. Ocular Pharmacology and Therapeutics, 29(10): 855-864, the disclosure of which is incorporated by reference herein.

[001229] In some embodiments, the siRNA or sdRNA oligonucleotides can be delivered to the TILs described herein using sterile electroporation. In certain embodiments, the method comprises sterile electroporation of a population of TILs to deliver siRNA or sdRNA oligonucleotides.

[001230] In some embodiments, the oligonucleotides can be delivered to the cells in combination with a transmembrane delivery system. In some embodimets, this transmembrane delivery system comprises lipids, viral vectors, and the like. In some embodiments, the oligonucleotide agent is a self-delivery RNAi agent, that does not require any delivery agents. In certain embodiments, the method comprises use of a transmembrane delivery system to deliver siRNA or sdRNA oligonucleotides to a population of TILs.

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[001231] Oligonucleotides and oligonucleotide compositions are contacted with (*e.g.*, brought into contact with, also referred to herein as administered or delivered to) and taken up by TILs described herein, including through passive uptake by TILs. The siRNA or sdRNA can be added to the TILs as described herein during the first expansion, for example Step B, after the first expansion, for example, during Step C, before or during the second expansion, for example before or during Step D, after Step D and before harvest in Step E, during or after harvest in Step F, before or during final formulation and/or transfer to infusion Bag in Step F, as well as before any optional cryopreservation step in Step F. Mroeover, siRNA or sdRNA can be added after thawing from any cryopreservation step in Step F. In some embodiments, one or more siRNA or sdRNAs targeting genes as described herein, including PD-1, LAG-3, TIM-3, CISH, and CBLB, may be added to cell culture media comprising TILs and other agents at concentrations selected from the group consisting of 100 nM to 20 mM, 200 nM to 10 mM, 500 nm to 1 mM, 1 μM to 100 μM, and 1 μM to 100 μM. In some embodiments, one or more siRNA or sdRNAs targeting genes as described herein, including PD-1, LAG-3, TIM-3, CISH, and CBLB, may be added to cell culture media comprising TILs and other agents at amounts selected from the group consisting of 0.1 μM siRNA or sdRNA/10,000 TILs/100 μL media, 0.5 μM siRNA or sdRNA/10,000 TILs /100 μL media, 0.75 μM siRNA or sdRNA/10,000 TILs /100 μL media, 1 μM siRNA or sdRNA/10,000 TILs /100 μL media, 1.25 μM siRNA or sdRNA/10,000 TILs /100 μL media, 1.5 μM siRNA or sdRNA/10,000 TILs /100 μL media, 2 μM siRNA or sdRNA/10,000 TILs /100 μL media, 5 μM siRNA or sdRNA/10,000 TILs /100 μL media, or 10 μM siRNA or sdRNA/10,000 TILs /100 μL media. In some embodiments, one or more sdRNAs targeting genes as described herein, including PD-1, LAG-3, TIM-3, CISH, and CBLB, may be added to TIL cultures during the pre-REP or REP stages twice a day, once a day, every two days, every three days, every four days, every five days, every six days, or every seven days.

[001232] Oligonucleotide compositions of the invention, including siRNA or sdRNA, can be contacted with TILs as described herein during the expansion process, for example by dissolving siRNA or sdRNA at high concentrations in cell culture media and allowing sufficient time for passive uptake to occur. In certain embodiments, the method of the present invention comprises contacting a population of TILs with an oligonucleotide composition as described herein. In certain embodiments, the method comprises dissolving an oligonucleotide *e.g.* siRNA or sdRNA in a cell culture media and contacting the cell

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culture media with a population of TILs. The TILs may be a first population, a second population and/or a third population as described herein.

[001233] In some embodiments, delivery of siRNA or sdRNA oligonucleotides into cells can be enhanced by suitable art recognized methods including calcium phosphate, DMSO, glycerol or dextran, electroporation, or by transfection, *e.g.*, using cationic, anionic, or neutral lipid compositions or liposomes using methods known in the art (*see*, *e.g.*, WO 90/14074; WO 91/16024; WO 91/17424; U.S. Pat. No. 4,897,355; Bergan et a 1993. Nucleic Acids Research. 21 :3567).

[001234] In some embodiments, more than one siRNA or sdRNA is used to reduce expression of a target gene. In some embodiments, one or more of PD-1, TIM-3, CBLB, LAG3 and/or CISH targeting siRNAs or sdRNAs are used together. In some embodiments, a PD-1 siRNA or sdRNA is used with one or more of TIM-3, CBLB, LAG3 and/or CISH in order to reduce expression of more than one gene target. In some embodiments, a LAG3 siRNA or sdRNA is used in combination with a CISH targeting siRNA or sdRNA to reduce gene expression of both targets. In some embodiments, the siRNAs or sdRNAs targeting one or more of PD-1, TIM-3, CBLB, LAG3 and/or CISH herein are commercially available from Advirna LLC, Worcester, MA, USA.

[001235] In some embodiments, the siRNA or sdRNA targets a gene selected from the group consisting of PD-1, LAG3, TIM3, CTLA-4, TIGIT, CISH, TGFβR2, PKA, CBLB, BAFF (BR3), and combinations thereof. In some embodiments, the siRNA or sdRNA targets a gene selected from the group consisting of PD-1, LAG3, TIM3, CTLA-4, TIGIT, CISH, TGFβR2, PKA, CBLB, BAFF (BR3), and combinations thereof. In some embodiments, one siRNA or sdRNA targets PD-1 and another siRNA or sdRNA targets a gene selected from the group consisting of LAG3, TIM3, CTLA-4, TIGIT, CISH, TGFβR2, PKA, CBLB, BAFF (BR3), and combinations thereof. In some embodiments, the siRNA or sdRNA targets a gene selected from PD-1, LAG-3, CISH, CBLB, TIM3, and combinations thereof. In some embodiments, the siRNA or sdRNA targets a gene selected from PD-1 and one of LAG3, CISH, CBLB, TIM3, and combinations thereof. In some embodiments, one siRNA or sdRNA targets PD-1 and one siRNA or sdRNA targets LAG3. In some embodiments, one siRNA or sdRNA targets PD-1 and one siRNA or sdRNA targets CISH. In some embodiments, one siRNA or sdRNA targets PD-1 and one siRNA or sdRNA targets CBLB. In some embodiments, one siRNA or sdRNA targets LAG3 and one siRNA or sdRNA targets CISH. In some embodiments, one siRNA or sdRNA targets LAG3 and one siRNA or

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sdRNA targets CBLB. In some embodiments, one siRNA or sdRNA targets CISH and one siRNA or sdRNA targets CBLB. In some embodiments, one siRNA or sdRNA targets TIM3 and one siRNA or sdRNA targets PD-1. In some embodiments, one siRNA or sdRNA targets TIM3 and one siRNA or sdRNA targets LAG3. In some embodiments, one siRNA or sdRNA targets TIM3 and one siRNA or sdRNA targets CISH. In some embodiments, one siRNA or sdRNA targets TIM3 and one siRNA or sdRNA targets CBLB.

[001236] As discussed above, embodiments of the present invention provide tumor infiltrating lymphocytes (TILs) that have been genetically modified via gene-editing to enhance their therapeutic effect. Embodiments of the present invention embrace genetic editing through nucleotide insertion (RNA or DNA) into a population of TILs for both promotion of the expression of one or more proteins and inhibition of the expression of one or more proteins, as well as combinations thereof. Embodiments of the present invention also provide methods for expanding TILs into a therapeutic population, wherein the methods comprise gene-editing the TILs. There are several gene-editing technologies that may be used to genetically modify a population of TILs, which are suitable for use in accordance with the present invention.

[001237] In some embodiments, the method comprises a method of genetically modifying a population of TILs *e.g.* a first population, a second population and/or a third population as described herein. In some embodiments, a method of genetically modifying a population of TILs includes the step of stable incorporation of genes for production or inhibition (*e.g.*, silencing) of one ore more proteins. In some embodiments, a method of genetically modifying a population of TILs includes the step of electroporation. Electroporation methods are known in the art and are described, *e.g.*, in Tsong, *Biophys. J.* **1991,** *60,* 297-306, and U.S. Patent Application Publication No. 2014/0227237 A1, the disclosures of each of which are incorporated by reference herein. Other electroporation methods known in the art, such as those described in U.S. Patent Nos. 5,019,034; 5,128,257; 5,137,817; 5,173,158; 5,232,856; 5,273,525; 5,304,120; 5,318,514; 6,010,613 and 6,078,490, the disclosures of which are incorporated by reference herein, may be used. In some embodiments, the electroporation method is a sterile electroporation method. In some embodiments, the electroporation method is a pulsed electroporation method. In some embodiments, the electroporation method is a pulsed electroporation method comprising the steps of treating TILs with pulsed electrical fields to alter, manipulate, or cause defined and controlled, permanent or temporary changes in the TILs, comprising the step of applying a sequence of

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at least three single, operator-controlled, independently programmed, DC electrical pulses, having field strengths equal to or greater than 100 V/cm, to the TILs, wherein the sequence of at least three DC electrical pulses has one, two, or three of the following characteristics: (1) at least two of the at least three pulses differ from each other in pulse amplitude; (2) at least two of the at least three pulses differ from each other in pulse width; and (3) a first pulse interval for a first set of two of the at least three pulses is different from a second pulse interval for a second set of two of the at least three pulses. In some embodiments, the electroporation method is a pulsed electroporation method comprising the steps of treating TILs with pulsed electrical fields to alter, manipulate, or cause defined and controlled, permanent or temporary changes in the TILs, comprising the step of applying a sequence of at least three single, operator-controlled, independently programmed, DC electrical pulses, having field strengths equal to or greater than 100 V/cm, to the TILs, wherein at least two of the at least three pulses differ from each other in pulse amplitude. In some embodiments, the electroporation method is a pulsed electroporation method comprising the steps of treating TILs with pulsed electrical fields to alter, manipulate, or cause defined and controlled, permanent or temporary changes in the TILs, comprising the step of applying a sequence of at least three single, operator-controlled, independently programmed, DC electrical pulses, having field strengths equal to or greater than 100 V/cm, to the TILs, wherein at least two of the at least three pulses differ from each other in pulse width. In some embodiments, the electroporation method is a pulsed electroporation method comprising the steps of treating TILs with pulsed electrical fields to alter, manipulate, or cause defined and controlled, permanent or temporary changes in the TILs, comprising the step of applying a sequence of at least three single, operator-controlled, independently programmed, DC electrical pulses, having field strengths equal to or greater than 100 V/cm, to the TILs, wherein a first pulse interval for a first set of two of the at least three pulses is different from a second pulse interval for a second set of two of the at least three pulses. In some embodiments, the electroporation method is a pulsed electroporation method comprising the steps of treating TILs with pulsed electrical fields to induce pore formation in the TILs, comprising the step of applying a sequence of at least three DC electrical pulses, having field strengths equal to or greater than 100 V/cm, to TILs, wherein the sequence of at least three DC electrical pulses has one, two, or three of the following characteristics: (1) at least two of the at least three pulses differ from each other in pulse amplitude; (2) at least two of the at least three pulses differ from each other in pulse width; and (3) a first pulse interval for a first set of two of the at least three pulses is different from a second pulse interval for a second set of two of the at least three pulses, such that

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induced pores are sustained for a relatively long period of time, and such that viability of the TILs is maintained. In some embodiments, a method of genetically modifying a population of TILs includes the step of calcium phosphate transfection. Calcium phosphate transfection methods (calcium phosphate DNA precipitation, cell surface coating, and endocytosis) are known in the art and are described in Graham and van der Eb, *Virology* **1973,** *52,* 456-467; Wigler, *et al.*, *Proc. Natl. Acad. Sci.* **1979,** *76,* 1373-1376; and Chen and Okayarea, *Mol. Cell. Biol.* **1987,** *7,* 2745-2752; and in U.S. Patent No. 5,593,875, the disclosures of each of which are incorporated by reference herein. In some embodiments, a method of genetically modifying a population of TILs includes the step of liposomal transfection. Liposomal transfection methods, such as methods that employ a 1:1 (w/w) liposome formulation of the cationic lipid *N*-[1-(2,3-dioleyloxy)propyl]-*n,n,n*-trimethylammonium chloride (DOTMA) and dioleoyl phophotidylethanolamine (DOPE) in filtered water, are known in the art and are described in Rose, *et al.*, *Biotechniques* **1991,** *10,* 520-525 and Felgner, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1987,** *84,* 7413-7417 and in U.S. Patent Nos. 5,279,833; 5,908,635; 6,056,938; 6,110,490; 6,534,484; and 7,687,070, the disclosures of each of which are incorporated by reference herein. In some embodiments, a method of genetically modifying a population of TILs includes the step of transfection using methods described in U.S. Patent Nos. 5,766,902; 6,025,337; 6,410,517; 6,475,994; and 7,189,705; the disclosures of each of which are incorporated by reference herein. The TILs may be a first population, a second population and/or a third population of TILs as described herein.

[001238] According to an embodiment, the gene-editing process may comprise the use of a programmable nuclease that mediates the generation of a double-strand or single-strand break at one or more immune checkpoint genes. Such programmable nucleases enable precise genome editing by introducing breaks at specific genomic loci, *i.e.,* they rely on the recognition of a specific DNA sequence within the genome to target a nuclease domain to this location and mediate the generation of a double-strand break at the target sequence. A double-strand break in the DNA subsequently recruits endogenous repair machinery to the break site to mediate genome editing by either non-homologous end-joining (NHEJ) or homology-directed repair (HDR). Thus, the repair of the break can result in the introduction of insertion/deletion mutations that disrupt (*e.g.*, silence, repress, or enhance) the target gene product.

[001239] Major classes of nucleases that have been developed to enable site-specific genomic editing include zinc finger nucleases (ZFNs), transcription activator-like nucleases

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(TALENs), and CRISPR-associated nucleases (*e.g.*, CRISPR/Cas9). These nuclease systems can be broadly classified into two categories based on their mode of DNA recognition: ZFNs and TALENs achieve specific DNA binding via protein-DNA interactions, whereas CRISPR systems, such as Cas9, are targeted to specific DNA sequences by a short RNA guide molecule that base-pairs directly with the target DNA and by protein-DNA interactions. *See, e.g.,* Cox *et al.*, *Nature Medicine,* 2015, Vol. 21, No. 2.

[001240] Non-limiting examples of gene-editing methods that may be used in accordance with TIL expansion methods of the present invention include CRISPR methods, TALE methods, and ZFN methods, which are described in more detail below. According to an embodiment, a method for expanding TILs into a therapeutic population may be carried out in accordance with any embodiment of the methods described herein (*e.g.*, Gen 3 process) or as described in U.S. Patent Application Publication Nos. US 2020/0299644 A1 and US 2020/0121719 A1 and U.S. Patent No. 10,925,900, the disclosures of which are incorporated by reference herein, wherein the method further comprises gene-editing at least a portion of the TILs by one or more of a CRISPR method, a TALE method or a ZFN method, in order to generate TILs that can provide an enhanced therapeutic effect. According to an embodiment, gene-edited TILs can be evaluated for an improved therapeutic effect by comparing them to non-modified TILs *in vitro, e.g.,* by evaluating *in vitro* effector function, cytokine profiles, etc. compared to unmodified TILs. In certain embodiments, the method comprises gene editing a population of TILs using CRISPR, TALE and/ or ZFN methods.

[001241] In some embodiments of the present invention, electroporation is used for delivery of a gene editing system, such as CRISPR, TALEN, and ZFN systems. In some embodiments of the present invention, the electroporation system is a flow electroporation system. An example of a suitable flow electroporation system suitable for use with some embodiments of the present invention is the commercially-available MaxCyte STX system. There are several alternative commercially-available electroporation instruments which may be suitable for use with the present invention, such as the AgilePulse system or ECM 830 available from BTX-Harvard Apparatus, Cellaxess Elektra (Cellectricon), Nucleofector (Lonza/Amaxa), GenePulser MXcell (BIORAD), iPorator-96 (Primax) or siPORTer96 (Ambion). In some embodiments of the present invention, the electroporation system forms a closed, sterile system with the remainder of the TIL expansion method. In some embodiments of the present invention, the electroporation system is a pulsed electroporation

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system as described herein, and forms a closed, sterile system with the remainder of the TIL expansion method.

[001242] A method for expanding TILs into a therapeutic population may be carried out in accordance with any embodiment of the methods described herein (*e.g.*, process Gen 3) or as described in U.S. Patent Application Publication Nos. US 2020/0299644 A1 and US 2020/0121719 A1 and U.S. Patent No. 10,925,900, the disclosures of which are incorporated by reference herein, wherein the method further comprises gene-editing at least a portion of the TILs by a CRISPR method (*e.g.*, CRISPR/Cas9 or CRISPR/Cpf1). According to particular embodiments, the use of a CRISPR method during the TIL expansion process causes expression of one or more immune checkpoint genes to be silenced or reduced in at least a portion of the therapeutic population of TILs. Alternatively, the use of a CRISPR method during the TIL expansion process causes expression of one or more immune checkpoint genes to be enhanced in at least a portion of the therapeutic population of TILs.

[001243] CRISPR stands for "Clustered Regularly Interspaced Short Palindromic Repeats." A method of using a CRISPR system for gene editing is also referred to herein as a CRISPR method. There are three types of CRISPR systems which incorporate RNAs and Cas proteins, and which may be used in accordance with the present invention: Types I, II, and III. The Type II CRISPR (exemplified by Cas9) is one of the most well-characterized systems.

[001244] CRISPR technology was adapted from the natural defense mechanisms of bacteria and archaea (the domain of single-celled microorganisms). These organisms use CRISPR-derived RNA and various Cas proteins, including Cas9, to foil attacks by viruses and other foreign bodies by chopping up and destroying the DNA of a foreign invader. A CRISPR is a specialized region of DNA with two distinct characteristics: the presence of nucleotide repeats and spacers. Repeated sequences of nucleotides are distributed throughout a CRISPR region with short segments of foreign DNA (spacers) interspersed among the repeated sequences. In the type II CRISPR/Cas system, spacers are integrated within the CRISPR genomic loci and transcribed and processed into short CRISPR RNA (crRNA). These crRNAs anneal to trans-activating crRNAs (tracrRNAs) and direct sequence-specific cleavage and silencing of pathogenic DNA by Cas proteins. Target recognition by the Cas9 protein requires a "seed" sequence within the crRNA and a conserved dinucleotide-containing protospacer adjacent motif (PAM) sequence upstream of the crRNA-binding region. The CRISPR/Cas system can thereby be retargeted to cleave virtually any DNA

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sequence by redesigning the crRNA. The crRNA and tracrRNA in the native system can be simplified into a single guide RNA (sgRNA) of approximately 100 nucleotides for use in genetic engineering. The CRISPR/Cas system is directly portable to human cells by co-delivery of plasmids expressing the Cas9 endo-nuclease and the necessary crRNA components. Different variants of Cas proteins may be used to reduce targeting limitations (*e.g.*, orthologs of Cas9, such as Cpf1).

[001245] Non-limiting examples of genes that may be silenced or inhibited by permanently gene-editing TILs via a CRISPR method include PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, PKA, CBL-B, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3, TOX, SOCS1, ANKRD11, and BCOR.

[001246] Non-limiting examples of genes that may be enhanced by permanently gene-editing TILs via a CRISPR method include CCR2, CCR4, CCR5, CXCR2, CXCR3, CX3CR1, IL-2, IL12, IL-15, and IL-21.

[001247] Examples of systems, methods, and compositions for altering the expression of a target gene sequence by a CRISPR method, and which may be used in accordance with embodiments of the present invention, are described in U.S. Patent Nos. 8,697,359; 8,993,233; 8,795,965; 8,771,945; 8,889,356; 8,865,406; 8,999,641; 8,945,839; 8,932,814; 8,871,445; 8,906,616; and 8,895,308, the disclosures of each of which are incorporated by reference herein. Resources for carrying out CRISPR methods, such as plasmids for expressing CRISPR/Cas9 and CRISPR/Cpf1, are commercially available from companies such as GenScript.

[001248] In some embodiments, genetic modifications of populations of TILs, as described herein, may be performed using the CRISPR/Cpf1 system as described in U.S. Patent No. US 9790490, the disclosure of which is incorporated by reference herein.

[001249] A method for expanding TILs into a therapeutic population may be carried out in accordance with any embodiment of the methods described herein (*e.g.*, process 2A) or as described in U.S. Patent Application Publication Nos. US 2020/0299644 A1 and US 2020/0121719 A1 and U.S. Patent No. 10,925,900, the disclosures of which are incorporated

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by reference herein, wherein the method further comprises gene-editing at least a portion of the TILs by a TALE method. According to particular embodiments, the use of a TALE method during the TIL expansion process causes expression of one or more immune checkpoint genes to be silenced or reduced in at least a portion of the therapeutic population of TILs. Alternatively, the use of a TALE method during the TIL expansion process causes expression of one or more immune checkpoint genes to be enhanced in at least a portion of the therapeutic population of TILs.

[001250] TALE stands for "Transcription Activator-Like Effector" proteins, which include TALENs ("Transcription Activator-Like Effector Nucleases"). A method of using a TALE system for gene editing may also be referred to herein as a TALE method. TALEs are naturally occurring proteins from the plant pathogenic bacteria genus *Xanthomonas*, and contain DNA-binding domains composed of a series of 33–35-amino-acid repeat domains that each recognizes a single base pair. TALE specificity is determined by two hypervariable amino acids that are known as the repeat-variable di-residues (RVDs). Modular TALE repeats are linked together to recognize contiguous DNA sequences. A specific RVD in the DNA-binding domain recognizes a base in the target locus, providing a structural feature to assemble predictable DNA-binding domains. The DNA binding domains of a TALE are fused to the catalytic domain of a type IIS FokI endonuclease to make a targetable TALE nuclease. To induce site-specific mutation, two individual TALEN arms, separated by a 14-20 base pair spacer region, bring FokI monomers in close proximity to dimerize and produce a targeted double-strand break.

[001251] Several large, systematic studies utilizing various assembly methods have indicated that TALE repeats can be combined to recognize virtually any user-defined sequence. Custom-designed TALE arrays are also commercially available through Cellectis Bioresearch (Paris, France), Transposagen Biopharmaceuticals (Lexington, KY, USA), and Life Technologies (Grand Island, NY, USA). TALE and TALEN methods suitable for use in the present invention are described in U.S. Patent Application Publication Nos. US 2011/0201118 A1; US 2013/0117869 A1; US 2013/0315884 A1; US 2015/0203871 A1 and US 2016/0120906 A1, the disclosures of which are incorporated by reference herein.

[001252] Non-limiting examples of genes that may be silenced or inhibited by permanently gene-editing TILs via a TALE method include PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, PKA, CBL-B, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A,

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CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3, TOX, SOCS1, ANKRD11, and BCOR..

[001253] Non-limiting examples of genes that may be enhanced by permanently gene-editing TILs via a TALE method include CCR2, CCR4, CCR5, CXCR2, CXCR3, CX3CR1, IL-2, IL12, IL-15, and IL-21.

[001254] Examples of systems, methods, and compositions for altering the expression of a target gene sequence by a TALE method, and which may be used in accordance with embodiments of the present invention, are described in U.S. Patent No. 8,586,526, which is incorporated by reference herein.

[001255] A method for expanding TILs into a therapeutic population may be carried out in accordance with any embodiment of the methods described herein (*e.g.*, process Gen 3) or as described in U.S. Patent Application Publication Nos. US 2020/0299644 A1 and US 2020/0121719 A1 and U.S. Patent No. 10,925,900, the disclosures of each of which are incorporated by reference herein, wherein the method further comprises gene-editing at least a portion of the TILs by a zinc finger or zinc finger nuclease method. According to particular embodiments, the use of a zinc finger method during the TIL expansion process causes expression of one or more immune checkpoint genes to be silenced or reduced in at least a portion of the therapeutic population of TILs. Alternatively, the use of a zinc finger method during the TIL expansion process causes expression of one or more immune checkpoint genes to be enhanced in at least a portion of the therapeutic population of TILs.

[001256] An individual zinc finger contains approximately 30 amino acids in a conserved $\beta\beta\alpha$ configuration. Several amino acids on the surface of the α-helix typically contact 3 bp in the major groove of DNA, with varying levels of selectivity. Zinc fingers have two protein domains. The first domain is the DNA binding domain, which includes eukaryotic transcription factors and contain the zinc finger. The second domain is the nuclease domain, which includes the FokI restriction enzyme and is responsible for the catalytic cleavage of DNA.

[001257] The DNA-binding domains of individual ZFNs typically contain between three and six individual zinc finger repeats and can each recognize between 9 and 18 base pairs. If the zinc finger domains are specific for their intended target site then even a pair of 3-finger

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ZFNs that recognize a total of 18 base pairs can, in theory, target a single locus in a mammalian genome. One method to generate new zinc-finger arrays is to combine smaller zinc-finger "modules" of known specificity. The most common modular assembly process involves combining three separate zinc fingers that can each recognize a 3 base pair DNA sequence to generate a 3-finger array that can recognize a 9 base pair target site. Alternatively, selection-based approaches, such as oligomerized pool engineering (OPEN) can be used to select for new zinc-finger arrays from randomized libraries that take into consideration context-dependent interactions between neighboring fingers. Engineered zinc fingers are available commercially; Sangamo Biosciences (Richmond, CA, USA) has developed a propriety platform (CompoZr®) for zinc-finger construction in partnership with Sigma–Aldrich (St. Louis, MO, USA).

[001258] Non-limiting examples of genes that may be silenced or inhibited by permanently gene-editing TILs via a zinc finger method include PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, PKA, CBL-B, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3, TOX, SOCS1, ANKRD11, and BCOR.

[001259] Non-limiting examples of genes that may be enhanced by permanently gene-editing TILs via a zinc finger method include CCR2, CCR4, CCR5, CXCR2, CXCR3, CX3CR1, IL-2, IL12, IL-15, and IL-21.

[001260] Examples of systems, methods, and compositions for altering the expression of a target gene sequence by a zinc finger method, which may be used in accordance with embodiments of the present invention, are described in U.S. Patent Nos. 6,534,261, 6,607,882, 6,746,838, 6,794,136, 6,824,978, 6,866,997, 6,933,113, 6,979,539, 7,013,219, 7,030,215, 7,220,719, 7,241,573, 7,241,574, 7,585,849, 7,595,376, 6,903,185, and 6,479,626, which are incorporated by reference herein.

[001261] Other examples of systems, methods, and compositions for altering the expression of a target gene sequence by a zinc finger method, which may be used in accordance with embodiments of the present invention, are described in Beane, *et al.*, *Mol. Therapy*, **2015,** 23 1380-1390, the disclosure of which is incorporated by reference herein.

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[001262] In some embodiments, the TILs are optionally genetically engineered to include additional functionalities, including, but not limited to, a high-affinity T cell receptor (TCR), *e.g.*, a TCR targeted at a tumor-associated antigen such as MAGE-1, HER2, or NY-ESO-1, or a chimeric antigen receptor (CAR) which binds to a tumor-associated cell surface molecule (*e.g.*, mesothelin) or lineage-restricted cell surface molecule (*e.g.*, CD19). In certain embodiments, the method comprises genetically engineering a population of TILs to include a high-affinity T cell receptor (TCR), *e.g.*, a TCR targeted at a tumor-associated antigen such as MAGE-1, HER2, or NY-ESO-1, or a chimeric antigen receptor (CAR) which binds to a tumor-associated cell surface molecule (*e.g.*, mesothelin) or lineage-restricted cell surface molecule (*e.g.*, CD19). Aptly, the population of TILs may be a first population, a second population and/or a third population as described herein.

K. Closed Systems for TIL Manufacturing

[001263] The present invention provides for the use of closed systems during the TIL culturing process. Such closed systems allow for preventing and/or reducing microbial contamination, allow for the use of fewer flasks, and allow for cost reductions. In some embodiments, the closed system uses two containers.

[001264] Such closed systems are well-known in the art and can be found, for example, at http://www.fda.gov/cber/guidelines.htm and https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm076779.htm.

[001265] Sterile connecting devices (STCDs) produce sterile welds between two pieces of compatible tubing. This procedure permits sterile connection of a variety of containers and tube diameters. In some embodiments, the closed systems include luer lock and heat sealed systems as described in for example, Example 12. In some embodiments, the closed system is accessed via syringes under sterile conditions in order to maintain the sterility and closed nature of the system. In some embodiments, a closed system as described in Example 12 is employed. In some embodiments, the TILs are formulated into a final product formulation container according to the method described in Example 12, section "Final Formulation and Fill".

[001266] In some embodiments, the closed system uses one container from the time the tumor fragments are obtained until the TILs are ready for administration to the patient or cryopreserving. In some embodiments when two containers are used, the first container is a

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closed G-container and the population of TILs is centrifuged and transferred to an infusion bag without opening the first closed G-container. In some embodiments, when two containers are used, the infusion bag is a HypoThermosol-containing infusion bag. A closed system or closed TIL cell culture system is characterized in that once the tumor sample and/or tumor fragments have been added, the system is tightly sealed from the outside to form a closed environment free from the invasion of bacteria, fungi, and/or any other microbial contamination.

[001267] In some embodiments, the reduction in microbial contamination is between about 5% and about 100%. In some embodiments, the reduction in microbial contamination is between about 5% and about 95%. In some embodiments, the reduction in microbial contamination is between about 5% and about 90%. In some embodiments, the reduction in microbial contamination is between about 10% and about 90%. In some embodiments, the reduction in microbial contamination is between about 15% and about 85%. In some embodiments, the reduction in microbial contamination is about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 97%, about 98%, about 99%, or about 100%.

[001268] The closed system allows for TIL growth in the absence and/or with a significant reduction in microbial contamination.

[001269] Moreover, pH, carbon dioxide partial pressure and oxygen partial pressure of the TIL cell culture environment each vary as the cells are cultured. Consequently, even though a medium appropriate for cell culture is circulated, the closed environment still needs to be constantly maintained as an optimal environment for TIL proliferation. To this end, it is desirable that the physical factors of pH, carbon dioxide partial pressure and oxygen partial pressure within the culture liquid of the closed environment be monitored by means of a sensor, the signal whereof is used to control a gas exchanger installed at the inlet of the culture environment, and the that gas partial pressure of the closed environment be adjusted in real time according to changes in the culture liquid so as to optimize the cell culture environment. In some embodiments, the present invention provides a closed cell culture system which incorporates at the inlet to the closed environment a gas exchanger equipped with a monitoring device which measures the pH, carbon dioxide partial pressure and oxygen partial pressure of the closed environment, and optimizes the cell culture environment by automatically adjusting gas concentrations based on signals from the monitoring device.

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[001270] In some embodiments, the pressure within the closed environment is continuously or intermittently controlled. That is, the pressure in the closed environment can be varied by means of a pressure maintenance device for example, thus ensuring that the space is suitable for growth of TILs in a positive pressure state, or promoting exudation of fluid in a negative pressure state and thus promoting cell proliferation. By applying negative pressure intermittently, moreover, it is possible to uniformly and efficiently replace the circulating liquid in the closed environment by means of a temporary shrinkage in the volume of the closed environment.

[001271] In some embodiments, optimal culture components for proliferation of the TILs can be substituted or added, and including factors such as IL-2 and/or OKT3, as well as combination, can be added.

L. Optional Cryopreservation of TILs

[001272] Either the bulk TIL population (for example the second population of TILs) or the expanded population of TILs (for example the third population of TILs) can be optionally cryopreserved. In some embodiments, cryopreservation occurs on the therapeutic TIL population. In some embodiments, cryopreservation occurs on the TILs harvested after the second expansion. In some embodiments, cryopreservation occurs on the TILs in exemplary Step F of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, the TILs are cryopreserved in the infusion bag. In some embodiments, the TILs are cryopreserved prior to placement in an infusion bag. In some embodiments, the TILs are cryopreserved and not placed in an infusion bag. In some embodiments, cryopreservation is performed using a cryopreservation medium. In some embodiments, the cryopreservation media contains dimethylsulfoxide (DMSO). This is generally accomplished by putting the TIL population into a freezing solution, *e.g.* 85% complement inactivated AB serum and 15% dimethyl sulfoxide (DMSO). The cells in solution are placed into cryogenic vials and stored for 24 hours at -80 °C, with optional transfer to gaseous nitrogen freezers for cryopreservation. See, Sadeghi, *et al., Acta Oncologica* **2013,** *52,* 978-986.

[001273] When appropriate, the cells are removed from the freezer and thawed in a 37 °C water bath until approximately 4/5 of the solution is thawed. The cells are generally resuspended in complete media and optionally washed one or more times. In some

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embodiments, the thawed TILs can be counted and assessed for viability as is known in the art.

[001274] In some embodiments, a population of TILs is cryopreserved using CS10 cryopreservation media (CryoStor 10, BioLife Solutions). In some embodiments, a population of TILs is cryopreserved using a cryopreservation media containing dimethylsulfoxide (DMSO). In some embodiments, a population of TILs is cryopreserved using a 1:1 (vol:vol) ratio of CS10 and cell culture media. In some embodiments, a population of TILs is cryopreserved using about a 1:1 (vol:vol) ratio of CS10 and cell culture media, further comprising additional IL-2.

[001275] As discussed above, and exemplified in Steps A through E as provided in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), cryopreservation can occur at numerous points throughout the TIL expansion process. In some embodiments, the expanded population of TILs after the second expansion (as provided for example, according to Step D of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) can be cryopreserved. Cryopreservation can be generally accomplished by placing the TIL population into a freezing solution, *e.g.*, 85% complement inactivated AB serum and 15% dimethyl sulfoxide (DMSO). The cells in solution are placed into cryogenic vials and stored for 24 hours at -80 °C, with optional transfer to gaseous nitrogen freezers for cryopreservation. See Sadeghi, *et al., Acta Oncologica* **2013,** *52,* 978-986. In some embodiments, the TILs are cryopreserved in 5% DMSO. In some embodiments, the TILs are cryopreserved in cell culture media plus 5% DMSO. In some embodiments, the TILs are cryopreserved according to the methods provided in Example D.

[001276] When appropriate, the cells are removed from the freezer and thawed in a 37 °C water bath until approximately 4/5 of the solution is thawed. The cells are generally resuspended in complete media and optionally washed one or more times. In some embodiments, the thawed TILs can be counted and assessed for viability as is known in the art.

[001277] In some cases, the Step B TIL population can be cryopreserved immediately, using the protocols discussed below. Alternatively, the bulk TIL population can be subjected to Step C and Step D and then cryopreserved after Step D. Similarly, in the case where

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genetically modified TILs will be used in therapy, the Step B or Step D TIL populations can be subjected to genetic modifications for suitable treatments.

M. Phenotypic Characteristics of Expanded TILs

[001278] In some embodiment, the TILs are analyzed for expression of numerous phenotype markers after expansion, including those described herein and in the Examples. In some embodiments, expression of one or more phenotypic markers is examined. In some embodiments, the phenotypic characteristics of the TILs are analyzed after the first expansion in Step B. In some embodiments, the phenotypic characteristics of the TILs are analyzed during the transition in Step C. In some embodiments, the phenotypic characteristics of the TILs are analyzed during the transition according to Step C and after cryopreservation. In some embodiments, the phenotypic characteristics of the TILs are analyzed after the second expansion according to Step D. In some embodiments, the phenotypic characteristics of the TILs are analyzed after two or more expansions according to Step D.

[001279] In some embodiments, the marker is selected from the group consisting of CD8 and CD28. In some embodiments, expression of CD8 is examined. In some embodiments, expression of CD28 is examined. In some embodiments, the expression of CD8 and/or CD28 is higher on TILs produced according the current invention process, as compared to other processes (*e.g.*, the Gen 3 process as provided for example in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as compared to the 2A process as provided for example in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, the expression of CD8 is higher on TILs produced according the current invention process, as compared to other processes (*e.g.*, the Gen 3 process as provided for example in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as compared to the 2A process as provided for example in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, the expression of CD28 is higher on TILs produced according the current invention process, as compared to other processes (*e.g.*, the Gen 3 process as provided for example in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as compared to the 2A process as provided for example in Figure 1 (in particular, *e.g.*, Figure 1A)). In some embodiments, high CD28 expression is indicative of a younger, more presisitent TIL phenotype. In some embodiments, expression of one or more regulatory markers is measured.

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[001280] In some embodiments, no selection of the first population of TILs, second population of TILs, third population of TILs, or harvested TIL population based on CD8 and/or CD28 expression is performed during any of the steps for the method for expanding tumor infiltrating lymphocytes (TILs) described herein.

[001281] In some embodiments, the percentage of central memory cells is higher on TILs produced according the current invention process, as compared to other processes (*e.g.*, the Gen 3 process as provided for example in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), as compared to the 2A process as provided for example in Figure 1 (in particular, *e.g.*, Figure 1A)). In some embodiments, the memory marker for central memory cells is selected from the group consisting of CCR7 and CD62L.

[001282] In some embodiments, the CD4+ and/or CD8+ TIL Memory subsets can be divided into different memory subsets. In some embodiments, the CD4+ and/or CD8+ TILs comprise the naïve (CD45RA+CD62L+) TILS. In some embodiments, the CD4+ and/or CD8+ TILs comprise the central memory (CM; CD45RA-CD62L+) TILs. In some embodiments, the CD4+ and/or CD8+ TILs comprise the effector memory (EM; CD45RA-CD62L-) TILs. In some embodiments, the CD4+ and/or CD8+ TILs comprise the, RA+ effector memory/effector (TEMRA/TEFF; CD45RA+CD62L+) TILs. In some embodiments, there is a higher % of CD8+ as compared to CD4+ population. In some embodiments, the TILs express one more markers selected from the group consisting of granzyme B, perforin, and granulysin. In some embodiments, the TILs express granzyme B. In some embodiments, the TILs express perforin. In some embodiments, the TILs express granulysin.

[001283] In some embodiments, restimulated TILs can also be evaluated for cytokine release, using cytokine release assays. In some embodiments, TILs can be evaluated for interferon-γ (IFN-γ) secretion. In some embodiments, the IFN-γ secretion is measured by an ELISA assay. In some embodiments, the IFN-γ secretion is measured by an ELISA assay after the rapid second expansion step, after Step D as provided in for example, Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, TIL health is measured by IFN-gamma (IFN-γ) secretion. In some embodiments, IFN-γ secretion is indicative of active TILs. In some embodiments, a potency assay for IFN-γ production is employed. IFN-γ production is another measure of cytotoxic potential. IFN-γ production can be measured by determining the levels of the cytokine IFN-γ in the media of TIL stimulated with antibodies to CD3, CD28, and CD137/4-1BB. IFN-γ

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levels in media from these stimulated TIL can be determined using by measuring IFN-γ release. In some embodiments, an increase in IFN-γ production in for example Step D in the Gen 3 process as provided in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) TILs as compared to for example Step D in the 2A process as provided in Figure 1 (in particular, *e.g.*, Figure 1A) is indicative of an increase in cytotoxic potential of the Step D TILs. In some embodiments, IFN-γ secretion is increased one-fold, two-fold, three-fold, four-fold, or five-fold or more. In some embodiments, IFN-γ secretion is increased one-fold. In some embodiments, IFN-γ secretion is increased two-fold. In some embodiments, IFN-γ secretion is increased three-fold. In some embodiments, IFN-γ secretion is increased four-fold. In some embodiments, IFN-γ secretion is increased five-fold. In some embodiments, IFN-γ is measured using a Quantikine ELISA kit. In some embodiments, IFN-γ is measured in TILs *ex vivo*. In some embodiments, IFN-γ is measured in TILs *ex vivo*, including TILs produced by the methods of the present invention, including, for example Figure 1B methods.

[001284] In some embodiments, TILs capable of at least one-fold, two-fold, three-fold, four-fold, or five-fold or more IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least one-fold more IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least two-fold more IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least three-fold more IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least four-fold more IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least five-fold more IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods.

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[001285] In some embodiments, TILs capable of at least 100 pg/ml to about 1000 pg/mL or more IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 200 pg/ml, at least 250 pg/ml, at least 300 pg/ml, at least 350 pg/ml, at least 400 pg/ml, at least 450 pg/ml, at least 500 pg/ml, at least 550 pg/ml, at least 600 pg/ml, at least 650 pg/ml, at least 700 pg/ml, at least 750 pg/ml, at least 800 pg/ml, at least 850 pg/ml, at least 900 pg/ml, at least 950 pg/ml, or at least 1000 pg/mL or more IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 200 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 200 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 300 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 400 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 500 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 600 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 700 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 800 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 900 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the

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present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 1000 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 2000 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 3000 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 4000 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 5000 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 6000 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 7000 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 8000 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 9000 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 10,000 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 15,000 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 20,000 pg/ml IFN-γ secretion are TILs produced

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by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 25,000 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 30,000 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 35,000 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 40,000 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 45,000 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 50,000 pg/ml IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods.

[001286] In some embodiments, TILs capable of at least 100 pg/ml/5e5 cells to about 1000 pg/ml/5e5 cells or more IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 200 pg/ml/5e5 cells, at least 250 pg/ml/5e5 cells, at least 300 pg/ml/5e5 cells, at least 350 pg/ml/5e5 cells, at least 400 pg/ml/5e5 cells, at least 450 pg/ml/5e5 cells, at least 500 pg/ml/5e5 cells, at least 550 pg/ml/5e5 cells, at least 600 pg/ml/5e5 cells, at least 650 pg/ml/5e5 cells, at least 700 pg/ml/5e5 cells, at least 750 pg/ml/5e5 cells, at least 800 pg/ml/5e5 cells, at least 850 pg/ml/5e5 cells, at least 900 pg/ml/5e5 cells, at least 950 pg/ml/5e5 cells, or at least 1000 pg/ml/5e5 cells or more IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 200 pg/ml/5e5 cells IFN-γ secretion are TILs produced

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by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 200 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 300 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 400 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 500 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 600 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 700 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 800 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 900 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 1000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 2000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 3000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure

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1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 4000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 5000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 6000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 7000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 8000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 9000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 10,000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 15,000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 20,000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 25,000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 30,000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some

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embodiments, TILs capable of at least 35,000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 40,000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 45,000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 50,000 pg/ml/5e5 cells IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods.

[001287] In some embodiments, TILs that exhibit greater than 3000 pg/10^6 TILs to 300000 pg/10^6 TILs or more Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILS that exhibit greater than 3000 pg/10^6 TILs greater than 5000 pg/10^6 TILs, greater than 7000 pg/10^6 TILs, greater than 9000 pg/10^6 TILs, greater than 11000 pg/10^6 TILs, greater than 13000 pg/10^6 TILs, greater than 15000 pg/10^6 TILs, greater than 17000 pg/10^6 TILs, greater than 19000 pg/10^6 TILs, greater than 20000 pg/10^6 TILs, greater than 40000 pg/10^6 TILs, greater than 60000 pg/10^6 TILs, greater than 80000 pg/10^6 TILs, greater than 100000 pg/10^6 TILs, greater than 120000 pg/10^6 TILs, greater than 140000 pg/10^6 TILs, greater than 160000 pg/10^6 TILs, greater than 180000 pg/10^6 TILs, greater than 200000 pg/10^6 TILs, greater than 220000 pg/10^6 TILs, greater than 240000 pg/10^6 TILs, greater than 260000 pg/10^6 TILs, greater than 280000 pg/10^6 TILs, greater than 300000 pg/10^6 TILsor more Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 3000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 5000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some

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embodiments, TILs that exhibit greater than 7000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 9000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 11000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 13000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 15000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 17000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 19000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 20000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 40000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 60000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 80000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 100000 pg/10^6 TILs Granzyme B secretion are

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TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 120000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 140000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 160000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 180000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 200000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 220000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 240000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 260000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 280000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 300000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 3000 pg/10^6 TILs to 300000 pg/10^6 TILs or more Granzyme B secretion are TILs produced by the expansion methods of the present

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invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILS that exhibit greater than 3000 pg/10^6 TILs greater than 5000 pg/10^6 TILs, greater than 7000 pg/10^6 TILs, greater than 9000 pg/10^6 TILs, greater than 11000 pg/10^6 TILs, greater than 13000 pg/10^6 TILs, greater than 15000 pg/10^6 TILs, greater than 17000 pg/10^6 TILs, greater than 19000 pg/10^6 TILs, greater than 20000 pg/10^6 TILs, greater than 40000 pg/10^6 TILs, greater than 60000 pg/10^6 TILs, greater than 80000 pg/10^6 TILs, greater than 100000 pg/10^6 TILs, greater than 120000 pg/10^6 TILs, greater than 140000 pg/10^6 TILs, greater than 160000 pg/10^6 TILs, greater than 180000 pg/10^6 TILs, greater than 200000 pg/10^6 TILs, greater than 220000 pg/10^6 TILs, greater than 240000 pg/10^6 TILs, greater than 260000 pg/10^6 TILs, greater than 280000 pg/10^6 TILs, greater than 300000 pg/10^6 TILsor more Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 3000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 5000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 7000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 9000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 11000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 13000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 15000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that

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exhibit greater than 17000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 19000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 20000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 40000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 60000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 80000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 100000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 120000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 140000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 160000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 180000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 200000 pg/10^6 TILs Granzyme B secretion are TILs produced by the

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expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 220000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 240000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 260000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 280000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 300000 pg/10^6 TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G.

[001288] In some embodiments, TILs that exhibit greater than 1000 pg/ml to 300000 pg/ml or more Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 1000 pg/ml, greater than 2000 pg/ml, greater than 3000 pg/ml, greater than 4000 pg/ml, greater than 5000 pg/ml, greater than 6000 pg/ml, greater than 7000 pg/ml, greater than 8000 pg/ml, greater than 9000 pg/ml, greater than 10000 pg/ml, greater than 20000 pg/ml, greater than 30000 pg/ml, greater than 40000 pg/ml, greater than 50000 pg/ml, greater than 60000 pg/ml, greater than 70000 pg/ml, greater than 80000 pg/ml, greater than 90000 pg/ml, greater than 100000 pg/ml or more Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILS that exhibit greater than 1000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 2000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including

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for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 3000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 4000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 5000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 6000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 7000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 8000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 9000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 10000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILS that exhibit greater than 20000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 30000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 40000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 50000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or

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Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 60000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 70000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 80000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 90000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 100000 pg/ml Granzyme B are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 120000 pg/ml Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 140000 pg/ml Granzyme B are TILs Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 160000 pg/ml Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 180000 pg/ml Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 200000 pg/ml Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 220000 pg/ml Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 240000 pg/ml Granzyme B secretion are TILs

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produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 260000 pg/ml Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 280000 pg/ml Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G. In some embodiments, TILs that exhibit greater than 300000 pg/ml Granzyme B secretion are TILs produced by the expansion methods of the present invention, including for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G.

[001289] In some embodiments, the expansion methods of the present invention produce an expanded population of TILs that exhibits increased Granzyme B secretion in vitro including for example TILs as provided in Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G, as compared to non-expanded population of TILs. In some embodiments, Granzyme B secretion of the expanded population of TILs of the present invention is increased by at least one-fold to fifty-fold or more as compared to non-expanded population of TILs. In some embodiments, IFN-γ secretion is increased by at least one-fold, at least two-fold, at least three-fold, at least four-fold, at least five-fold, at least six-fold, at least seven-fold, at least eight-fold, at least nine-fold, at least ten-fold, at least twenty-fold, at least thirty-fold, at least forty-fold, at least fifty-fold or more as compared to non-expanded population of TILs. In some embodiments, Granzyme B secretion of the expanded population of TILs of the present invention is increased by at least one-fold as compared to non-expanded population of TILs. In some embodiments, Granzyme B secretion of the expanded population of TILs of the present invention is increased by at least two-fold as compared to non-expanded population of TILs. In some embodiments, Granzyme B secretion of the expanded population of TILs of the present invention is increased by at least three-fold as compared to non-expanded population of TILs. In some embodiments, Granzyme B secretion of the expanded population of TILs of the present invention is increased by at least four-fold as compared to non-expanded population of TILs. In some embodiments, Granzyme B secretion of the expanded population of TILs of the present invention is increased by at least five-fold as compared to non-expanded population of TILs. In some embodiments, Granzyme B secretion of the expanded population of TILs of the present invention is increased by at

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least six-fold as compared to non-expanded population of TILs. In some embodiments, Granzyme B secretion of the expanded population of TILs of the present invention is increased by at least seven-fold as compared to non-expanded population of TILs. In some embodiments, Granzyme B secretion of the expanded population of TILs of the present invention is increased by at least eight-fold as compared to non-expanded population of TILs. In some embodiments, Granzyme B secretion of the expanded population of TILs of the present invention is increased by at least nine-fold as compared to non-expanded population of TILs. In some embodiments, Granzyme B secretion of the expanded population of TILs of the present invention is increased by at least ten-fold as compared to non-expanded population of TILs. In some embodiments, Granzyme B secretion of the expanded population of TILs of the present invention is increased by at least twenty-fold as compared to non-expanded population of TILs. In some embodiments, Granzyme B secretion of the expanded population of TILs of the present invention is increased by at least thirty-fold as compared to non-expanded population of TILs. In some embodiments, Granzyme B secretion of the expanded population of TILs of the present invention is increased by at least forty-fold as compared to non-expanded population of TILs. In some embodiments, Granzyme B secretion of the expanded population of TILs of the present invention is increased by at least fifty-fold as compared to non-expanded population of TILs.

[001290] In some embodiments, TILs capable of at least one-fold, two-fold, three-fold, four-fold, or five-fold or more lower levels of TNF-α (*i.e.*, TNF-alpha) secretion as compared to IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least one-fold lower levels of TNF-α secretion as compared to IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least two-fold lower levels of TNF-α secretion as compared to IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least three-fold lower levels of TNF-α secretion as compared to IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least four-fold

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lower levels of TNF-α secretion as compared to IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least five-fold lower levels of TNF-α secretion as compared to IFN-γ secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods.

[001291] In some embodiments, TILs capable of at least 200 pg/ml/5e5 cells to about 10,000 pg/ml/5e5 cells or more TNF-α (*i.e.*, TNF-alpha) secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 500 pg/ml/5e5 cells to about 10,000 pg/ml/5e5 cells or more TNF-α secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 1000 pg/ml/5e5 cells to about 10,000 pg/ml/5e5 cells or more TNF-α secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 2000 pg/ml/5e5 cells to about 10,000 pg/ml/5e5 cells or more TNF-α secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 3000 pg/ml/5e5 cells to about 10,000 pg/ml/5e5 cells or more TNF-α secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 4000 pg/ml/5e5 cells to about 10,000 pg/ml/5e5 cells or more TNF-α secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 5000 pg/ml/5e5 cells to about 10,000 pg/ml/5e5 cells or more TNF-α secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 6000 pg/ml/5e5 cells to about 10,000 pg/ml/5e5 cells or more TNF-α secretion are TILs produced

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by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 7000 pg/ml/5e5 cells to about 10,000 pg/ml/5e5 cells or more TNF-α secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 8000 pg/ml/5e5 cells to about 10,000 pg/ml/5e5 cells or more TNF-α secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, TILs capable of at least 9000 pg/ml/5e5 cells to about 10,000 pg/ml/5e5 cells or more TNF-α secretion are TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods.

[001292] In some embodiments, IFN-γ and granzyme B levels are measured to determine the phenotypic characteristics of the TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, IFN-γ and TNF-α levels are measured to determine the phenotypic characteristics of the TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, granzyme B and TNF-α levels are measured to determine the phenotypic characteristics of the TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods. In some embodiments, IFN-γ, granzyme B and TNF-α levels are measured to determine the phenotypic characteristics of the TILs produced by the expansion methods of the present invention, including, for example Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G methods.

[001293] The diverse antigen receptors of T and B lymphocytes are produced by somatic recombination of a limited, but large number of gene segments. These gene segments: V (variable), D (diversity), J (joining), and C (constant), determine the binding specificity and downstream applications of immunoglobulins and T-cell receptors (TCRs). The present invention provides a method for generating TILs which exhibit and increase the T-cell repertoire diversity. In some embodiments, the TILs obtained by the present method

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exhibit an increase in the T-cell repertoire diversity. In some embodiments, the TILs obtained by the present method exhibit an increase in the T-cell repertoire diversity as compared to freshly harvested TILs and/or TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, the TILs obtained by the present method exhibit an increase in the T-cell repertoire diversity as compared to freshly harvested TILs and/or TILs prepared using methods referred to as process 2A, as exemplified in Figure 1 (in particular, *e.g.*, Figure 1A). In some embodiments, the TILs obtained in the first expansion exhibit an increase in the T-cell repertoire diversity. In some embodiments, the increase in diversity is an increase in the immunoglobulin diversity and/or the T-cell receptor diversity. In some embodiments, the diversity is in the immunoglobulin is in the immunoglobulin heavy chain. In some embodiments, the diversity is in the immunoglobulin is in the immunoglobulin light chain. In some embodiments, the diversity is in the T-cell receptor. In some embodiments, the diversity is in one of the T-cell receptors selected from the group consisting of alpha, beta, gamma, and delta receptors. In some embodiments, there is an increase in the expression of T-cell receptor (TCR) alpha and/or beta. In some embodiments, there is an increase in the expression of T-cell receptor (TCR) alpha. In some embodiments, there is an increase in the expression of T-cell receptor (TCR) beta. In some embodiments, there is an increase in the expression of TCRαβ (*i.e.*, TCRα/β).

[001294] In some embodiments, the activation and exhaustion of TILs can be determined by examining one or more markers. In some embodiments, the activation and exhaustion can be determined using multicolor flow cytometry. In some embodiments, the activation and exhaustion of markers include but not limited to one or more markers selected from the group consisting of CD3, PD-1, 2B4/CD244, CD8, CD25, BTLA, KLRG, TIM-3, CD194/CCR4, CD4, TIGIT, CD183, CD69, CD95, CD127, CD103, and/or LAG-3). In some embodiments, the activation and exhaustion of markers include but not limited to one or more markers selected from the group consisting of BTLA, CTLA-4, ICOS, Ki67, LAG-3, PD-1, TIGIT, and/or TIM-3. In some embodiments, the activation and exhaustion of markers include but not limited to one or more markers selected from the group consisting of BTLA, CTLA-4, ICOS, Ki67, LAG-3, CD103+/CD69+, CD103+/CD69-, PD-1, TIGIT, and/or TIM-3. In some embodiments, the T-cell markers (including activation and exhaustion markers) can be determined and/or analyzed to examine T-cell activation, inhibition, or function. In

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some embodiments, the T-cell markers can include but are not limited to one or more markers selected from the group consisting of TIGIT, CD3, FoxP3, Tim-3, PD-1, CD103, CTLA-4, LAG-3, BTLA-4, ICOS, Ki67, CD8, CD25, CD45, CD4, and/or CD59.

[001295] In some embodiments, the phenotypic characterization is examined after cryopreservation.

N. Additional Process Embodiments

[001296] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (a) obtaining a first population of TILs from a tumor resected from a 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 first cell culture medium comprising IL-2 and OKT-3 to produce a second population of TILs, wherein the priming first expansion is performed for about 1 to 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 second cell culture medium comprising IL-2, OKT-3 and exogenous antigen presenting cells (APCs) to produce a third population of TILs, wherein the rapid second expansion is performed for about 1 to 10 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).

[001297] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (a) obtaining a first population of TILs from a tumor resected from a 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 first cell culture medium comprising IL-2 and a culture supernatant obtained from a culture of antigen-presenting cells (APCs) cultured in a culture medium supplemented with IL-2 and OKT-3 to produce a second population of TILs, wherein the first cell culture medium is not supplemented with APCs, wherein the priming first expansion is performed for about 1 to 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 second cell culture medium comprising IL-2, OKT-3 and exogenous antigen presenting cells (APCs) to produce a third population of TILs, wherein the

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rapid second expansion is performed for about 1 to 10 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).

[001298] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (a) obtaining a first population of TILs from a tumor resected from a 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 first cell culture medium comprising IL-2, OKT-3 and exogenous antigen-presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed for about 1 to 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 second cell culture medium comprising IL-2 and a culture supernatant obtained from a culture of APCs cultured in a cell culture medium supplemented with IL-2 and OKT-3 to produce a third population of TILs, wherein the second cell culture medium is not supplemented with APCs, wherein the rapid second expansion is performed for about 1 to 10 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).

[001299] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (a) obtaining a first population of TILs from a tumor resected from a 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 first cell culture medium comprising IL-2 and a first culture supernatant obtained from a first culture of antigen-presenting cells (APCs) cultured in a culture medium supplemented with IL-2 and OKT-3 to produce a second population of TILs, wherein the first cell culture medium is not supplemented with APCs, wherein the priming first expansion is performed for about 1 to 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 second cell culture medium comprising IL-2 and a second culture supernatant obtained from a second culture of APCs cultured in a cell culture medium supplemented with IL-2 and OKT-3 to produce a third population of

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TILs, wherein the second cell culture medium is not supplemented with APCs, wherein the rapid second expansion is performed for about 1 to 10 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).

[001300] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (a) obtaining a first population of TILs from a tumor resected from a 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 first cell culture medium comprising IL-2 and OKT-3 to produce a second population of TILs, wherein the priming first expansion is performed for about 1 to 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 second cell culture medium comprising IL-2, OKT-3 and exogenous antigen presenting cells (APCs) to produce a third population of TILs, wherein the rapid second expansion is performed for about 1 to 8 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).

[001301] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (a) obtaining a first population of TILs from a tumor resected from a 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 first cell culture medium comprising IL-2 and a culture supernatant obtained from a culture of antigen presenting cells (APCs) cultured in a cell culture medium supplemented with IL-2 and OKT-3 to produce a second population of TILs, wherein the first cell culture medium is not supplemented with APCs, wherein the priming first expansion is performed for about 1 to 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 second cell culture medium comprising IL-2, OKT-3 and exogenous antigen presenting cells (APCs) to produce a third population of TILs, wherein the rapid second expansion is performed for about 1 to 8 days to obtain the third population of

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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).

[001302] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (a) obtaining a first population of TILs from a tumor resected from a 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 first cell culture medium comprising exogenous antigen presenting cells (APCs), IL-2 and OKT-3 to produce a second population of TILs, wherein the priming first expansion is performed for about 1 to 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 second cell culture medium comprising IL-2 and a culture supernatant obtained from a culture of APCs cultured in a culture medium supplemented with IL-2 and OKT-3 to produce a third population of TILs, wherein the second cell culture medium is not supplemented with APCs, wherein the rapid second expansion is performed for about 1 to 8 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).

[001303] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (a) obtaining a first population of TILs from a tumor resected from a 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 first cell culture medium comprising IL-2 and a first culture supernatant obtained from a first culture of antigen presenting cells (APCs) cultured in a cell culture medium supplemented with IL-2 and OKT-3 to produce a second population of TILs, wherein the first cell culture medium is not supplemented with APCs, wherein the priming first expansion is performed for about 1 to 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 second cell culture medium comprising IL-2 and a second culture supernatant obtained from a second culture of APCs cultured in a cell culture medium supplemented with IL-2 and OKT-3 to produce a third population of TILs, wherein the second cell culture medium is not supplemented with APCs, wherein the

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rapid second expansion is performed for about 1 to 8 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).

[001304] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (a) obtaining a first population of TILs from a tumor resected from a 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 first cell culture medium comprising IL-2 and OKT-3 to produce a second population of TILs, wherein the priming first expansion is performed for about 1 to 7 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs; (c) performing a rapid second expansion by contacting the second population of TILs with a second cell culture medium comprising IL-2, OKT-3 and exogenous antigen presenting cells (APCs) to produce a third population of TILs, 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; and (d) harvesting the therapeutic population of TILs obtained from step (c).

[001305] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (a) obtaining a first population of TILs from a tumor resected from a 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 first cell culture medium comprising IL-2 and OKT-3 to produce a second population of TILs, wherein the priming first expansion is performed for 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; (c) performing a rapid second expansion by contacting the second population of TILs with a second cell culture medium comprising IL-2, OKT-3 and exogenous antigen presenting cells (APCs) to produce a third population of TILs, wherein the rapid second expansion is performed for about 8 to 10 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).

[001306] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (a)

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obtaining 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 first cell culture medium comprising IL-2 and a cell culture supernatant obtained from a culture of antigen presenting cells (APCs) cultured in a cell culture medium supplemented with IL-2 and OKT-3 to produce a second population of TILs, wherein the first cell culture medium is not supplemented with APCs, wherein the priming first expansion is performed for 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; (c) performing a rapid second expansion by contacting the second population of TILs with a second cell culture medium comprising IL-2, OKT-3 and exogenous antigen presenting cells (APCs) to produce a third population of TILs, wherein the rapid second expansion is performed for about 8 to 10 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).

[001307] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (a) obtaining a first population of TILs from a tumor resected from a 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 first cell culture medium supplemented with antigen presenting cells (APCs), IL-2 and OKT-3 to produce a second population of TILs, wherein the priming first expansion is performed for 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; (c) performing a rapid second expansion by contacting the second population of TILs with a second cell culture medium comprising IL-2 and a culture supernatant obtained from a culture of APCs cultured in a culture medium supplemented with IL-2 and OKT-3 to produce a third population of TILs, wherein the second cell culture medium is not supplemented with APCs, wherein the rapid second expansion is performed for about 8 to 10 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).

[001308] In some embodiments, the invention provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (a) obtaining a first population of TILs from a tumor resected from a subject by processing a

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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 first cell culture medium supplemented with IL-2 and a first culture supernatant obtained from a first culture of antigen presenting cells (APCs) cultured in a cell culture medium supplemented with IL-2 and OKT-3 to produce a second population of TILs, wherein the first cell culture medium is not supplemented with APCs, wherein the priming first expansion is performed for 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; (c) performing a rapid second expansion by contacting the second population of TILs with a second cell culture medium comprising IL-2 and a second culture supernatant obtained from a second culture of APCs cultured in a culture medium supplemented with IL-2 and OKT-3 to produce a third population of TILs, wherein the second cell culture medium is not supplemented with APCs, wherein the rapid second expansion is performed for about 8 to 10 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).

[001309] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of rapid second expansion is split into a plurality of steps to achieve a scaling up of the culture by: (1) performing the rapid second expansion by culturing the second population of TILs in a small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 2 to 4 days, and then (2) effecting the transfer of the second population of TILs from the small scale culture to a second container larger than the first container, *e.g.*, a G-REX 500MCS container, wherein in the second container the second population of TILs from the small scale culture is cultured in a larger scale culture for a period of about 4 to 8 days. In some embodiments, the step of rapid expansion is split into a plurality of steps to achieve a scaling out of the culture by: (1) performing the rapid second expansion by culturing the second population of TILs in a first small scale culture in a first container, *e.g.*, a G-REX 100MCS container or a G-REX 10M container, for a period of about 3 to 4 days, and then (2) effecting the transfer and apportioning of the second population of TILs from the first small scale culture into and amongst at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 second containers that are equal in size to the first container, wherein in each second container the portion of the second population of TILs from the first small scale culture transferred to such second container is cultured in a second small scale culture for a period of

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about 4 to 8 days. In some embodiments, the step of rapid expansion is split into a plurality of steps to achieve a scaling out of the culture by: (1) performing the rapid second expansion by culturing the second population of TILs in a first small scale culture in a first container, *e.g.*, a G-REX 10M container or a G-REX 100M container, for a period of about 3 to 4 days, and then (2) effecting the transfer and apportioning of the second population of TILs from the first small scale culture into and amongst 5 second containers that are equal in size to the first container, wherein in each second container the portion of the second population of TILs from the first small scale culture transferred to such second container is cultured in a second small scale culture for a period of about 6 to 7 days. In some embodiments, the step of rapid expansion is split into a plurality of steps to achieve a scaling out and scaling up of the culture by: (1) performing the rapid second expansion by culturing the second population of TILs in a small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 2 to 4 days, and then (2) effecting the transfer and apportioning of the second population of TILs from the first small scale culture into and amongst at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 second containers that are larger in size than the first container, *e.g.*, G-REX 500MCS containers, wherein in each second container the portion of the second population of TILs transferred from the small scale culture to such second container is cultured in a larger scale culture for a period of about 4 to 8 days. In some embodiments, the step of rapid expansion is split into a plurality of steps to achieve a scaling out and scaling up of the culture by: (1) performing the rapid second expansion by culturing the second population of TILs in a small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 3 to 4 days, and then (2) effecting the transfer and apportioning of the second population of TILs from the first small scale culture into and amongst 2, 3 or 4 second containers that are larger in size than the first container, *e.g.*, G-REX 500MCS containers, wherein in each second container the portion of the second population of TILs transferred from the small scale culture to such second container is cultured in a larger scale culture for a period of about 5 to 7 days.

[001310] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by contacting the first population of TILs with a first culture medium which further comprises exogenous antigen-presenting cells (APCs), wherein the number of APCs in the second culture medium in step (c) is greater than the number of APCs in the first culture medium in step (b).

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[001311] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (c) the second culture medium is supplemented with additional exogenous APCs.

[001312] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 20:1.

[001313] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 10:1.

[001314] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 9:1.

[001315] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 8:1.

[001316] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 7:1.

[001317] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 6:1.

[001318] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 5:1.

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[001319] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 4:1.

[001320] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 3:1.

[001321] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 2.9:1.

[001322] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 2.8:1.

[001323] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 2.7:1.

[001324] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 2.6:1.

[001325] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 2.5:1.

[001326] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 2.4:1.

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[001327] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 2.3:1.

[001328] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 2.2:1.

[001329] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 2.1:1.

[001330] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 1.1:1 to at or about 2:1.

[001331] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 2:1 to at or about 10:1.

[001332] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 2:1 to at or about 5:1.

[001333] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 2:1 to at or about 4:1.

[001334] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 2:1 to at or about 3:1.

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[001335] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 2:1 to at or about 2.9:1.

[001336] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 2:1 to at or about 2.8:1.

[001337] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 2:1 to at or about 2.7:1.

[001338] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 2:1 to at or about 2.6:1.

[001339] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 2:1 to at or about 2.5:1.

[001340] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 2:1 to at or about 2.4:1.

[001341] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 2:1 to at or about 2.3:1.

[001342] In other embodiments, the invention provides the method described in any of the preceding paragraph as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 2:1 to at or about 2.2:1.

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[001343] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is in a range of from at or about 2:1 to at or about 2.1:1.

[001344] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is at or about 2:1.

[001345] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the ratio of number of APCs added in the rapid second expansion to the number of APCs added in step (b) is at or about 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1, 1.6:1, 1.7:1, 1.8:1, 1.9:1, 2:1, 2.1:1, 2.2:1, 2.3:1, 2.4:1, 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, 3.5:1, 3.6:1, 3.7:1, 3.8:1, 3.9:1, 4:1, 4.1:1, 4.2:1, 4.3:1, 4.4:1, 4.5:1, 4.6:1, 4.7:1, 4.8:1, 4.9:1, or 5:1.

[001346] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the number of APCs added in the primary first expansion is at or about 1×10^8, 1.1×10^8, 1.2×10^8, 1.3×10^8, 1.4×10^8, 1.5×10^8, 1.6×10^8, 1.7×10^8, 1.8×10^8, 1.9×10^8, 2×10^8, 2.1×10^8, 2.2×10^8, 2.3×10^8, 2.4×10^8, 2.5×10^8, 2.6×10^8, 2.7×10^8, 2.8×10^8, 2.9×10^8, 3×10^8, 3.1×10^8, 3.2×10^8, 3.3×10^8, 3.4×10^8 or 3.5×10^8 APCs, and such that the number of APCs added in the rapid second expansion is at or about 3.5×10^8, 3.6×10^8, 3.7×10^8, 3.8×10^8, 3.9×10^8, 4×10^8, 4.1×10^8, 4.2×10^8, 4.3×10^8, 4.4×10^8, 4.5×10^8, 4.6×10^8, 4.7×10^8, 4.8×10^8, 4.9×10^8, 5×10^8, 5.1×10^8, 5.2×10^8, 5.3×10^8, 5.4×10^8, 5.5×10^8, 5.6×10^8, 5.7×10^8, 5.8×10^8, 5.9×10^8, 6×10^8, 6.1×10^8, 6.2×10^8, 6.3×10^8, 6.4×10^8, 6.5×10^8, 6.6×10^8, 6.7×10^8, 6.8×10^8, 6.9×10^8, 7×10^8, 7.1×10^8, 7.2×10^8, 7.3×10^8, 7.4×10^8, 7.5×10^8, 7.6×10^8, 7.7×10^8, 7.8×10^8, 7.9×10^8, 8×10^8, 8.1×10^8, 8.2×10^8, 8.3×10^8, 8.4×10^8, 8.5×10^8, 8.6×10^8, 8.7×10^8, 8.8×10^8, 8.9×10^8, 9×10^8, 9.1×10^8, 9.2×10^8, 9.3×10^8, 9.4×10^8, 9.5×10^8, 9.6×10^8, 9.7×10^8, 9.8×10^8, 9.9×10^8 or 1×10^9 APCs.

[001347] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the number of APCs added in the primary first expansion is in the range of at or about 1×10^8 APCs to at or about 3.5×10^8 APCs, and wherein the number of APCs added in the rapid second expansion is in the range of at or about 3.5×10^8 APCs to at or about 1×10^9 APCs.

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[001348] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the number of APCs added in the primary first expansion is in the range of at or about 1.5×10^8 APCs to at or about 3×10^8 APCs, and wherein the number of APCs added in the rapid second expansion is in the range of at or about 4×10^8 APCs to at or about 7.5×10^8 APCs.

[001349] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the number of APCs added in the primary first expansion is in the range of at or about 2×10^8 APCs to at or about 2.5×10^8 APCs, and wherein the number of APCs added in the rapid second expansion is in the range of at or about 4.5×10^8 APCs to at or about 5.5×10^8 APCs.

[001350] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that at or about 2.5×10^8 APCs are added to the primary first expansion and at or about 5×10^8 APCs are added to the rapid second expansion.

[001351] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the antigen-presenting cells added in the primary first expansion are peripheral blood mononuclear cells (PBMCs).

[001352] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the antigen-presenting cells added in the rapid second expansion are peripheral blood mononuclear cells (PBMCs).

[001353] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in the priming first expansion the first cell culture medium comprises a culture supernatant obtained from a culture of peripheral blood mononuclear cells (PBMCs).

[001354] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in the rapid second expansion the second cell culture medium comprises a culture supernatant obtained from a culture of peripheral blood mononuclear cells (PBMCs).

[001355] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in the priming first expansion the first cell culture medium comprises a first culture supernatant obtained from a first culture of peripheral blood mononuclear cells (PBMCs) and in the rapid second expansion the

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second cell culture medium comprises a second culture supernatant obtained from a second culture of PBMCs.

[001356] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in the priming first expansion the first cell culture medium comprises a culture supernatant obtained from a culture of peripheral blood mononuclear cells (PBMCs), wherein the culture supernatant is obtained after the rate of growth of cells in the culture begins to decline. In some embodiments, the culture supernatant is obtained 3 or 4 days after the initiation of the culture of PBMCs. In some embodiments, the culture supernatant is obtained after the rate of growth of cells in the culture has declined at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more from the peak rate of growth of cells in the culture. In some embodiments, the culture supernatant is obtained after the cell culture medium in which the PMBCs were cultured is spent or exhausted.

[001357] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in the rapid second expansion the second cell culture medium comprises a culture supernatant obtained from a culture of peripheral blood mononuclear cells (PBMCs). In some embodiments, the culture supernatant is obtained 3 or 4 days after the initiation of the culture of PBMCs. In some embodiments, the culture supernatant is obtained after the rate of growth of cells in the culture has declined at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more from the peak rate of growth of cells in the culture. In some embodiments, the culture supernatant is obtained after the cell culture medium in which the PMBCs were cultured is spent or exhausted.

[001358] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in the priming first expansion the first cell culture medium comprises a first culture supernatant obtained from a first culture of peripheral blood mononuclear cells (PBMCs) and in the rapid second expansion the second cell culture medium comprises a second culture supernatant obtained from a second culture of PBMCs. In some embodiments, the first culture supernatant is obtained 3 or 4 days after the initiation of the first culture of PBMCs. In some embodiments, the second culture supernatant is obtained 3 or 4 days after the initiation of the second culture of PBMCs. In some embodiments, the first culture supernatant is obtained 3 or 4 days after the initiation of the first culture of PBMCs and the second culture supernatant is obtained 3 or 4 days after the

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initiation of the second culture of PBMCs. In some embodiments, the first culture supernatant is obtained after the rate of growth of cells in the first culture of PBMCs has declined at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more from the peak rate of growth of cells in the first culture of PBMCs. In some embodiments, the second culture supernatant is obtained after the rate of growth of cells in the second culture of PBMCs has declined at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more from the peak rate of growth of cells in the second culture of PBMCs. In some embodiments, the first culture supernatant is obtained after the rate of growth of cells in the first culture of PBMCs has declined at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more from the peak rate of growth of cells in the first culture of PBMCs and the second culture supernatant is obtained after the rate of growth of cells in the second culture of PBMCs has declined at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more from the peak rate of growth of cells in the second culture of PBMCs. In some embodiments, the first culture supernatant is obtained after the cell culture medium in which the first culture of PMBCs was cultured is spent or exhausted. In some embodiments, the second culture supernatant is obtained after the cell culture medium in which the second culture of PMBCs was cultured is spent or exhausted. In some embodiments, the first culture supernatant is obtained after the cell culture medium in which the first culture of PMBCs was cultured is spent or exhausted and the second culture supernatant is obtained after the cell culture medium in which the second culture of PMBCs was cultured is spent or exhausted.

[001359] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in the priming first expansion the first cell culture medium comprises a culture supernatant obtained from a culture of peripheral blood mononuclear cells (PBMCs) initiated with about $1x10^7$ to about $1x10^9$ PBMCs. In other embodiments, the first cell culture medium comprises a culture supernatant obtained from a culture of peripheral blood mononuclear cells (PBMCs) initiated with about $5x10^7$ to about $5x10^8$ PBMCs.

[001360] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in the rapid second expansion the second cell culture medium comprises a culture supernatant obtained from a culture of

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peripheral blood mononuclear cells (PBMCs) initiated with about $1x10^7$ to about $1x10^9$ PBMCs. In other embodiments, the second cell culture medium comprises a culture supernatant obtained from a culture of peripheral blood mononuclear cells (PBMCs) initiated with about $5x10^7$ to about $5x10^8$ PBMCs.

[001361] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in the priming first expansion the first cell culture medium comprises a first culture supernatant obtained from a first culture of peripheral blood mononuclear cells (PBMCs) initiated with about $1x10^7$ to about $1x10^9$ PBMCs and in the rapid second expansion the second cell culture medium comprises a second culture supernatant obtained from a second culture of PBMCs initiated with about $1x10^7$ to about $1x10^9$ PBMCs. In other embodiments, the first cell culture medium comprises a first culture supernatant obtained from a first culture of peripheral blood mononuclear cells (PBMCs) initiated with about $5x10^7$ to about $5x10^8$ PBMCs and the second cell culture medium comprises a second culture supernatant obtained from a second culture of PBMCs initiated with about $5x10^7$ to about $5x10^8$ PBMCs.

[001362] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the multiple tumor fragments are distributed into a plurality of separate containers, in each of which separate containers the first population of TILs is obtained in step (a), the second population of TILs is obtained in step (b), and the third population of TILs is obtained in step (c), and the therapeutic populations of TILs from the plurality of containers in step (c) are combined to yield the harvested TIL population from step (d).

[001363] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the multiple tumors are evenly distributed into the plurality of separate containers.

[001364] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the plurality of separate containers comprises at least two separate containers.

[001365] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the plurality of separate containers comprises from two to twenty separate containers.

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[001366] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the plurality of separate containers comprises from two to fifteen separate containers.

[001367] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the plurality of separate containers comprises from two to ten separate containers.

[001368] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the plurality of separate containers comprises from two to five separate containers.

[001369] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the plurality of separate containers comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 separate containers.

[001370] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that for each container in which the priming first expansion is performed on a first population of TILs in step (b) the rapid second expansion in step (c) is performed in the same container on the second population of TILs produced from such first population of TILs.

[001371] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that each of the separate containers comprises a first gas-permeable surface area.

[001372] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the multiple tumor fragments are distributed in a single container.

[001373] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the single container comprises a first gas-permeable surface area.

[001374] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added

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in step (c) is greater than the number of APCs added in step (b), and wherein in step (b) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about one cell layer to at or about three cell layers.

[001375] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about 1.5 cell layers to at or about 2.5 cell layers.

[001376] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about 2 cell layers.

[001377] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 or 3 cell layers.

[001378] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (c) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about 3 cell layers to at or about 10 cell layers.

[001379] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (c) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about 4 cell layers to at or about 8 cell layers.

[001380] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (c) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about 3, 4, 5, 6, 7, 8, 9 or 10 cell layers.

[001381] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (c) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9 or 8 cell layers.

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[001382] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the priming first expansion is performed in a first container comprising a first gas-permeable surface area and in step (c) the rapid second expansion is performed in a second container comprising a second gas-permeable surface area.

[001383] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the second container is larger than the first container.

[001384] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein in step (b) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about one cell layer to at or about three cell layers.

[001385] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about 1.5 cell layers to at or about 2.5 cell layers.

[001386] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about 2 cell layers.

[001387] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable modified such that in step (b) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 or 3 cell layers.

[001388] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (c) the APCs are layered onto the second gas-permeable surface area at an average thickness of at or about 3 cell layers to at or about 10 cell layers.

[001389] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (c) the APCs are layered

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onto the second gas-permeable surface area at an average thickness of at or about 4 cell layers to at or about 8 cell layers.

[001390] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (c) the APCs are layered onto the second gas-permeable surface area at an average thickness of at or about 3, 4, 5, 6, 7, 8, 9 or 10 cell layers.

[001391] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (c) the APCs are layered onto the second gas-permeable surface area at an average thickness of at or about 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9 or 8 cell layers.

[001392] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the priming first expansion is performed in a first container comprising a first gas-permeable surface area and in step (c) the rapid second expansion is performed in the first container.

[001393] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein in step (b) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about one cell layer to at or about three cell layers.

[001394] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about 1.5 cell layers to at or about 2.5 cell layers.

[001395] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about 2 cell layers.

[001396] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the APCs are layered

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onto the first gas-permeable surface area at an average thickness of at or about 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 or 3 cell layers.

[001397] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (c) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about 3 cell layers to at or about 10 cell layers.

[001398] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (c) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about 4 cell layers to at or about 8 cell layers.

[001399] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (c) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about 3, 4, 5, 6, 7, 8, 9 or 10 cell layers.

[001400] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (c) the APCs are layered onto the first gas-permeable surface area at an average thickness of at or about 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9 or 8 cell layers.

[001401] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:1.1 to at or about 1:10.

[001402] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the

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average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:1.1 to at or about 1:9.

[001403] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:1.1 to at or about 1:8.

[001404] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:1.1 to at or about 1:7.

[001405] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:1.1 to at or about 1:6.

[001406] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:1.1 to at or about 1:5.

[001407] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first

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expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:1.1 to at or about 1:4.

[001408] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:1.1 to at or about 1:3.

[001409] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:1.1 to at or about 1:2.

[001410] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:1.2 to at or about 1:8.

[001411] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the

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average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:1.3 to at or about 1:7.

[001412] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:1.4 to at or about 1:6.

[001413] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:1.5 to at or about 1:5.

[001414] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:1.6 to at or about 1:4.

[001415] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:1.7 to at or about 1:3.5.

[001416] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first

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expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:1.8 to at or about 1:3.

[001417] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:1.9 to at or about 1:2.5.

[001418] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is in the range of at or about 1:2.

[001419] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the primary first expansion is performed by supplementing the first cell culture medium of the first population of TILs with additional antigen-presenting cells (APCs), wherein the number of APCs added in step (c) is greater than the number of APCs added in step (b), and wherein the ratio of the average number of layers of APCs layered in step (b) to the average number of layers of APCs layered in step (c) is selected from at or about 1:1.1, 1:1.2, 1:1.3, 1:1.4, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1:1.9, 1:2, 1:2.1, 1:2.2, 1:2.3, 1:2.4, 1:2.5, 1:2.6, 1:2.7, 1:2.8, 1:2.9, 1:3, 1:3.1, 1:3.2, 1:3.3, 1:3.4, 1:3.5, 1:3.6, 1:3.7, 1:3.8, 1:3.9, 1:4, 1:4.1, 1:4.2, 1:4.3, 1:4.4, 1:4.5, 1:4.6, 1:4.7, 1:4.8, 1:4.9, 1:5, 1:5.1, 1:5.2, 1:5.3, 1:5.4, 1:5.5, 1:5.6, 1:5.7, 1:5.8, 1:5.9, 1:6, 1:6.1, 1:6.2, 1:6.3, 1:6.4, 1:6.5, 1:6.6, 1:6.7, 1:6.8, 1:6.9, 1:7, 1:7.1, 1:7.2, 1:7.3, 1:7.4, 1:7.5, 1:7.6, 1:7.7, 1:7.8, 1:7.9, 1:8, 1:8.1, 1:8.2, 1:8.3, 1:8.4, 1:8.5, 1:8.6, 1:8.7, 1:8.8, 1:8.9, 1:9, 1:9.1, 1:9.2, 1:9.3, 1:9.4, 1:9.5, 1:9.6, 1:9.7, 1:9.8, 1:9.9 or 1:10.

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[001420] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that 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 at or about 1.5:1 to at or about 100:1.

[001421] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that 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 at or about 50:1.

[001422] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that 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 at or about 25:1.

[001423] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that 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 at or about 20:1.

[001424] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that 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 at or about 10:1.

[001425] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the second population of TILs is at least at or about 50-fold greater in number than the first population of TILs.

[001426] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the second population of TILs is at least at or about 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, 15-, 16-, 17-, 18-, 19-, 20-, 21-, 22-, 23-, 24-, 25-, 26-, 27-, 28-, 29-, 30-, 31-, 32-, 33-, 34-, 35-, 36-, 37-, 38-, 39-, 40-, 41-, 42-, 43-, 44-, 45-, 46-, 47-, 48-, 49- or 50-fold greater in number than the first population of TILs.

[001427] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that at or about 2 days or at or about

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3 days after the commencement of the second period in step (c), the cell culture medium is supplemented with additional IL-2.

[001428] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified to further comprise the step of cryopreserving the harvested TIL population in step (d) using a cryopreservation process.

[001429] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified to comprise performing after step (d) the additional step of (e) transferring the harvested TIL population from step (d) to an infusion bag that optionally contains HypoThermosol.

[001430] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified to comprise the step of cryopreserving the infusion bag comprising the harvested TIL population in step (e) using a cryopreservation process.

[001431] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.

[001432] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).

[001433] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the PBMCs are irradiated and allogeneic.

[001434] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the total number of APCs added to the first cell culture medium in step (b) is 2.5×10^8.

[001435] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the total number of APCs added to the second cell culture medium in step (c) is 5×10^8.

[001436] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the APCs are PBMCs.

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[001437] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the PBMCs are irradiated and allogeneic.

[001438] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the antigen-presenting cells are artificial antigen-presenting cells.

[001439] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the harvesting in step (d) is performed using a membrane-based cell processing system.

[001440] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the harvesting in step (d) is performed using a LOVO cell processing system.

[001441] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the multiple fragments comprise at or about 5 to at or about 60 fragments per container in step (b).

[001442] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the multiple fragments comprise at or about 10 to at or about 60 fragments per container in step (b).

[001443] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the multiple fragments comprise at or about 15 to at or about 60 fragments per container in step (b).

[001444] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the multiple fragments comprise at or about 20 to at or about 60 fragments per container in step (b).

[001445] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the multiple fragments comprise at or about 25 to at or about 60 fragments per container in step (b).

[001446] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the multiple fragments comprise at or about 30 to at or about 60 fragments per container in step (b).

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[001447] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the multiple fragments comprise at or about 35 to at or about 60 fragments per container in step (b).

[001448] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the multiple fragments comprise at or about 40 to at or about 60 fragments per container in step (b).

[001449] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the multiple fragments comprise at or about 45 to at or about 60 fragments per container in step (b).

[001450] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the multiple fragments comprise at or about 50 to at or about 60 fragments per container in step (b).

[001451] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the multiple fragments comprise at or about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 or 60 fragment(s) per container in step (b).

[001452] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that each fragment has a volume of at or about 27 mm^3.

[001453] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that each fragment has a volume of at or about 20 mm^3 to at or about 50 mm^3.

[001454] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that each fragment has a volume of at or about 21 mm^3 to at or about 30 mm^3.

[001455] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that each fragment has a volume of at or about 22 mm^3 to at or about 29.5 mm^3.

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[001456] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that each fragment has a volume of at or about 23 mm^3 to at or about 29 mm^3.

[001457] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that each fragment has a volume of at or about 24 mm^3 to at or about 28.5 mm^3.

[001458] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that each fragment has a volume of at or about 25 mm^3 to at or about 28 mm^3.

[001459] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that each fragment has a volume of at or about 26.5 mm^3 to at or about 27.5 mm^3.

[001460] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that each fragment has a volume of at or about 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 mm^3.

[001461] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the multiple fragments comprise at or about 30 to at or about 60 fragments with a total volume of at or about 1300 mm^3 to at or about 1500 mm^3.

[001462] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the multiple fragments comprise at or about 50 fragments with a total volume of at or about 1350 mm^3.

[001463] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the multiple fragments comprise at or about 50 fragments with a total mass of at or about 1 gram to at or about 1.5 grams.

[001464] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first cell culture medium is provided in a container that is a G-container or a Xuri cellbag.

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[001465] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the second cell culture medium is provided in a container that is a G-container or a Xuri cellbag.

[001466] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that each of the first cell culture medium and the second cell culture medium is provided in a container that is a G-container or a Xuri cellbag.

[001467] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the IL-2 concentration in the first cell culture medium is about 10,000 IU/mL to about 5,000 IU/mL.

[001468] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the IL-2 concentration in the second cell culture medium is about 10,000 IU/mL to about 5,000 IU/mL.

[001469] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the IL-2 concentration in each of the first cell culture medium and the second cell culture medium is about 10,000 IU/mL to about 5,000 IU/mL.

[001470] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the IL-2 concentration in the first cell culture medium is about 6,000 IU/mL.

[001471] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the IL-2 concentration in the second cell culture medium is about 6,000 IU/mL.

[001472] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the IL-2 concentration in each of the first cell culture medium and the second cell culture medium is about 6,000 IU/mL.

[001473] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the cryopreservation media comprises dimethlysulfoxide (DMSO).

[001474] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the cryopreservation media comprises 7% to 10% DMSO.

[001475] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first period in step (b) is performed within a period of at or about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days.

[001476] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the second period in step (c) is performed within a period of at or about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days or 11 days.

[001477] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first period in step (b) and the second period in step (c) are each individually performed within a period of at or about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days.

[001478] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first period in step (b) and the second period in step (c) are each individually performed within a period of at or about 5 days, 6 days, or 7 days.

[001479] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first period in step (b) and the second period in step (c) are each individually performed within a period of at or about 7 days.

[001480] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 14 days to at or about 18 days.

[001481] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 15 days to at or about 18 days.

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[001482] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 16 days to at or about 18 days.

[001483] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 17 days to at or about 18 days.

[001484] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 14 days to at or about 17 days.

[001485] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 15 days to at or about 17 days.

[001486] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 16 days to at or about 17 days.

[001487] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 14 days to at or about 16 days.

[001488] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 15 days to at or about 16 days.

[001489] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 14 days.

[001490] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 15 days.

[001491] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 16 days.

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[001492] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 17 days.

[001493] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 18 days.

[001494] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 14 days or less.

[001495] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 15 days or less.

[001496] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 16 days or less.

[001497] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 17 days or less.

[001498] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that steps (a) through (d) are performed in a total of at or about 18 days or less.

[001499] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the therapeutic population of TILs harvested in step (d) comprises sufficient TILs for a therapeutically effective dosage of the TILs.

[001500] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the number of TILs sufficient for a therapeutically effective dosage is from at or about 2.3×10^{10} to at or about 13.7×10^{10}.

[001501] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the third population of TILs in

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step (c) provides for increased efficacy, increased interferon-gamma production, and/or increased polyclonality.

[001502] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that 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.

[001503] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that 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 17 days.

[001504] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that 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 18 days.

[001505] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the effector T cells and/or central memory T cells obtained from the third population of TILs 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 step (b).

[001506] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that each container recited in the method is a closed container.

[001507] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that each container recited in the method is a G-container.

[001508] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that each container recited in the method is a GREX-10.

[001509] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that each container recited in the method is a GREX-100.

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[001510] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that each container recited in the method is a GREX-500.

[001511] In other embodiments, the invention provides the therapeutic population of tumor infiltrating lymphocytes (TILs) made by the method described in any of the preceding paragraphs as applicable above.

[001512] In other embodiments, the invention provides a 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 compared to TILs prepared by a process in which the first expansion of TILs is performed without any added antigen-presenting cells (APCs) or OKT3.

[001513] In other embodiments, the invention provides a 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 compared to TILs prepared by a process in which the first expansion of TILs is performed without any added antigen-presenting cells (APCs).

[001514] In other embodiments, the invention provides a 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 compared to TILs prepared by a process in which the first expansion of TILs is performed without any added OKT3.

[001515] In other embodiments, the invention provides a 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 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.

[001516] In other embodiments, the invention provides a 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

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production, and/or increased polyclonality compared to TILs prepared by a process by a process longer than 16 days.

[001517] In other embodiments, the invention provides a 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 compared to TILs prepared by a process by a process longer than 17 days.

[001518] In other embodiments, the invention provides a 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 compared to TILs prepared by a process by a process longer than 18 days.

[001519] In other embodiments, the invention provides for the therapeutic population of TILs described in any of the preceding paragraphs as applicable above that provides for increased interferon-gamma production.

[001520] In other embodiments, the invention provides for the therapeutic population of TILs described in any of the preceding paragraphs as applicable above that provides for increased polyclonality.

[001521] In other embodiments, the invention provides for the therapeutic population of TILs described in any of the preceding paragraphs as applicable above that provides for increased efficacy.

[001522] In other embodiments, the invention provides for the therapeutic population of TILs described in any of the preceding paragraphs as applicable above modified such that the therapeutic population of TILs is capable of at least one-fold more interferon-gamma production as compared to TILs prepared by a process longer than 16 days. In other embodiments, the invention provides for the therapeutic population of TILs described in any of the preceding paragraphs as applicable above modified such that the therapeutic population of TILs is capable of at least one-fold more interferon-gamma production as compared to TILs prepared by a process longer than 17 days. In other embodiments, the invention provides for the therapeutic population of TILs described in any of the preceding paragraphs as applicable above modified such that the therapeutic population of TILs is capable of at least one-fold more interferon-gamma production as compared to TILs prepared by a process

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longer than 18 days. In some embodiments, the TILs are rendered capable of the at least one-fold more interferon-gamma production due to the expansion process described herein, for example as described in Steps A through F above or according to Steps A through F above (also as shown, for example, in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G).

[001523] In other embodiments, the invention provides for the therapeutic population of TILs described in any of the preceding paragraphs as applicable above modified such that the therapeutic population of TILs is capable of at least two-fold more interferon-gamma production as compared to TILs prepared by a process longer than 16 days. In other embodiments, the invention provides for the therapeutic population of TILs described in any of the preceding paragraphs as applicable above modified such that the therapeutic population of TILs is capable of at least two-fold more interferon-gamma production as compared to TILs prepared by a process longer than 17 days. In other embodiments, the invention provides for the therapeutic population of TILs described in any of the preceding paragraphs as applicable above modified such that the therapeutic population of TILs is capable of at least two-fold more interferon-gamma production as compared to TILs prepared by a process longer than 18 days. In some embodiments, the TILs are rendered capable of the at least two-fold more interferon-gamma production due to the expansion process described herein, for example as described in Steps A through F above or according to Steps A through F above (also as shown, for example, in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G).

[001524] In other embodiments, the invention provides for the therapeutic population of TILs described in any of the preceding paragraphs as applicable above modified such that the therapeutic population of TILs is capable of at least three-fold more interferon-gamma production as compared to TILs prepared by a process longer than 16 days. In other embodiments, the invention provides for the therapeutic population of TILs described in any of the preceding paragraphs as applicable above modified such that the therapeutic population of TILs is capable of at least three-fold more interferon-gamma production as compared to TILs prepared by a process longer than 17 days. In other embodiments, the invention provides for the therapeutic population of TILs described in any of the preceding paragraphs as applicable above modified such that the therapeutic population of TILs is capable of at least three-fold more interferon-gamma production as compared to TILs prepared by a process longer than 18 days. In some embodiments, the TILs are rendered capable of the at

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least three-fold more interferon-gamma production due to the expansion process described herein, for example as described in Steps A through F above or according to Steps A through F above (also as shown, for example, in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G).

[001525] In other embodiments, the invention provides for a therapeutic population of tumor infiltrating lymphocytes (TILs) that 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). In some embodiments, the TILs are rendered capable of the at least one-fold more interferon-gamma production due to the expansion process described herein, for example as described in Steps A through F above or according to Steps A through F above (also as shown, for example, in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G).

[001526] In other embodiments, the invention provides for a therapeutic population of tumor infiltrating lymphocytes (TILs) that 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. In some embodiments, the TILs are rendered capable of the at least one-fold more interferon-gamma production due to the expansion process described herein, for example as described in Steps A through F above or according to Steps A through F above (also as shown, for example, in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G).

[001527] In other embodiments, the invention provides for a therapeutic population of TILs that 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. In some embodiments, the TILs are rendered capable of the at least two-fold more interferon-gamma production due to the expansion process described herein, for example as described in Steps A through F above or according to Steps A through F above (also as shown, for example, in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G).

[001528] In other embodiments, the invention provides for a therapeutic population of TILs that 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

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OKT3. In some embodiments, the TILs are rendered capable of the at least two-fold more interferon-gamma production due to the expansion process described herein, for example as described in Steps A through F above or according to Steps A through F above (also as shown, for example, in Figure 1 (in particular, *e.g.,* Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G).

[001529] In other embodiments, the invention provides for a therapeutic population of TILs that 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. In some embodiments, the TILs are rendered capable of the at least one-fold more interferon-gamma production due to the expansion process described herein, for example as described in Steps A through F above or according to Steps A through F above (also as shown, for example, in Figure 1 (in particular, *e.g.,* Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G).

[001530] In other embodiments, the invention provides for a therapeutic population of TILs that 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. In some embodiments, the TILs are rendered capable of the at least three-fold more interferon-gamma production due to the expansion process described herein, for example as described in Steps A through F above or according to Steps A through F above (also as shown, for example, in Figure 1 (in particular, *e.g.,* Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G).

[001531] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the tumor fragments are small biopsies (including, for example, a punch biopsy), core biopsies, core needle biopsies or fine needle aspirates.

[001532] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the tumor fragments are core biopsies.

[001533] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the tumor fragments are fine needle aspirates.

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[001534] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the tumor fragments are small biopsies (including, for example, a punch biopsy).

[001535] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the tumor fragments are core needle biopsies.

[001536] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that (i) the method comprises obtaining the first population of TILs from one or more small biopsies (including, for example, a punch biopsy), core biopsies, core needle biopsies or fine needle aspirates of tumor tissue from the subject, (ii) the method comprises performing the step of culturing the first population of TILs in a cell culture medium comprising IL-2 for a period of about 3 days prior to performing the step of the priming first expansion, (iii) the method comprises performing the priming first expansion for a period of about 8 days, and (iv) the method comprises performing the rapid second expansion for a period of about 11 days. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

[001537] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that (i) the method comprises obtaining the first population of TILs from one or more small biopsies (including, for example, a punch biopsy), core biopsies, core needle biopsies or fine needle aspirates of tumor tissue from the subject, (ii) the method comprises performing the step of culturing the first population of TILs in a cell culture medium comprising IL-2 for a period of about 3 days prior to performing the step of the priming first expansion, (iii) the method comprises performing the priming first expansion for a period of about 8 days, and (iv) the method comprises performing the rapid second expansion by culturing the culture of the second population of TILs for about 5 days, splitting the culture into up to 5 subcultures and culturing the subcultures for about 6 days. In some of the foregoing embodiments, the up to 5 subcultures are each cultured in a container that is the same size or larger than the container in which the culture of the second population of TILs is commenced in the rapid second expansion. In some of the foregoing embodiments, the culture of the second population of TILs is equally divided amongst the up to 5 subcultures. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

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[001538] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1 to about 20 small biopsies (including, for example, a punch biopsy), core biopsies, core needle biopsies or fine needle aspirates of tumor tissue from the subject.

[001539] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1 to about 10 small biopsies (including, for example, a punch biopsy), core biopsies, core needle biopsies or fine needle aspirates of tumor tissue from the subject.

[001540] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 small biopsies (including, for example, a punch biopsy), core biopsies, core needle biopsies or fine needle aspirates of tumor tissue from the subject.

[001541] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 small biopsies (including, for example, a punch biopsy), core biopsies, core needle biopsies or fine needle aspirates of tumor tissue from the subject.

[001542] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1 to about 20 core biopsies of tumor tissue from the subject.

[001543] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1 to about 10 core biopsies of tumor tissue from the subject.

[001544] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 core biopsies of tumor tissue from the subject.

[001545] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 core biopsies of tumor tissue from the subject.

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[001546] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1 to about 20 fine needle aspirates of tumor tissue from the subject.

[001547] praragraphs as applicable above modified such that the first population of TILs is obtained from 1 to about 10 fine needle aspirates of tumor tissue from the subject.

[001548] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 fine needle aspirates of tumor tissue from the subject.

[001549] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 fine needle aspirates of tumor tissue from the subject.

[001550] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1 to about 20 core needle biopsies of tumor tissue from the subject.

[001551] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1 to about 10 core needle biopsies of tumor tissue from the subject.

[001552] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 core needle biopsies of tumor tissue from the subject.

[001553] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 core needle biopsies of tumor tissue from the subject.

[001554] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1 to about 20 small biopsies (including, for example, a punch biopsy) of tumor tissue from the subject.

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[001555] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1 to about 10 small biopsies (including, for example, a punch biopsy) of tumor tissue from the subject.

[001556] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 small biopsies (including, for example, a punch biopsy) of tumor tissue from the subject.

[001557] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that the first population of TILs is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 small biopsies (including, for example, a punch biopsy) of tumor tissue from the subject.

[001558] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that (i) the method comprises obtaining the first population of TILs from 1 to about 10 core biopsies of tumor tissue from the subject, (ii) the method comprises performing the step of culturing the first population of TILs in a first cell culture medium comprising IL-2 for a period of about 3 days prior to performing the step of the priming first expansion, (iii) the method comprises performing the priming first expansion step by culturing the first population of TILs in a second cell culture medium comprising IL-2, OKT-3 and antigen presenting cells (APCs) for a period of about 8 days to obtain the second population of TILs, and (iv) the method comprises performing the rapid second expansion step by culturing the second population of TILs in a third cell culture medium comprising IL-2, OKT-3 and APCs for a period of about 11 days. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

[001559] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that (i) the method comprises obtaining the first population of TILs from 1 to about 10 core biopsies of tumor tissue from the subject, (ii) the method comprises performing the step of culturing the first population of TILs in a first cell culture medium comprising IL-2 for a period of about 3 days prior to performing the step of the priming first expansion, (iii) the method comprises performing the priming first expansion step by culturing the first population of TILs in a second cell culture medium comprising IL-2 and a culture supernatant obtained from a culture of antigen

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presenting cells (APCs) cultured in a cell culture medium supplemented with IL-2 and OKT-3 for a period of about 8 days to obtain the second population of TILs, and (iv) the method comprises performing the rapid second expansion step by culturing the second population of TILs in a third cell culture medium comprising IL-2, OKT-3 and APCs for a period of about 11 days. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

[001560] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that (i) the method comprises obtaining the first population of TILs from 1 to about 10 core biopsies of tumor tissue from the subject, (ii) the method comprises performing the step of culturing the first population of TILs in a first cell culture medium comprising IL-2 for a period of about 3 days prior to performing the step of the priming first expansion, (iii) the method comprises performing the priming first expansion step by culturing the first population of TILs in a second cell culture medium comprising IL-2, OKT-3 and antigen presenting cells (APCs) for a period of about 8 days to obtain the second population of TILs, and (iv) the method comprises performing the rapid second expansion step by culturing the second population of TILs for a period of about 11 days in a third culture medium comprising IL-2 and a culture supernatant obtained from a culture of APCs cultured in a cell culture medium supplemented with IL-2 and OKT-3. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

[001561] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that (i) the method comprises obtaining the first population of TILs from 1 to about 10 core biopsies of tumor tissue from the subject, (ii) the method comprises performing the step of culturing the first population of TILs in a first cell culture medium comprising IL-2 for a period of about 3 days prior to performing the step of the priming first expansion, (iii) the method comprises performing the priming first expansion step by culturing the first population of TILs for a period of about 8 days in a second cell culture medium comprising IL-2 and a first culture supernatant obtained from a first culture of antigen presenting cells (APCs) cultured in a cell culture medium supplemented with IL-2 and OKT-3 to obtain the second population of TILs, and (iv) the method comprises performing the rapid second expansion step by culturing the second population of TILs for a period of about 11 days in a third culture medium comprising IL-2 and a second culture supernatant obtained from a second culture of APCs cultured in a cell

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culture medium supplemented with IL-2 and OKT-3. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

[001562] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that (i) the method comprises obtaining the first population of TILs from 1 to about 10 core biopsies of tumor tissue from the subject, (ii) the method comprises performing the step of culturing the first population of TILs in a first cell culture medium comprising IL-2 for a period of about 3 days prior to performing the step of the priming first expansion, (iii) the method comprises performing the priming first expansion step by culturing the first population of TILs in a second cell culture medium comprising IL-2, OKT-3 and antigen presenting cells (APCs) for a period of about 8 days to obtain the second population of TILs, and (iv) the method comprises performing the rapid second expansion by culturing the culture of the second population of TILs in a third cell culture medium comprising IL-2, OKT-3 and APCs for about 5 days, splitting the culture into up to 5 subcultures and culturing each of the subcultures in a fourth cell culture medium comprising IL-2 for about 6 days. In some of the foregoing embodiments, the up to 5 subcultures are each cultured in a container that is the same size or larger than the container in which the culture of the second population of TILs is commenced in the rapid second expansion. In some of the foregoing embodiments, the culture of the second population of TILs is equally divided amongst the up to 5 subcultures. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

[001563] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that (i) the method comprises obtaining the first population of TILs from 1 to about 10 core biopsies of tumor tissue from the subject, (ii) the method comprises performing the step of culturing the first population of TILs in a first cell culture medium comprising IL-2 for a period of about 3 days prior to performing the step of the priming first expansion, (iii) the method comprises performing the priming first expansion step by culturing the first population of TILs for a period of about 8 days in a second cell culture medium comprising a culture supernatant obtained from a culture of antigen presenting cells (APCs) cultured in a cell culture medium supplemented with IL-2 and OKT-3 to obtain the second population of TILs, and (iv) the method comprises performing the rapid second expansion by culturing the culture of the second population of TILs in a third cell culture medium comprising IL-2, OKT-3 and APCs for about 5 days, splitting the culture into up to 5 subcultures and culturing each of the subcultures in a fourth

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cell culture medium comprising IL-2 for about 6 days. In some of the foregoing embodiments, the up to 5 subcultures are each cultured in a container that is the same size or larger than the container in which the culture of the second population of TILs is commenced in the rapid second expansion. In some of the foregoing embodiments, the culture of the second population of TILs is equally divided amongst the up to 5 subcultures. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

[001564] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that (i) the method comprises obtaining the first population of TILs from 1 to about 10 core biopsies of tumor tissue from the subject, (ii) the method comprises performing the step of culturing the first population of TILs in a first cell culture medium comprising IL-2 for a period of about 3 days prior to performing the step of the priming first expansion, (iii) the method comprises performing the priming first expansion step by culturing the first population of TILs in a second cell culture medium comprising IL-2, OKT-3 and antigen presenting cells (APCs) for a period of about 8 days to obtain the second population of TILs, and (iv) the method comprises performing the rapid second expansion by culturing the culture of the second population of TILs for about 5 days in a third cell culture medium comprising IL-2 and a culture supernatant obtained from a culture of APCs cultured in a cell culture medium supplemented with IL-2 and OKT-3, and then splitting the culture of the second population of TILs into up to 5 subcultures and culturing each of the subcultures in a fourth cell culture medium comprising IL-2 for about 6 days. In some of the foregoing embodiments, the up to 5 subcultures are each cultured in a container that is the same size or larger than the container in which the culture of the second population of TILs is commenced in the rapid second expansion. In some of the foregoing embodiments, the culture of the second population of TILs is equally divided amongst the up to 5 subcultures. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

[001565] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that (i) the method comprises obtaining the first population of TILs from 1 to about 10 core biopsies of tumor tissue from the subject, (ii) the method comprises performing the step of culturing the first population of TILs in a first cell culture medium comprising IL-2 for a period of about 3 days prior to performing the step of the priming first expansion, (iii) the method comprises performing the priming first expansion step by culturing the first population of TILs for a period of about 8

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days in a second cell culture medium comprising a first culture supernatant obtained from a first culture of antigen presenting cells (APCs) cultured in a cell culture medium supplemented with IL-2 and OKT-3 to obtain the second population of TILs, and (iv) the method comprises performing the rapid second expansion by culturing the culture of the second population of TILs for about 5 days in a third cell culture medium comprising IL-2 and a second culture supernatant obtained from a second culture of APCs cultured in a cell culture medium supplemented with IL-2 and OKT-3, and then splitting the culture of the second population of TILs into up to 5 subcultures and culturing each of the subcultures in a fourth cell culture medium comprising IL-2 for about 6 days. In some of the foregoing embodiments, the up to 5 subcultures are each cultured in a container that is the same size or larger than the container in which the culture of the second population of TILs is commenced in the rapid second expansion. In some of the foregoing embodiments, the culture of the second population of TILs is equally divided amongst the up to 5 subcultures. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

[001566] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that (i) the method comprises obtaining the first population of TILs from 1 to about 10 core biopsies of tumor tissue from the subject, (ii) the method comprises performing the step of culturing the first population of TILs in a cell culture medium comprising 6000 IU IL-2/ml in 0.5 L of CM1 culture medium in a G-Rex 100M flask for a period of about 3 days prior to performing the step of the priming first expansion, (iii) the method comprises performing the priming first expansion by adding 0.5 L of CM1 culture medium containing 6000 IU/ml IL-2, 30 ng/ml OKT-3, and about 10^8 feeder cells and culturing for a period of about 8 days, and (iv) the method comprises performing the rapid second expansion by (a) transferring the second population of TILs to a G-Rex 500MCS flask containing 5 L of CM2 culture medium with 3000 IU/ml IL-2, 30 ng/ml OKT-3, and $5x10^9$ feeder cells and culturing for about 5 days (b) splitting the culture into up to 5 subcultures by transferring 10^9 TILs into each of up to 5 G-Rex 500MCS flasks containing 5 L of AIM-V medium with 3000 IU/ml IL-2, and culturing the subcultures for about 6 days. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

[001567] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that (i) the method comprises obtaining the first population of TILs from 1 to about 10 core biopsies of tumor tissue from

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the subject, (ii) the method comprises performing the step of culturing the first population of TILs in a cell culture medium comprising 6000 IU IL-2/ml in 0.25 L of DM1 culture medium in a first G-Rex 100M flask for a period of about 3 days prior to performing the step of the priming first expansion, (iii) the method comprises performing the priming first expansion by adding 0.25 L of DM1 culture medium containing 6000 IU/ml IL-2, 30 ng/ml OKT-3, and about $2.5x10^8$ PBMC feeder cells and culturing for a period of about 8 days, and (iv) the method comprises performing the rapid second expansion by (a) adding 0.5 L of DM1 culture medium supplemented with 6000 IU/ml IL-2, 30 ng/ml OKT-3, and $5x10^8$ PBMC feeder cells and culturing for about 5 days (b) splitting the culture into up to 5 subcultures by transferring one-fifth of the culture into each of up to 5 G-Rex 500MCS flasks containing 5 L of DM2 with 3000 IU/ml IL-2, and culturing the subcultures for about 6 days. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

[001568] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that (i) the method comprises obtaining the first population of TILs from 1 to about 10 core biopsies of tumor tissue from the subject, (ii) the method comprises performing the step of culturing the first population of TILs in a first cell culture medium comprising 6000 IU IL-2/ml in 0.15 L of DM1 culture medium in a first G-Rex 100M flask for a period of about 3 days prior to performing the step of the priming first expansion, (iii) the method comprises performing the priming first expansion by adding 0.15 L of DM1 culture medium containing 6000 IU/ml IL-2 and 0.15 L of a culture supernatant obtained from a culture of $5x10^8$ PBMCs that has been cultured in a second G-Rex 100M flask for a period of about 3 days in 1 L of DM1 supplemented with 6000 IU IL-2/ml and 30 ng OKT-3/ml to the culture of the first population of TILs and culturing for a period of about 8 days, and (iv) the method comprises performing the rapid second expansion by (a) adding 0.5 L of DM1 culture medium supplemented with 6000 IU/ml IL-2, 30 ng/ml OKT-3, and $5x10^8$ PBMC feeder cells and culturing for about 5 days (b) splitting the culture into up to 5 subcultures by transferring one-fifth of the culture into each of up to 5 G-Rex 500MCS flasks containing 5 L of DM2 with 3000 IU/ml IL-2, and culturing the subcultures for about 6 days. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

[001569] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that (i) the method comprises obtaining the first population of TILs from 1 to about 10 core biopsies of tumor tissue from

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the subject, (ii) the method comprises performing the step of culturing the first population of TILs in a cell culture medium comprising 6000 IU IL-2/ml in 0.25 L of DM1 culture medium in a first G-Rex 100M flask for a period of about 3 days prior to performing the step of the priming first expansion, (iii) the method comprises performing the priming first expansion by adding 0.25 L of DM1 culture medium containing 6000 IU/ml IL-2, 30 ng/ml OKT-3, and about $2.5x10^8$ PBMC feeder cells and culturing for a period of about 8 days, and (iv) the method comprises performing the rapid second expansion by (a) adding 0.25 L of DM1 culture medium with 6000 IU/ml IL-2 and 0.25 L of a culture supernatant obtained from a culture of $5x10^8$ PBMCs cultured in a second G-Rex 100M flask for a period of about 3 days in 1L DM1 culture medium supplemented with 6000 IU IL-2/ml and 30 ng OKT-3/ml to the culture of the second population of TILs and culturing for about 5 days (b) splitting the culture of the second population of TILs into up to 5 subcultures by transferring one-fifth of the culture into each of up to 5 G-Rex 500MCS flasks containing 5 L of DM2 with 3000 IU/ml IL-2, and culturing the subcultures for about 6 days. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

[001570] In other embodiments, the invention provides the method described in any of the preceding praragraphs as applicable above modified such that (i) the method comprises obtaining the first population of TILs from 1 to about 10 core biopsies of tumor tissue from the subject, (ii) the method comprises performing the step of culturing the first population of TILs in a first cell culture medium comprising 6000 IU IL-2/ml in 0.15 L of DM1 culture medium in a first G-Rex 100M flask for a period of about 3 days prior to performing the step of the priming first expansion, (iii) the method comprises performing the priming first expansion by adding 0.15 L of DM1 culture medium containing 6000 IU/ml IL-2 and 0.15 L of a first culture supernatant obtained from a first culture of $5x10^8$ PBMCs that has been cultured in a second G-Rex 100M flask for a period of about 3 days in 1 L of DM1 supplemented with 6000 IU IL-2/ml and 30 ng OKT-3/ml to the culture of the first population of TILs and culturing for a period of about 8 days, and (iv) the method comprises performing the rapid second expansion by (a) adding 0.25 L of DM1 culture medium with 6000 IU/ml IL-2 and 0.25 L of a second culture supernatant obtained from a second culture of $5x10^8$ PBMCs cultured in a second G-Rex 100M flask for a period of about 3 days in 1L DM1 culture medium supplemented with 6000 IU IL-2/ml and 30 ng OKT-3/ml to the culture of the second population of TILs and culturing for about 5 days (b) splitting the culture of the second population of TILs into up to 5 subcultures by transferring one-fifth of the culture into

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each of up to 5 G-Rex 500MCS flasks containing 5 L of DM2 with 3000 IU/ml IL-2, and culturing the subcultures for about 6 days. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

[001571] In other embodiments, the invention 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. In other embodiments, the step of rapid second expansion is split into a plurality of steps to achieve a scaling up of the culture by: (a) performing the rapid second expansion by culturing the first population of T cells in a small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 3 to 4 days, and then (b) effecting the transfer of the first population of T cells from the small scale culture to a second container larger than the first container, *e.g.*, a G-REX 500MCS container, and culturing the first population of T cells from the small scale culture in a larger scale culture in the second container for a period of about 4 to 7 days. In other embodiments, the step of rapid expansion is split into a plurality of steps to achieve a scaling out of the culture by: (a) performing the rapid second expansion by culturing the first population of T cells in a first small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 3 to 4 days, and then (b) effecting the transfer and apportioning of the first population of T cells from the first small scale culture into and amongst at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 second containers that are equal in size to the first container, wherein in each second container the portion of the first population of T cells from first small scale culture transferred to such second container is cultured in a second small scale culture for a period of about 4 to 7 days. In other embodiments, the step of rapid expansion is split into a plurality of steps to achieve a scaling out and scaling up of the culture by: (a) performing the rapid second expansion by culturing the first population of T cells in a small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 3 to 4 days, and then (b) effecting the transfer and apportioning of the first population of T cells from the small scale culture into and amongst at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 second containers that are larger in size than the

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first container, *e.g.*, G-REX 500MCS containers, wherein in each second container the portion of the first population of T cells from the small scale culture transferred to such second container is cultured in a larger scale culture for a period of about 4 to 7 days. In other embodiments, the step of rapid expansion is split into a plurality of steps to achieve a scaling out and scaling up of the culture by: (a) performing the rapid second expansion by culturing the first population of T cells in a small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 4 days, and then (b) effecting the transfer and apportioning of the first population of T cells from the small scale culture into and amongst 2, 3 or 4 second containers that are larger in size than the first container, *e.g.*, G-REX 500MCS containers, wherein in each second container the portion of the first population of T cells from the small scale culture transferred to such second container is cultured in a larger scale culture for a period of about 5 days.

[001572] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of rapid second expansion is split into a plurality of steps to achieve a scaling up of the culture by: (a) performing the rapid second expansion by culturing the first population of T cells in a small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 2 to 4 days, and then (b) effecting the transfer of the first population of T cells from the small scale culture to a second container larger than the first container, *e.g.*, a G-REX 500MCS container, and culturing the first population of T cells from the small scale culture in a larger scale culture in the second container for a period of about 5 to 7 days.

[001573] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of rapid expansion is split into a plurality of steps to achieve a scaling out of the culture by: (a) performing the rapid second expansion by culturing the first population of T cells in a first small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 2 to 4 days, and then (b) effecting the transfer and apportioning of the first population of T cells from the first small scale culture into and amongst at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 second containers that are equal in size to the first container, wherein in each second container the portion of the first population of T cells from first small scale culture transferred to such second container is cultured in a second small scale culture for a period of about 5 to 7 days.

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[001574] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of rapid expansion is split into a plurality of steps to achieve a scaling out and scaling up of the culture by: (a) performing the rapid second expansion by culturing the first population of T cells in a small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 2 to 4 days, and then (b) effecting the transfer and apportioning of the first population of T cells from the small scale culture into and amongst at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 second containers that are larger in size than the first container, *e.g.*, G-REX 500MCS containers, wherein in each second container the portion of the first population of T cells from the small scale culture transferred to such second container is cultured in a larger scale culture for a period of about 5 to 7 days.

[001575] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of rapid expansion is split into a plurality of steps to achieve a scaling out and scaling up of the culture by: (a) performing the rapid second expansion by culturing the first population of T cells in a small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 3 to 4 days, and then (b) effecting the transfer and apportioning of the first population of T cells from the small scale culture into and amongst 2, 3 or 4 second containers that are larger in size than the first container, *e.g.*, G-REX 500MCS containers, wherein in each second container the portion of the first population of T cells from the small scale culture transferred to such second container is cultured in a larger scale culture for a period of about 5 to 6 days.

[001576] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of rapid expansion is split into a plurality of steps to achieve a scaling out and scaling up of the culture by: (a) performing the rapid second expansion by culturing the first population of T cells in a small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 3 to 4 days, and then (b) effecting the transfer and apportioning of the first population of T cells from the small scale culture into and amongst 2, 3 or 4 second containers that are larger in size than the first container, *e.g.*, G-REX 500MCS containers, wherein in each second container the portion of the first population of T cells from the small scale culture transferred to such second container is cultured in a larger scale culture for a period of about 5 days.

[001577] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of rapid expansion

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is split into a plurality of steps to achieve a scaling out and scaling up of the culture by: (a) performing the rapid second expansion by culturing the first population of T cells in a small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 3 to 4 days, and then (b) effecting the transfer and apportioning of the first population of T cells from the small scale culture into and amongst 2, 3 or 4 second containers that are larger in size than the first container, *e.g.*, G-REX 500MCS containers, wherein in each second container the portion of the first population of T cells from the small scale culture transferred to such second container is cultured in a larger scale culture for a period of about 6 days.

[001578] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of rapid expansion is split into a plurality of steps to achieve a scaling out and scaling up of the culture by: (a) performing the rapid second expansion by culturing the first population of T cells in a small scale culture in a first container, *e.g.*, a G-REX 100MCS container, for a period of about 3 to 4 days, and then (b) effecting the transfer and apportioning of the first population of T cells from the small scale culture into and amongst 2, 3 or 4 second containers that are larger in size than the first container, *e.g.*, G-REX 500MCS containers, wherein in each second container the portion of the first population of T cells from the small scale culture transferred to such second container is cultured in a larger scale culture for a period of about 7 days.

[001579] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the priming first expansion of step (a) is performed during a period of up to 7 days.

[001580] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the rapid second expansion of step (b) is performed during a period of up to 8 days.

[001581] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the rapid second expansion of step (b) is performed during a period of up to 9 days.

[001582] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the rapid second expansion of step (b) is performed during a period of up to 10 days.

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[001583] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the rapid second expansion of step (b) is performed during a period of up to 11 days.

[001584] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the priming first expansion in step (a) is performed during a period of 7 days and the rapid second expansion of step (b) is performed during a period of up to 9 days.

[001585] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the priming first expansion in step (a) is performed during a period of 7 days and the rapid second expansion of step (b) is performed during a period of up to 10 days.

[001586] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the priming first expansion in step (a) is performed during a period of 7 days or 8 days and the rapid second expansion of step (b) is performed during a period of up to 9 days.

[001587] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the priming first expansion in step (a) is performed during a period of 7 days or 8 days and the rapid second expansion of step (b) is performed during a period of up to 10 days.

[001588] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the priming first expansion in step (a) is performed during a period of 8 days and the rapid second expansion of step (b) is performed during a period of up to 9 days.

[001589] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the priming first expansion in step (a) is performed during a period of 8 days and the rapid second expansion of step (b) is performed during a period of up to 8 days.

[001590] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (a) the first population of T cells is cultured in a first culture medium comprising OKT-3 and IL-2.

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[001591] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first culture medium comprises 4-1BB agonist, OKT-3 and IL-2.

[001592] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first culture medium comprises OKT-3, IL-2 and antigen-presenting cells (APCs).

[001593] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first culture medium comprises 4-1BB agonist, OKT-3, IL-2 and antigen-presenting cells (APCs).

[001594] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that 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).

[001595] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the second culture medium comprises 4-1BB agonist, OKT-3, IL-2 and antigen-presenting cells (APCs).

[001596] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that 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 OKT-3, IL-2 and 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.

[001597] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that 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 4-1BB agonist, OKT-3,

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IL-2 and 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.

[001598] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that 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 OKT-3, IL-2 and 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 4-1BB agonist, 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.

[001599] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that 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 4-1BB agonist, OKT-3, IL-2 and 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 4-1BB agonist, 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.

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[001600] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that 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.

[001601] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the number of APCs in the first population of APCs is about 2.5×10^8 and the number of APCs in the second population of APCs is about 5×10^8.

[001602] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that 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.

[001603] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the second population of APCs is layered onto the first gas-permeable surface at an average thickness in the range of 4 to 8 layers of APCs.

[001604] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that 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.

[001605] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (a) the first population of APCs is seeded on the first gas permeable surface at a density in the range of at or about 1.0×10^6 APCs/cm^2 to at or about 4.5×10^6 APCs/cm^2.

[001606] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (a) the first population of APCs is seeded on the first gas permeable surface at a density in the range of at or about 1.5×10^6 APCs/cm^2 to at or about 3.5×10^6 APCs/cm^2.

[001607] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (a) the first population of APCs is seeded on the first gas permeable surface at a density in the range of at or about 2.0×10^6 APCs/cm^2 to at or about 3.0×10^6 APCs/cm^2.

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[001608] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (a) the first population of APCs is seeded on the first gas permeable surface at a density of at or about 2.0×10^6 APCs/cm^2.

[001609] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the second population of APCs is seeded on the first gas permeable surface at a density in the range of at or about 2.5×10^6 APCs/cm^2 to at or about 7.5×10^6 APCs/cm^2.

[001610] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the second population of APCs is seeded on the first gas permeable surface at a density in the range of at or about 3.5×10^6 APCs/cm^2 to at or about 6.0×10^6 APCs/cm^2.

[001611] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the second population of APCs is seeded on the first gas permeable surface at a density in the range of at or about 4.0×10^6 APCs/cm^2 to at or about 5.5×10^6 APCs/cm^2.

[001612] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (b) the second population of APCs is seeded on the first gas permeable surface at a density of at or about 4.0×10^6 APCs/cm^2.

[001613] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (a) the first population of APCs is seeded on the first gas permeable surface at a density in the range of at or about 1.0×10^6 APCs/cm^2 to at or about 4.5×10^6 APCs/cm^2 and in step (b) the second population of APCs is seeded on the first gas permeable surface at a density in the range of at or about 2.5×10^6 APCs/cm^2 to at or about 7.5×10^6 APCs/cm^2.

[001614] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable modified such that in step (a) the first population of APCs is seeded on the first gas permeable surface at a density in the range of at or about 1.5×10^6 APCs/cm^2 to at or about 3.5×10^6 APCs/cm^2 and in step (b) the second population of APCs is seeded on the first gas permeable surface at a density in the range of at or about 3.5×10^6 APCs/cm^2 to at or about 6.0×10^6 APCs/cm^2.

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[001615] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (a) the first population of APCs is seeded on the first gas permeable surface at a density in the range of at or about 2.0×10^6 APCs/cm^2 to at or about 3.0×10^6 APCs/cm^2 and in step (b) the second population of APCs is seeded on the first gas permeable surface at a density in the range of at or about 4.0×10^6 APCs/cm^2 to at or about 5.5×10^6 APCs/cm^2.

[001616] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that in step (a) the first population of APCs is seeded on the first gas permeable surface at a density of at or about 2.0×10^6 APCs/cm^2 and in step (b) the second population of APCs is seeded on the first gas permeable surface at a density of at or about 4.0×10^6 APCs/cm^2.

[001617] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the APCs are peripheral blood mononuclear cells (PBMCs).

[001618] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the PBMCs are irradiated and exogenous to the donor of the first population of T cells.

[001619] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the T cells are tumor infiltrating lymphocytes (TILs).

[001620] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the T cells are marrow infiltrating lymphocytes (MILs).

[001621] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the T cells are peripheral blood lymphocytes (PBLs).

[001622] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained by separation from the whole blood of the donor.

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[001623] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained by separation from the apheresis product of the donor.

[001624] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is separated from the whole blood or apheresis product of the donor by positive or negative selection of a T cell phenotype.

[001625] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the T cell phenotype is CD3+ and CD45+.

[001626] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that before performing the priming first expansion of the first population of T cells the T cells are separated from NK cells. In other embodiments, the T cells are separated from NK cells in the first population of T cells by removal of CD3- CD56+ cells from the first population of T cells. In other embodiments, the CD3- CD56+ cells are removed from the first population of T cells by subjecting the first population of T cells to cell sorting using a gating strategy that removes the CD3- CD56+ cell fraction and recovers the negative fraction. In other embodiments, the foregoing method is utilized for the expansion of T cells in a first population of T cells characterized by a high percentage of NK cells. In other embodiments, the foregoing method is utilized for the expansion of T cells in a first population of T cells characterized by a high percentage of CD3- CD56+ cells. In other embodiments, the foregoing method is utilized for the expansion of T cells in tumor tissue characterized by the present of a high number of NK cells. In other embodiments, the foregoing method is utilized for the expansion of T cells in tumor tissue characterized by a high number of CD3- CD56+ cells. In other embodiments, the foregoing method is utilized for the expansion of T cells in tumor tissue obtained from a patient suffering from a tumor characterized by the presence of a high number of NK cells. In other embodiments, the foregoing method is utilized for the expansion of T cells in tumor tissue obtained from a patient suffering from a tumor characterized by the presence of a high number of CD3- CD56+ cells. In other embodiments, the foregoing method is utilized for the expansion of T cells in tumor tissue obtained from a patient suffering from ovarian cancer.

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[001627] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that at or about 1×10^7 T cells from the first population of T cells are seeded in a container to initiate the primary first expansion culture in such container.

[001628] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is distributed into a plurality of containers, and in each container at or about 1×10^7 T cells from the first population of T cells are seeded to initiate the primary first expansion culture in such container.

[001629] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the second population of T cells harvested in step (c) is a therapeutic population of TILs.

[001630] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from one or more small biopsies (including, for example, a punch biopsy), core biopsies, core needle biopsies or fine needle aspirates of tumor tissue from the donor.

[001631] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1 to 20 small biopsies (including, for example, a punch biopsy), core biopsies, core needle biopsies or fine needle aspirates of tumor tissue from the donor.

[001632] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1 to 10 small biopsies (including, for example, a punch biopsy), core biopsies, core needle biopsies or fine needle aspirates of tumor tissue from the donor.

[001633] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 small biopsies (including, for example, a punch biopsy), core biopsies, core needle biopsies or fine needle aspirates of tumor tissue from the donor.

[001634] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 small biopsies (including, for example, a punch

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biopsy), core biopsies, core needle biopsies or fine needle aspirates of tumor tissue from the donor.

[001635] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from one or more core biopsies of tumor tissue from the donor.

[001636] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1 to 20 core biopsies of tumor tissue from the donor.

[001637] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1 to 10 core biopsies of tumor tissue from the donor.

[001638] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 core biopsies of tumor tissue from the donor.

[001639] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 core biopsies of tumor tissue from the donor.

[001640] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from one or more fine needle aspirates of tumor tissue from the donor.

[001641] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1 to 20 fine needle aspirates of tumor tissue from the donor.

[001642] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1 to 10 fine needle aspirates of tumor tissue from the donor.

[001643] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 fine needle aspirates of tumor tissue from the donor.

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[001644] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 fine needle aspirates of tumor tissue from the donor.

[001645] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from one or more small biopsies (including, for example, a punch biopsy) of tumor tissue from the donor.

[001646] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1 to 20 small biopsies (including, for example, a punch biopsy) of tumor tissue from the donor.

[001647] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1 to 10 small biopsies (including, for example, a punch biopsy) of tumor tissue from the donor.

[001648] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 small biopsies (including, for example, a punch biopsy) of tumor tissue from the donor.

[001649] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 small biopsies (including, for example, a punch biopsy) of tumor tissue from the donor.

[001650] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from one or more core needle biopsies of tumor tissue from the donor.

[001651] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1 to 20 core needle biopsies of tumor tissue from the donor.

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[001652] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1 to 10 core needle biopsies of tumor tissue from the donor.

[001653] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 core needle biopsies of tumor tissue from the donor.

[001654] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the first population of T cells is obtained from 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 core needle biopsies of tumor tissue from the donor.

[001655] In other embodiments, the invention provides 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 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.

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[001656] In other embodiments, the invention provides 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 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).

[001657] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that (i) the method comprises performing the priming first expansion step by culturing the first population of TILs for a period of about 8 days in the second cell culture medium comprising a culture supernatant obtained from a culture of antigen presenting cells (APCs) cultured in a cell culture medium supplemented with IL-2 and OKT-3 to obtain the second population of TILs, wherein the culture of the first population of TILs is not supplemented with APCs, and (ii) the method comprises performing the rapid second expansion by culturing the second population of TILs in the third cell culture medium comprising IL-2, OKT-3 and APCs for about 5 days, splitting the culture into up to 5 subcultures and culturing each of the subcultures in a fourth cell culture medium comprising IL-2 for about 6 days. In some of the foregoing embodiments, the up to 5 subcultures are each cultured in a container that is the same size or larger than the container in which the culture of the second population of TILs is commenced in the rapid second expansion. In some of the foregoing embodiments, the culture of the second population of TILs is equally divided amongst the up to 5 subcultures. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

[001658] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that (i) the method comprises

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performing the priming first expansion step by culturing the first population of TILs in the second cell culture medium comprising IL-2, OKT-3 and antigen presenting cells (APCs) for a period of about 8 days to obtain the second population of TILs, and (ii) the method comprises performing the rapid second expansion by culturing the second population of TILs for about 5 days in a third cell culture medium comprising IL-2 and a culture supernatant obtained from a culture of APCs cultured in a cell culture medium supplemented with IL-2 and OKT-3, wherein the culture of the second population of TILs is not supplemented with APCs, and then splitting the culture of the second population of TILs into up to 5 subcultures and culturing each of the subcultures in a fourth cell culture medium comprising IL-2 for about 6 days. In some of the foregoing embodiments, the up to 5 subcultures are each cultured in a container that is the same size or larger than the container in which the culture of the second population of TILs is commenced in the rapid second expansion. In some of the foregoing embodiments, the culture of the second population of TILs is equally divided amongst the up to 5 subcultures. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

[001659] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that (i) the method comprises performing the priming first expansion step by culturing the first population of TILs for a period of about 8 days in the second cell culture medium comprising a first culture supernatant obtained from a first culture of antigen presenting cells (APCs) cultured in a cell culture medium supplemented with IL-2 and OKT-3 to obtain the second population of TILs, wherein the culture of the first population of TILs is not supplemented with APCs, and (iii) the method comprises performing the rapid second expansion by culturing the second population of TILs for about 5 days in a third cell culture medium comprising IL-2 and a second culture supernatant obtained from a second culture of APCs cultured in a cell culture medium supplemented with IL-2 and OKT-3, wherein the culture of the second population of TILs is not supplemented with APCs, and then splitting the culture of the second population of TILs into up to 5 subcultures and culturing each of the subcultures in a fourth cell culture medium comprising IL-2 for about 6 days. In some of the foregoing embodiments, the up to 5 subcultures are each cultured in a container that is the same size or larger than the container in which the culture of the second population of TILs is commenced in the rapid second expansion. In some of the foregoing embodiments, the culture of the second population of

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TILs is equally divided amongst the up to 5 subcultures. In some of the foregoing embodiments, the steps of the method are completed in about 22 days.

[001660] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that after day 5 of the second period the culture is split into 2 or more subcultures, and each subculture is supplemented with an additional quantity of the third culture medium and cultured for about 6 days.

[001661] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that 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.

[001662] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that after day 5 of the second period the culture is split into up to 5 subcultures.

[001663] In other embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that all steps in the method are completed in about 22 days.

[001664] In other embodiments, the invention provides a method of expanding T cells comprising: (i) performing a priming first expansion of a first population of T cells from a tumor sample 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. In some embodiments, the tumor sample is obtained from a plurality of core biopsies. In some embodiments, the plurality of core biopsies is selected from the group consisting of 2, 3, 4, 5, 6, 7, 8, 9 and 10 core biopsies.

[001665] In some embodiments, the rapid second expansion occurs as two periods, comprising an activation II period followed by a split or division and further growth period within the rapid second expansion. In some embodiments, the rapid second expansion occurs for for a period of 1 to 11 days. In some embodiments, rapid second expansion occurs for for a period of 1 to 10 days, resulting in a bulk TIL population. In some embodiments, the rapid

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second expansion occurs for for a period of 1 to 9 days. In some embodiments, rapid second expansion occurs for for a period of 1 to 8 days. In some embodiments, rapid second expansion comprises an activation II period of 1 to 4 days followed by a split or division and further growth period of 1 to 7 days. In some embodiments, rapid second expansion comprises an activation II period of 1 to 3 days followed by a split or division and further growth period of 1 to 6 days. In some embodiments, rapid second expansion comprises an activation II period of 1 to 4 days followed by a split and further growth period of 1 to 6 days. In some embodiments, rapid second expansion comprises an activation II period of 1 to 7 days followed by a split or division and further growth period of 1 to 7 days. In some embodiments, rapid second expansion comprises an activation II period of 1 to 3 days followed by a split or division and further growth period of 1 to 7 days. In some embodiments, rapid second expansion comprises an activation II period of 1 day, 2 days, 3 days, or 4 days followed by a split or division and further growth period of 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days. In some embodiments, the split or division can also include a scale-up to an increased number of containers (including, for example, bags and/or GREX containers). In some embodiments, the split or division can also include a scale-up to an increased number of containers (including, for example, bags and/or GREX containers) from the number of containers during the activation II step to the increased number of containers during the further growth period.

[001666] In some embodiments, the method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprises:

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

obtaining a first population of TILs from a tumor resected from a patient by processing a tumor sample obtained from the patient into a tumor digest;

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

(c) performing a first expansion or priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or priming first expansion is optionally performed in a closed container providing a first gas-permeable surface

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area, wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about to about 1 to 5 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 4 to 8 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

(f) harvesting the second plurality of subpopulations of TILs obtained from step (e), wherein the transition from step (e) to step (f) occurs without opening the system when optionally performed in a closed system; and

(g) transferring the harvested subpopulations of TILs from step (g) to one or more infusion bags, wherein the transition from step (f) to (g) occurs without opening the system when optionally performed in a closed system.

[001667] In some embodiments, the invention provides a tumor infiltrating lymphocyte (TIL) composition comprising a therapeutic population of infiltrating lymphocytes (TILs), wherein the TIL composition is produced by a method comprising:

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

obtaining a first population of TILs from a tumor resected from a patient by processing a tumor sample obtained from the patient into a tumor digest;

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

(c) performing a first expansion or priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about to about 1 to 5 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 4 to 8 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

(f) harvesting the second plurality of subpopulations of TILs obtained from step (e), wherein the transition from step (e) to step (f) occurs without opening the system when optionally performed in a closed system; and

(g) transferring the harvested subpopulations of TILs from step (g) to one or more infusion bags, wherein the transition from step (f) to (g) occurs without opening the system when optionally performed in a closed system.

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[001668] In some embodiments, the TIL composition is a cryopreserved composition and wherein the method further comprises (h) cryopreserving the infusion bag comprising the harvested TIL population from step (g) using a cryopreservation process.

[001669] In some embodiments, the invention provides a method for treating a subject with cancer, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

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

obtaining a first population of TILs from a tumor resected from a patient by processing a tumor sample obtained from the patient into a tumor digest;

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

(c) performing a first expansion or priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about to about 1 to 5 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 4 to 8 days, wherein optionally each separate container is a closed container

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providing a third gas-permeable surface area, and wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

(f) harvesting the second plurality of subpopulations of TILs obtained from step (e), wherein the transition from step (e) to step (f) occurs without opening the system when optionally performed in a closed system;

(g) transferring the harvested subpopulations of TILs from step (g) to one or more infusion bags, wherein the transition from step (f) to (g) occurs without opening the system when optionally performed in a closed system; and

(h) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (g) to the subject.

[001670] In some embodiments, the first expansion or priming first expansion, rapid second expansion, third expansion, and the third expansion are each performed for about 5-7 days, about 5-6 days, or about 6-7 days. In some embodiments, the first expansion or priming first expansion, rapid second expansion, third expansion, and the third expansion are each performed for about 5 days, 6 days, or 7 days. In some embodiments, the first expansion or priming first expansion, rapid second expansion, third expansion, and the third expansion are each performed for about 5 days. In some embodiments, the first expansion or priming first expansion, rapid second expansion, third expansion, and the third expansion are each performed for about 6 days. In some embodiments, the first expansion or priming first expansion, rapid second expansion, third expansion, and the third expansion are each performed for about 7 days. In some embodiments, the first plurality of subpopulations comprises about 2-10 subpopulations, about 2-9 subpopulations, about 2-8 subpopulations, about 2-7 subpopulations, about 2-6 subpopulations, about 2-5 subpopulations, about 2-4 subpopulations, or about 2-3 subpopulations.

III. Pharmaceutical Compositions, Dosages, and Dosing Regimens

[001671] In some embodiments, TILs expanded using the methods of the present disclosure are administered to a patient as a pharmaceutical composition. In some embodiments, the pharmaceutical composition is a suspension of TILs in a sterile buffer. TILs expanded using PBMCs of the present disclosure may be administered by any suitable route as known in the art. In some embodiments, the T-cells are administered as a single intra-arterial or intravenous infusion, which preferably lasts approximately 30 to 60 minutes. Other suitable

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routes of administration include intraperitoneal, intrathecal, and intralymphatic administration.

[001672] Any suitable dose of TILs can be administered. In some embodiments, from about 2.3×10^{10} to about 13.7×10^{10} TILs are administered, with an average of around 7.8×10^{10} TILs, particularly if the cancer is melanoma. In some embodiments, about 1.2×10^{10} to about 4.3×10^{10} of TILs are administered. In some embodiments, about 3×10^{10} to about 12×10^{10} TILs are administered. In some embodiments, about 4×10^{10} to about 10×10^{10} TILs are administered. In some embodiments, about 5×10^{10} to about 8×10^{10} TILs are administered. In some embodiments, about 6×10^{10} to about 8×10^{10} TILs are administered. In some embodiments, about 7×10^{10} to about 8×10^{10} TILs are administered. In some embodiments, the therapeutically effective dosage is about 2.3×10^{10} to about 13.7×10^{10}. In some embodiments, the therapeutically effective dosage is about 7.8×10^{10} TILs, particularly of the cancer is melanoma. In some embodiments, the therapeutically effective dosage is about 1.2×10^{10} to about 4.3×10^{10} of TILs. In some embodiments, the therapeutically effective dosage is about 3×10^{10} to about 12×10^{10} TILs. In some embodiments, the therapeutically effective dosage is about 4×10^{10} to about 10×10^{10} TILs. In some embodiments, the therapeutically effective dosage is about 5×10^{10} to about 8×10^{10} TILs. In some embodiments, the therapeutically effective dosage is about 6×10^{10} to about 8×10^{10} TILs. In some embodiments, the therapeutically effective dosage is about 7×10^{10} to about 8×10^{10} TILs.

[001673] In some embodiments, the number of the TILs provided in the pharmaceutical compositions of the invention is about 1×10^{6}, 2×10^{6}, 3×10^{6}, 4×10^{6}, 5×10^{6}, 6×10^{6}, 7×10^{6}, 8×10^{6}, 9×10^{6}, 1×10^{7}, 2×10^{7}, 3×10^{7}, 4×10^{7}, 5×10^{7}, 6×10^{7}, 7×10^{7}, 8×10^{7}, 9×10^{7}, 1×10^{8}, 2×10^{8}, 3×10^{8}, 4×10^{8}, 5×10^{8}, 6×10^{8}, 7×10^{8}, 8×10^{8}, 9×10^{8}, 1×10^{9}, 2×10^{9}, 3×10^{9}, 4×10^{9}, 5×10^{9}, 6×10^{9}, 7×10^{9}, 8×10^{9}, 9×10^{9}, 1×10^{10}, 2×10^{10}, 3×10^{10}, 4×10^{10}, 5×10^{10}, 6×10^{10}, 7×10^{10}, 8×10^{10}, 9×10^{10}, 1×10^{11}, 2×10^{11}, 3×10^{11}, 4×10^{11}, 5×10^{11}, 6×10^{11}, 7×10^{11}, 8×10^{11}, 9×10^{11}, 1×10^{12}, 2×10^{12}, 3×10^{12}, 4×10^{12}, 5×10^{12}, 6×10^{12}, 7×10^{12}, 8×10^{12}, 9×10^{12}, 1×10^{13}, 2×10^{13}, 3×10^{13}, 4×10^{13}, 5×10^{13}, 6×10^{13}, 7×10^{13}, 8×10^{13}, and 9×10^{13}. In some embodiments, the number of the TILs provided in the pharmaceutical compositions of the invention is in the range of 1×10^{6} to 5×10^{6}, 5×10^{6} to 1×10^{7}, 1×10^{7} to 5×10^{7}, 5×10^{7} to 1×10^{8}, 1×10^{8} to 5×10^{8}, 5×10^{8} to 1×10^{9}, 1×10^{9} to 5×10^{9}, 5×10^{9} to 1×10^{10}, 1×10^{10} to 5×10^{10}, 5×10^{10} to 1×10^{11}, 5×10^{11} to 1×10^{12}, 1×10^{12} to 5×10^{12}, and 5×10^{12} to 1×10^{13}.

[001674] In some embodiments, the concentration of the TILs provided in the pharmaceutical compositions of the invention is less than, for example, 100%, 90%, 80%,

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70%, 60%, 50%, 40%, 30%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.09%, 0.08%, 0.07%, 0.06%, 0.05%, 0.04%, 0.03%, 0.02%, 0.01%, 0.009%, 0.008%, 0.007%, 0.006%, 0.005%, 0.004%, 0.003%, 0.002%, 0.001%, 0.0009%, 0.0008%, 0.0007%, 0.0006%, 0.0005%, 0.0004%, 0.0003%, 0.0002% or 0.0001% w/w, w/v or v/v of the pharmaceutical composition.

[001675] In some embodiments, the concentration of the TILs provided in the pharmaceutical compositions of the invention is greater than 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 19.75%, 19.50%, 19.25% 19%, 18.75%, 18.50%, 18.25% 18%, 17.75%, 17.50%, 17.25% 17%, 16.75%, 16.50%, 16.25% 16%, 15.75%, 15.50%, 15.25% 15%, 14.75%, 14.50%, 14.25% 14%, 13.75%, 13.50%, 13.25% 13%, 12.75%, 12.50%, 12.25% 12%, 11.75%, 11.50%, 11.25% 11%, 10.75%, 10.50%, 10.25% 10%, 9.75%, 9.50%, 9.25% 9%, 8.75%, 8.50%, 8.25% 8%, 7.75%, 7.50%, 7.25% 7%, 6.75%, 6.50%, 6.25% 6%, 5.75%, 5.50%, 5.25% 5%, 4.75%, 4.50%, 4.25%, 4%, 3.75%, 3.50%, 3.25%, 3%, 2.75%, 2.50%, 2.25%, 2%, 1.75%, 1.50%, 125%, 1%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.09%, 0.08%, 0.07%, 0.06%, 0.05%, 0.04%, 0.03%, 0.02%, 0.01%, 0.009%, 0.008%, 0.007%, 0.006%, 0.005%, 0.004%, 0.003%, 0.002%, 0.001%, 0.0009%, 0.0008%, 0.0007%, 0.0006%, 0.0005%, 0.0004%, 0.0003%, 0.0002% or 0.0001% w/w, w/v, or v/v of the pharmaceutical composition.

[001676] In some embodiments, the concentration of the TILs provided in the pharmaceutical compositions of the invention is in the range from about 0.0001% to about 50%, about 0.001% to about 40%, about 0.01% to about 30%, about 0.02% to about 29%, about 0.03% to about 28%, about 0.04% to about 27%, about 0.05% to about 26%, about 0.06% to about 25%, about 0.07% to about 24%, about 0.08% to about 23%, about 0.09% to about 22%, about 0.1% to about 21%, about 0.2% to about 20%, about 0.3% to about 19%, about 0.4% to about 18%, about 0.5% to about 17%, about 0.6% to about 16%, about 0.7% to about 15%, about 0.8% to about 14%, about 0.9% to about 12% or about 1% to about 10% w/w, w/v or v/v of the pharmaceutical composition.

[001677] In some embodiments, the concentration of the TILs provided in the pharmaceutical compositions of the invention is in the range from about 0.001% to about 10%, about 0.01% to about 5%, about 0.02% to about 4.5%, about 0.03% to about 4%, about 0.04% to about 3.5%, about 0.05% to about 3%, about 0.06% to about 2.5%, about 0.07% to

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about 2%, about 0.08% to about 1.5%, about 0.09% to about 1%, about 0.1% to about 0.9% w/w, w/v or v/v of the pharmaceutical composition.

[001678] In some embodiments, the amount of the TILs provided in the pharmaceutical compositions of the invention is equal to or less than 10 g, 9.5 g, 9.0 g, 8.5 g, 8.0 g, 7.5 g, 7.0 g, 6.5 g, 6.0 g, 5.5 g, 5.0 g, 4.5 g, 4.0 g, 3.5 g, 3.0 g, 2.5 g, 2.0 g, 1.5 g, 1.0 g, 0.95 g, 0.9 g, 0.85 g, 0.8 g, 0.75 g, 0.7 g, 0.65 g, 0.6 g, 0.55 g, 0.5 g, 0.45 g, 0.4 g, 0.35 g, 0.3 g, 0.25 g, 0.2 g, 0.15 g, 0.1 g, 0.09 g, 0.08 g, 0.07 g, 0.06 g, 0.05 g, 0.04 g, 0.03 g, 0.02 g, 0.01 g, 0.009 g, 0.008 g, 0.007 g, 0.006 g, 0.005 g, 0.004 g, 0.003 g, 0.002 g, 0.001 g, 0.0009 g, 0.0008 g, 0.0007 g, 0.0006 g, 0.0005 g, 0.0004 g, 0.0003 g, 0.0002 g, or 0.0001 g.

[001679] In some embodiments, the amount of the TILs provided in the pharmaceutical compositions of the invention is more than 0.0001 g, 0.0002 g, 0.0003 g, 0.0004 g, 0.0005 g, 0.0006 g, 0.0007 g, 0.0008 g, 0.0009 g, 0.001 g, 0.0015 g, 0.002 g, 0.0025 g, 0.003 g, 0.0035 g, 0.004 g, 0.0045 g, 0.005 g, 0.0055 g, 0.006 g, 0.0065 g, 0.007 g, 0.0075 g, 0.008 g, 0.0085 g, 0.009 g, 0.0095 g, 0.01 g, 0.015 g, 0.02 g, 0.025 g, 0.03 g, 0.035 g, 0.04 g, 0.045 g, 0.05 g, 0.055 g, 0.06 g, 0.065 g, 0.07 g, 0.075 g, 0.08 g, 0.085 g, 0.09 g, 0.095 g, 0.1 g, 0.15 g, 0.2 g, 0.25 g, 0.3 g, 0.35 g, 0.4 g, 0.45 g, 0.5 g, 0.55 g, 0.6 g, 0.65 g, 0.7 g, 0.75 g, 0.8 g, 0.85 g, 0.9 g, 0.95 g, 1 g, 1.5 g, 2 g, 2.5, 3 g, 3.5, 4 g, 4.5 g, 5 g, 5.5 g, 6 g, 6.5 g, 7 g, 7.5 g, 8 g, 8.5 g, 9 g, 9.5 g, or 10 g.

[001680] The TILs provided in the pharmaceutical compositions of the invention are effective over a wide dosage range. The exact dosage will depend upon the route of administration, the form in which the compound is administered, the gender and age of the subject to be treated, the body weight of the subject to be treated, and the preference and experience of the attending physician. The clinically-established dosages of the TILs may also be used if appropriate. The amounts of the pharmaceutical compositions administered using the methods herein, such as the dosages of TILs, will be dependent on the human or mammal being treated, the severity of the disorder or condition, the rate of administration, the disposition of the active pharmaceutical ingredients and the discretion of the prescribing physician.

[001681] In some embodiments, TILs may be administered in a single dose. Such administration may be by injection, *e.g.*, intravenous injection. In some embodiments, TILs may be administered in multiple doses. Dosing may be once, twice, three times, four times, five times, six times, or more than six times per year. Dosing may be once a month, once

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every two weeks, once a week, or once every other day. Administration of TILs may continue as long as necessary.

[001682] In some embodiments, an effective dosage of TILs is about 1×10^6, 2×10^6, 3×10^6, 4×10^6, 5×10^6, 6×10^6, 7×10^6, 8×10^6, 9×10^6, 1×10^7, 2×10^7, 3×10^7, 4×10^7, 5×10^7, 6×10^7, 7×10^7, 8×10^7, 9×10^7, 1×10^8, 2×10^8, 3×10^8, 4×10^8, 5×10^8, 6×10^8, 7×10^8, 8×10^8, 9×10^8, 1×10^9, 2×10^9, 3×10^9, 4×10^9, 5×10^9, 6×10^9, 7×10^9, 8×10^9, 9×10^9, 1×10^{10}, 2×10^{10}, 3×10^{10}, 4×10^{10}, 5×10^{10}, 6×10^{10}, 7×10^{10}, 8×10^{10}, 9×10^{10}, 1×10^{11}, 2×10^{11}, 3×10^{11}, 4×10^{11}, 5×10^{11}, 6×10^{11}, 7×10^{11}, 8×10^{11}, 9×10^{11}, 1×10^{12}, 2×10^{12}, 3×10^{12}, 4×10^{12}, 5×10^{12}, 6×10^{12}, 7×10^{12}, 8×10^{12}, 9×10^{12}, 1×10^{13}, 2×10^{13}, 3×10^{13}, 4×10^{13}, 5×10^{13}, 6×10^{13}, 7×10^{13}, 8×10^{13}, and 9×10^{13}. In some embodiments, an effective dosage of TILs is in the range of 1×10^6 to 5×10^6, 5×10^6 to 1×10^7, 1×10^7 to 5×10^7, 5×10^7 to 1×10^8, 1×10^8 to 5×10^8, 5×10^8 to 1×10^9, 1×10^9 to 5×10^9, 5×10^9 to 1×10^{10}, 1×10^{10} to 5×10^{10}, 5×10^{10} to 1×10^{11}, 5×10^{11} to 1×10^{12}, 1×10^{12} to 5×10^{12}, and 5×10^{12} to 1×10^{13}.

[001683] In some embodiments, an effective dosage of TILs is in the range of about 0.01 mg/kg to about 4.3 mg/kg, about 0.15 mg/kg to about 3.6 mg/kg, about 0.3 mg/kg to about 3.2 mg/kg, about 0.35 mg/kg to about 2.85 mg/kg, about 0.15 mg/kg to about 2.85 mg/kg, about 0.3 mg to about 2.15 mg/kg, about 0.45 mg/kg to about 1.7 mg/kg, about 0.15 mg/kg to about 1.3 mg/kg, about 0.3 mg/kg to about 1.15 mg/kg, about 0.45 mg/kg to about 1 mg/kg, about 0.55 mg/kg to about 0.85 mg/kg, about 0.65 mg/kg to about 0.8 mg/kg, about 0.7 mg/kg to about 0.75 mg/kg, about 0.7 mg/kg to about 2.15 mg/kg, about 0.85 mg/kg to about 2 mg/kg, about 1 mg/kg to about 1.85 mg/kg, about 1.15 mg/kg to about 1.7 mg/kg, about 1.3 mg/kg mg to about 1.6 mg/kg, about 1.35 mg/kg to about 1.5 mg/kg, about 2.15 mg/kg to about 3.6 mg/kg, about 2.3 mg/kg to about 3.4 mg/kg, about 2.4 mg/kg to about 3.3 mg/kg, about 2.6 mg/kg to about 3.15 mg/kg, about 2.7 mg/kg to about 3 mg/kg, about 2.8 mg/kg to about 3 mg/kg, or about 2.85 mg/kg to about 2.95 mg/kg.

[001684] In some embodiments, an effective dosage of TILs is in the range of about 1 mg to about 500 mg, about 10 mg to about 300 mg, about 20 mg to about 250 mg, about 25 mg to about 200 mg, about 1 mg to about 50 mg, about 5 mg to about 45 mg, about 10 mg to about 40 mg, about 15 mg to about 35 mg, about 20 mg to about 30 mg, about 23 mg to about 28 mg, about 50 mg to about 150 mg, about 60 mg to about 140 mg, about 70 mg to about 130 mg, about 80 mg to about 120 mg, about 90 mg to about 110 mg, or about 95 mg to about 105 mg, about 98 mg to about 102 mg, about 150 mg to about 250 mg, about 160 mg to about

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240 mg, about 170 mg to about 230 mg, about 180 mg to about 220 mg, about 190 mg to about 210 mg, about 195 mg to about 205 mg, or about 198 to about 207 mg.

[001685] An effective amount of the TILs may be administered in either single or multiple doses by any of the accepted modes of administration of agents having similar utilities, including intranasal and transdermal routes, by intra-arterial injection, intravenously, intraperitoneally, parenterally, intramuscularly, subcutaneously, topically, by transplantation, or by inhalation.

[001686] In other embodiments, the invention provides an infusion bag comprising the therapeutic population of TILs described in any of the preceding paragraphs as applicable above.

[001687] In other embodiments, the invention provides a tumor infiltrating lymphocyte (TIL) composition comprising the therapeutic population of TILs described in any of the preceding paragraphs as applicable above and a pharmaceutically acceptable carrier.

[001688] In other embodiments, the invention provides an infusion bag comprising the TIL composition described in any of the preceding paragraphs as applicable above.

[001689] In other embodiments, the invention provides a cryopreserved preparation of the therapeutic population of TILs described in any of the preceding paragraphs as applicable above.

[001690] In other embodiments, the invention provides a tumor infiltrating lymphocyte (TIL) composition comprising the therapeutic population of TILs described in any of the preceding paragraphs as applicable above and a cryopreservation media.

[001691] In other embodiments, the invention provides the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cryopreservation media contains DMSO.

[001692] In other embodiments, the invention provides the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cryopreservation media contains 7-10% DMSO.

[001693] In some embodiments, the invention provides the TIL compositions comprising TILs in serum-free medium or a defined medium. In some embodiments, the serum-free or defined medium comprises a basal cell medium and a serum supplement and/or a serum replacement. In some embodiments, the serum-free or defined medium is used to prevent

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and/or decrease experimental variation due in part to the lot-to-lot variation of serum-containing media.

[001694] In some embodiments, the serum-free or defined medium comprises a basal cell medium and a serum supplement and/or serum replacement. In some embodiments, 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.

[001695] In some embodiments, the serum supplement or serum replacement includes, but is not limited to one or more of CTS™ OpTmizer T-Cell Expansion Serum Supplement, CTS™ Immune Cell Serum Replacement, 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 one or more trace elements. In some embodiments, the defined 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+}. In some embodiments, the defined medium further comprises L-glutamine, sodium bicarbonate and/or 2-mercaptoethanol.

[001696] In some embodiments, the CTS™OpTmizer™ T-cell Immune Cell Serum Replacement is used with conventional growth media, including but not limited to CTS™ OpTmizer™ T-cell Expansion Basal Medium, CTS™ OpTmizer™ T-cell Expansion SFM, CTS™ AIM-V Medium, CST™ 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.

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[001697] In some embodiments, the total serum replacement concentration (vol%) in the serum-free or defined medium is 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 total serum-free or defined medium. In some embodiments, the total serum replacement concentration is about 3% of the total volume of the serum-free or defined medium. In some embodiments, the total serum replacement concentration is about 5% of the total volume of the serum-free or defined medium. In some embodiments, the total serum replacement concentration is about 10% of the total volume of the serum-free or defined medium.

[001698] In some embodiments, the serum-free or defined medium is CTS™ OpTmizer™ T-cell Expansion SFM (ThermoFisher Scientific). Any formulation of CTS™ OpTmizer™ is useful in the present invention. CTS™ OpTmizer™ T-cell Expansion SFM is a combination of 1L CTS™ OpTmizer™ T-cell Expansion Basal Medium and 26 mL CTS™ OpTmizer™ T-Cell Expansion Supplement, which are mixed together prior to use. In some embodiments, the CTS™ OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), along with 2-mercaptoethanol at 55mM.

[001699] In some embodiments, the defined medium is CTS™ OpTmizer™ T-cell Expansion SFM (ThermoFisher Scientific). Any formulation of CTS™ OpTmizer™ is useful in the present invention. CTS™ OpTmizer™ T-cell Expansion SFM is a combination of 1L CTS™ OpTmizer™ T-cell Expansion Basal Medium and 26 mL CTS™ OpTmizer™ T-Cell Expansion Supplement, which are mixed together prior to use. In some embodiments, the CTS™ OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), along with 2-mercaptoethanol at 55mM. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), 55mM of 2-mercaptoethanol, and 2mM of L-glutamine. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), 55mM of 2-mercaptoethanol, and 2mM of L-glutamine, and further comprises about 1000 IU/mL to about 8000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), 55mM of 2-mercaptoethanol, and 2mM of L-glutamine, and further comprises about 3000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™

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T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific), 55mM of 2-mercaptoethanol, and 2mM of L-glutamine, and further comprises about 6000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and 55mM of 2-mercaptoethanol, and further comprises about 1000 IU/mL to about 8000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and 55mM of 2-mercaptoethanol, and further comprises about 3000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and 55mM of 2-mercaptoethanol, and further comprises about 1000 IU/mL to about 6000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and about 2mM glutamine, and further comprises about 1000 IU/mL to about 8000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and about 2mM glutamine, and further comprises about 3000 IU/mL of IL-2. In some embodiments, the CTS™OpTmizer™ T-cell Expansion SFM is supplemented with about 3% of the CTS™ Immune Cell Serum Replacement (SR) (ThermoFisher Scientific) and about 2mM glutamine, and further comprises about 6000 IU/mL of IL-2.

[001700] In some embodiments, the serum-free medium or defined medium is supplemented with 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. In some embodiments, the serum-free medium or defined medium is supplemented with glutamine (*i.e.*, GlutaMAX®) at a concentration of about 2mM.

[001701] In some embodiments, the serum-free medium or defined medium is supplemented with 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

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70mM, or about 65mM. In some embodiments, the serum-free medium or defined medium is supplemented with 2-mercaptoethanol at a concentration of about 55mM.

[001702] In some embodiments, the defined media described in International PCT Publication No. WO/1998/030679, which is herein incorporated by reference, are useful in the present invention. In that publication, serum-free eukaryotic cell culture media are described. The serum-free, eukaryotic cell culture medium includes a basal cell culture medium supplemented with a serum-free supplement capable of supporting the growth of cells in serum- free culture. The serum-free eukaryotic cell culture medium supplement comprises or is obtained by combining one or more ingredients selected from the group consisting of 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 trace elements, and one or more antibiotics. In some embodiments, the defined medium further comprises L-glutamine, sodium bicarbonate and/or beta-mercaptoethanol. In some embodiments, the defined medium comprises an albumin or an albumin substitute and one or more ingredients selected from group consisting of 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, and one or more trace elements. In some embodiments, the defined 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^{3m}, Ge^{4+}, Se^{4+}, Br, T, Mn^{2+}, P, Si^{4+}, V^{5+}, Mo^{6+}, Ni^{2+}, Rb^{+}, Sn^{2+} and Zr^{4+}. In some embodiments, the basal cell media is selected from the group consisting of 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.

[001703] In some embodiments, the concentration of glycine in the defined medium is in the range of from about 5-200 mg/L, the concentration of L- histidine is about 5-250 mg/L, the concentration of L-isoleucine is about 5-300 mg/L, the concentration of L-methionine is about 5-200 mg/L, the concentration of L-phenylalanine is about 5-400 mg/L, the

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concentration of L-proline is about 1-1000 mg/L, the concentration of L- hydroxyproline is about 1-45 mg/L, the concentration of L-serine is about 1-250 mg/L, the concentration of L-threonine is about 10-500 mg/L, the concentration of L-tryptophan is about 2-110 mg/L, the concentration of L-tyrosine is about 3-175 mg/L, the concentration of L-valine is about 5-500 mg/L, the concentration of thiamine is about 1-20 mg/L, the concentration of reduced glutathione is about 1-20 mg/L, the concentration of L-ascorbic acid-2-phosphate is about 1-200 mg/L, the concentration of iron saturated transferrin is about 1-50 mg/L, the concentration of insulin is about 1-100 mg/L, the concentration of sodium selenite is about 0.000001-0.0001 mg/L, and the concentration of albumin (*e.g.*, AlbuMAX® I) is about 5000-50,000 mg/L.

[001704] In some embodiments, 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 4. In other embodiments, the non-trace element moiety ingredients in the defined medium are present in the final concentrations listed in the column under the heading "A Preferred Embodiment of the 1X Medium" in Table 4. In other embodiments, the defined medium is a basal cell medium comprising a serum free supplement. In some of these embodiments, the serum free supplement comprises non-trace moiety ingredients of the type and in the concentrations listed in the column under the heading "A Preferred Embodiment in Supplement" in Table 4.

[001705] In some embodiments, the osmolarity of the defined medium is between about 260 and 350 mOsmol. In some embodiments, the osmolarity is between about 280 and 310 mOsmol. In some embodiments, the defined medium is supplemented with up to about 3.7 g/L, or about 2.2 g/L sodium bicarbonate. The defined medium can be further supplemented with L-glutamine (final concentration of about 2 mM), one or more antibiotics, non-essential amino acids (NEAA; final concentration of about 100 μM), 2-mercaptoethanol (final concentration of about 100 μM).

[001706] In some embodiments, the defined media described in Smith, *et al.*, "Ex vivo expansion of human T cells for adoptive immunotherapy using the novel Xeno-free CTS Immune Cell Serum Replacement," *Clin Transl Immunology*, 4(1) 2015 (doi: 10.1038/cti.2014.31) are useful in the present invention. Briefly, RPMI or CTS™ OpTmizer™ was used as the basal cell medium, and supplemented with either 0, 2%, 5%, or 10% CTS™ Immune Cell Serum Replacement.

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[001707] In some embodiments, the cell medium in the first and/or second gas permeable container is unfiltered. The use of unfiltered cell medium may simplify the procedures necessary to expand the number of cells. In some embodiments, the cell 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).

[001708] In other embodiments, the invention provides a cryopreserved preparation of the TIL composition described in any of the preceding paragraphs as applicable above.

IV. Methods of Treating Patients

[001709] Methods of treatment begin with the initial TIL collection and culture of TILs. Such methods have been both described in the art by, for example, Jin *et al., J. Immunotherapy*, **2012**, 35(3):283-292, incorporated by reference herein in its entirety. Embodiments of methods of treatment are described throughout the sections below, including the Examples.

[001710] The expanded TILs produced according the methods described herein, including, for example as described in Steps A through F above or according to Steps A through F above (also as shown, for example, in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) find particular use in the treatment of patients with cancer (for example, as described in Goff, et al., *J. Clinical Oncology*, **2016**, 34(20):2389-239, as well as the supplemental content; incorporated by reference herein in its entirety. In some embodiments, TIL were grown from resected deposits of metastatic melanoma as previously described (see, Dudley, et al., *J Immunother.*, **2003**, 26:332-342; incorporated by reference herein in its entirety). Fresh tumor can be dissected under sterile conditions. A representative sample can be collected for formal pathologic analysis. Single fragments of 2 mm^3 to 3 mm^3 may be used. In some embodiments, 5, 10, 15, 20, 25 or 30 samples per patient are obtained. In some embodiments, 20, 25, or 30 samples per patient are obtained. In some embodiments, 20, 22, 24, 26, or 28 samples per patient are obtained. In some embodiments, 24 samples per patient are obtained. Samples can be placed in individual wells of a 24-well plate, maintained in growth media with high-dose IL-2 (6,000 IU/mL), and monitored for destruction of tumor and/or proliferation of TIL. Any tumor with viable cells remaining after processing can be enzymatically digested into a single cell suspension and cryopreserved, as described herein.

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[001711] In some embodiments, successfully grown TIL can be sampled for phenotype analysis (CD3, CD4, CD8, and CD56) and tested against autologous tumor when available. TIL can be considered reactive if overnight coculture yielded interferon-gamma (IFN-γ) levels > 200 pg/mL and twice background. (Goff, et al., *J Immunother.*, **2010**, 33:840-847; incorporated by reference herein in its entirety). In some embodiments, cultures with evidence of autologous reactivity or sufficient growth patterns can be selected for a second expansion (for example, a second expansion as provided in according to Step D of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), including second expansions that are sometimes referred to as rapid expansion (REP). In some embodiments, expanded TILs with high autologous reactivity (for example, high proliferation during a second expansion), are selected for an additional second expansion. In some embodiments, TILs with high autologous reactivity (for example, high proliferation during second expansion as provided in Step D of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), are selected for an additional second expansion according to Step D of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G).

[001712] In some embodiments, the patient is not moved directly to ACT (adoptive cell transfer), for example, in some embodiments, after tumor harvesting and/or a first expansion, the cells are not utilized immediately. In some embodiments, TILs can be cryopreserved and thawed 2 days before administration to a patient. In some embodiments, TILs can be cryopreserved and thawed 1 day before administration to a patient. In some embodiments, the TILs can be cryopreserved and thawed immediately before the administration to a patient.

[001713] Cell phenotypes of cryopreserved samples of infusion bag TIL can be analyzed by flow cytometry (*e.g.*, FlowJo) for surface markers CD3, CD4, CD8, CCR7, and CD45RA (BD BioSciences), as well as by any of the methods described herein. Serum cytokines can be measured by using standard enzyme-linked immunosorbent assay techniques. A rise in serum IFN-g can be defined as >100 pg/mL and at least 4-fold or at least 3-fold or at least 2-fold or at least 1-fold greater than baseline levels of serum IFN-g. In some embodiments, a rise in serum IFN-g is defined as >1000 pg/mL. In some embodiments, a rise in serum IFN-g is defined as >200 pg/mL. In some embodiments, a rise in serum IFN-g is defined as >250 pg/mL. In some embodiments, a rise in serum IFN-g is defined as >300 pg/mL. In some embodiments, a rise in serum IFN-g is defined as >350 pg/mL. In some embodiments, a rise in serum IFN-g is defined as >400 pg/mL. In some embodiments, a rise in serum IFN-g is

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defined as >450 pg/mL. In some embodiments, a rise in serum IFN-g is defined as >500 pg/mL. In some embodiments, a rise in serum IFN-g is defined as >550 pg/mL. In some embodiments, a rise in serum IFN-g is defined as >600 pg/mL. In some embodiments, a rise in serum IFN-g is defined as >650 pg/mL. In some embodiments, a rise in serum IFN-g is defined as >700 pg/mL. In some embodiments, a rise in serum IFN-g is defined as >750 pg/mL. In some embodiments, a rise in serum IFN-g is defined as >800 pg/mL. In some embodiments, a rise in serum IFN-g is defined as >850 pg/mL. In some embodiments, a rise in serum IFN-g is defined as >900 pg/mL. In some embodiments, a rise in serum IFN-g is defined as >950 pg/mL. In some embodiments, a rise in serum IFN-g is defined as >1000 pg/mL.

[001714] In some embodiments, the TILs produced by the methods provided herein, for example those exemplified in Figure 1 (in particular, *e.g.,* Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), provide for a surprising improvement in clinical efficacy of the TILs. In some embodiments, the TILs produced by the methods provided herein, for example those exemplified in Figure 1 (in particular, *e.g.,* Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), exhibit increased clinical efficacy as compared to TILs produced by methods other than those described herein, including, for example, methods other than those exemplified in Figure 1 (in particular, *e.g.,* Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, the methods other than those described herein include methods referred to as process 1C and/or Generation 1 (Gen 1). In some embodiments, the increased efficacy is measured by DCR, ORR, and/or other clinical responses. In some embodiments, the TILS produced by the methods provided herein, for example those exemplified in Figure 1 (in particular, *e.g.,* Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), exhibit a similar time to response and safety profile compared to TILs produced by methods other than those described herein, including, for example, methods other than those exemplified in Figure 1 (in particular, *e.g.,* Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), for example the Gen 1 process.

[001715] In some embodiments, IFN-gamma (IFN-γ) is indicative of treatment efficacy and/or increased clinical efficacy. In some embodiments, IFN-γ in the blood of subjects treated with TILs is indicative of active TILs. In some embodiments, a potency assay for IFN-γ production is employed. IFN-γ production is another measure of cytotoxic potential. IFN-γ production can be measured by determining the levels of the cytokine IFN-γ in the

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blood, serum, or TILs *ex vivo* of a subject treated with TILs prepared by the methods of the present invention, including those as described for example in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, an increase in IFN-γ is indicative of treatment efficacy in a patient treated with the TILs produced by the methods of the present invention. In some embodiments, IFN-γ is increased one-fold, two-fold, three-fold, four-fold, five-fold, six-fold, seven-fold, eight-fold, nine-fold, ten-fold or more as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, IFN-γ secretion is increased one-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, IFN-γ secretion is increased two-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, IFN-γ secretion is increased three-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, IFN-γ secretion is increased four-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, IFN-γ secretion is increased five-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, IFN-γ is measured using a Quantikine ELISA kit. In some embodiments, IFN-γ is measured in TILs *ex vivo* of a subject treated with TILs prepared by the methods of the present invention, including those as described for example in Figure 1 (in particular, *e.g.*,

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Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, IFN-γ is measured in blood of a subject treated with TILs prepared by the methods of the present invention, including those as described for example in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, IFN-γ is measured in TILs serum of a subject treated with TILs prepared by the methods of the present invention, including those as described for example in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G).

[001716] In some embodiments, the TILs prepared by the methods of the present invention, including those as described for example in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), exhibit increased polyclonality as compared to TILs produced by other methods, including those not exemplified in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), such as for example, methods referred to as process 1C methods. In some embodiments, significantly improved polyclonality and/or increased polyclonality is indicative of treatment efficacy and/or increased clinical efficacy. In some embodiments, polyclonality refers to the T-cell repertoire diversity. In some embodiments, an increase in polyclonality can be indicative of treatment efficacy with regard to administration of the TILs produced by the methods of the present invention. In some embodiments, polyclonality is increased one-fold, two-fold, ten-fold, 100-fold, 500-fold, or 1000-fold as compared to TILs prepared using methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, polyclonality is increased one-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, polyclonality is increased two-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, polyclonality is increased ten-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those

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provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, polyclonality is increased 100-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, polyclonality is increased 500-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, polyclonality is increased 1000-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G).

[001717] Measures of efficacy can include the disease control rate (DCR) as well as overall response rate (ORR), as known in the art as well as described herein.

1. Methods of Treating Cancers and Other Diseases

[001718] The compositions and methods described herein can be used in a method for treating diseases. In some embodiments, they are for use in treating hyperproliferative disorders. They may also be used in treating other disorders as described herein and in the following paragraphs.

[001719] In some embodiments, the hyperproliferative disorder is cancer. In some embodiments, the hyperproliferative disorder is a solid tumor cancer. In some embodiments, the solid tumor cancer is selected from the group consisting of glioblastoma (GBM), gastrointestinal cancer, melanoma, ovarian cancer, endometrial cancer, thyroid cancer, colorectal cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, triple negative breast cancer, cancer caused by human papilloma virus, head and neck cancer (including head and neck squamous cell carcinoma (HNSCC)), renal cancer, and renal cell carcinoma. In some embodiments, the hyperproliferative disorder is a hematological malignancy. In some embodiments, the solid tumor cancer is selected from the group consisting of chronic lymphocytic leukemia, acute lymphoblastic leukemia, diffuse

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large B cell lymphoma, non-Hodgkin's lymphoma, Hodgkin's lymphoma, follicular lymphoma, and mantle cell lymphoma.

[001720] In some embodiments, the cancer is a hypermutated cancer phenotype. Hypermutated cancers are extensively described in Campbell, et al. (Cell, 171:1042-1056 (2017); incorporated by reference herein in its entirety for all purposes). In some embodiments, a hypermutated tumors comprise between 9 and 10 mutations per megabase (Mb). In some embodiments, pediatric hypermutated tumors comprise 9.91 mutations per megabase (Mb). In some embodiments, adult hypermutated tumors comprise 9 mutations per megabase (Mb). In some embodiments, enhanced hypermutated tumors comprise between 10 and 100 mutations per megabase (Mb). In some embodiments, enhanced pediatric hypermutated tumors comprise between 10 and 100 mutations per megabase (Mb). In some embodiments, enhanced adult hypermutated tumors comprise between 10 and 100 mutations per megabase (Mb). In some embodiments, an ultra-hypermutated tumors comprise greater than 100 mutations per megabase (Mb). In some embodiments, pediatric ultra-hypermutated tumors comprise greater than 100 mutations per megabase (Mb). In some embodiments, adult ultra-hypermutated tumors comprise greater than 100 mutations per megabase (Mb).

[001721] In some embodiments, the hypermutated tumors have mutations in replication repair pathways. In some embodiments, the hypermutated tumors have mutations in replication repair associated DNA polymerases. In some embodiments, the hypermutated tumors have microsatellite instability. In some embodiments, the ultra-hypermutated tumors have mutations in replication repair associated DNA polymerases and have microsatellite instability. In some embodiments, hypermutation in the tumor is correlated with response to immune checkpoint inhibitors. In some embodiments, hypermutated tumors are resistant to treatment with immune checkpoint inhibitors. In some embodiments, hypermutated tumors can be treated using the TILs of the present invention. In some embodiments, hypermutation in the tumor is caused by environmental factors (extrinsic exposures). For example, UV light can be the primary cause of the high numbers of mutations in malignant melanoma (*see*, for example, Pfeifer, G.P., You, Y.H., and Besaratinia, A. (2005). Mutat. Res. 571, 19–31.; Sage, E. (1993). Photochem. Photobiol. 57, 163–174.). In some embodiments, hypermutation in the tumor can be caused by the greater than 60 carcinogens in tobacco smoke for tumors of the lung and larynx, as well as other tumors, due to direct mutagen exposure (*see*, for example, Pleasance, E.D., Stephens, P.J., O'Meara, S., McBride, D.J., Meynert, A., Jones, D., Lin, M.L., Beare, D., Lau, K.W., Greenman, C., et al. (2010). Nature 463, 184–190). In some

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embodiments, hypermutation in the tumor is caused by dysregulation of apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) family members, which has been shown to result in increased levels of C to T transitions in a wide range of cancers (see, for example, Roberts, S.A., Lawrence, M.S., Klimczak, L.J., Grimm, S.A., Fargo, D., Stojanov, P., Kiezun, A., Kryukov, G.V., Carter, S.L., Saksena, G., et al. (2013). Nat. Genet. 45, 970–976). In some embodiments, hypermutation in the tumor is caused by defective DNA replication repair by mutations that compromise proofreading, which is performed by the major replicative enzymes Pol3 and Pold1. In some embodiments, hypermutation in the tumor is caused by defects in DNA mismatch repair, which are associated with hypermutation in colorectal, endometrial, and other cancers (see, for example, Kandoth, C., Schultz, N., Cherniack, A.D., Akbani, R., Liu, Y., Shen, H., Robertson, A.G., Pashtan, I., Shen, R., Benz, C.C., et al.; (2013). Nature 497, 67–73.; Muzny, D.M., Bainbridge, M.N., Chang, K., Dinh, H.H., Drummond, J.A., Fowler, G., Kovar, C.L., Lewis, L.R., Morgan, M.B., Newsham, I.F., et al.; (2012). Nature 487, 330–337). In some embodiments, DNA replication repair mutations are also found in cancer predisposition syndromes, such as constitutional or biallelic mismatch repair deficiency (CMMRD), Lynch syndrome, and polymerase proofreading-associated polyposis (PPAP).

[001722] In some embodiments, the invention includes a method of treating a cancer with a population of TILs, wherein the cancer is a hypermutated cancer. In some embodiments, the invention includes a method of treating a cancer with a population of TILs, wherein the cancer is an enhanced hypermutated cancer. In some embodiments, the invention includes a method of treating a cancer with a population of TILs, wherein the cancer is an ultra-hypermutated cancer.

[001723] In some embodiments, the invention includes a method of treating a cancer with a population of TILs, wherein a patient is pre-treated with non-myeloablative chemotherapy prior to an infusion of TILs according to the present disclosure. In some embodiments, the non-myeloablative chemotherapy is cyclophosphamide 60 mg/kg/d for 2 days (days 27 and 26 prior to TIL infusion) and fludarabine 25 mg/m^2/d for 5 days (days 27 to 23 prior to TIL infusion). In some embodiments, after non-myeloablative chemotherapy and TIL infusion (at day 0) according to the present disclosure, the patient receives an intravenous infusion of IL-2 intravenously at 720,000 IU/kg every 8 hours to physiologic tolerance.

[001724] Efficacy of the compounds and combinations of compounds described herein in treating, preventing and/or managing the indicated diseases or disorders can be tested using

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various models known in the art, which provide guidance for treatment of human disease. For example, models for determining efficacy of treatments for ovarian cancer are described, *e.g.*, in Mullany, *et al.*, *Endocrinology* **2012,** *153,* 1585-92; and Fong, *et al.*, *J. Ovarian Res.* **2009,** *2,* 12. Models for determining efficacy of treatments for pancreatic cancer are described in Herreros-Villanueva, *et al.*, *World J. Gastroenterol.* **2012,** *18,* 1286-1294. Models for determining efficacy of treatments for breast cancer are described, *e.g.*, in Fantozzi, *Breast Cancer Res.* **2006,** *8,* 212. Models for determining efficacy of treatments for melanoma are described, *e.g.*, in Damsky, *et al.*, *Pigment Cell & Melanoma Res.* **2010,** *23,* 853–859. Models for determining efficacy of treatments for lung cancer are described, *e.g.*, in Meuwissen, *et al.*, *Genes & Development*, **2005,** *19,* 643-664. Models for determining efficacy of treatments for lung cancer are described, *e.g.*, in Kim, *Clin. Exp. Otorhinolaryngol.* **2009,** *2,* 55-60; and Sano, *Head Neck Oncol.* **2009,** *1,* 32.

[001725] In some embodiments, IFN-gamma (IFN-γ) is indicative of treatment efficacy for hyperproliferative disorder treatment. In some embodiments, IFN-γ in the blood of subjects treated with TILs is indicative of active TILs. In some embodiments, a potency assay for IFN-γ production is employed. IFN-γ production is another measure of cytotoxic potential. IFN-γ production can be measured by determining the levels of the cytokine IFN-γ in the blood of a subject treated with TILs prepared by the methods of the present invention, including those as described for example in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, the TILs obtained by the present method provide for increased IFN-γ in the blood of subjects treated with the TILs of the present method as compared subjects treated with TILs prepared using methods referred to as the Gen 3 process, as exemplified Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G) and throughout this application. In some embodiments, an increase in IFN-γ is indicative of treatment efficacy in a patient treated with the TILs produced by the methods of the present invention. In some embodiments, IFN-γ is increased one-fold, two-fold, three-fold, four-fold, or five-fold or more as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, IFN-γ secretion is increased one-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including,

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for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, IFN-γ secretion is increased two-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, IFN-γ secretion is increased three-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, IFN-γ secretion is increased four-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, IFN-γ secretion is increased five-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, IFN-γ is measured using a Quantikine ELISA kit. In some embodiments, IFN-γ is measured using a Quantikine ELISA kit. In some embodiments, IFN-γ is measured in TILs *ex vivo* from a patient treated with the TILs produced by the methods of the present invention. In some embodiments, IFN-γ is measured in blood in a patient treated with the TILs produced by the methods of the present invention. In some embodiments, IFN-γ is measured in serum in a patient treated with the TILs produced by the methods of the present invention.

[001726] In some embodiments, the TILs prepared by the methods of the present invention, including those as described for example in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), exhibit increased polyclonality as compared to TILs produced by other methods, including those not exemplified in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), such as for example, methods referred to as process 1C methods. In some embodiments, significantly improved polyclonality and/or increased polyclonality is indicative of treatment efficacy and/or increased clinical efficacy for cancer

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treatment. In some embodiments, polyclonality refers to the T-cell repertoire diversity. In some embodiments, an increase in polyclonality can be indicative of treatment efficacy with regard to administration of the TILs produced by the methods of the present invention. In some embodiments, polyclonality is increased one-fold, two-fold, ten-fold, 100-fold, 500-fold, or 1000-fold as compared to TILs prepared using methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, polyclonality is increased one-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, polyclonality is increased two-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, polyclonality is increased ten-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, polyclonality is increased 100-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, polyclonality is increased 500-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). In some embodiments, polyclonality is increased 1000-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including, for example, methods other than those embodied in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G).

2. Methods of co-administration

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[001727] In some embodiments, the TILs produced as described herein, including, for example TILs derived from a method described in Steps A through F of Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G), can be administered in combination with one or more immune checkpoint regulators, such as the antibodies described below. For example, antibodies that target PD-1 and which can be co-administered with the TILs of the present invention include, *e.g.*, but are not limited to nivolumab (BMS-936558, Bristol-Myers Squibb; Opdivo®), pembrolizumab (lambrolizumab, MK03475 or MK-3475, Merck; Keytruda®), humanized anti-PD-1 antibody JS001 (ShangHai JunShi), monoclonal anti-PD-1 antibody TSR-042 (Tesaro, Inc.), Pidilizumab (anti-PD-1 mAb CT-011, Medivation), anti-PD-1 monoclonal Antibody BGB-A317 (BeiGene), and/or anti-PD-1 antibody SHR-1210 (ShangHai HengRui), human monoclonal antibody REGN2810 (Regeneron), human monoclonal antibody MDX-1106 (Bristol-Myers Squibb), and/or humanized anti-PD-1 IgG4 antibody PDR001 (Novartis). In some embodiments, the PD-1 antibody is from clone: RMP1-14 (rat IgG) - BioXcell cat# BP0146. Other suitable antibodies suitable for use in co-administration methods with TILs produced according to Steps A through F as described herein are anti-PD-1 antibodies disclosed in U.S. Patent No. 8,008,449, herein incorporated by reference. In some embodiments, the antibody or antigen-binding portion thereof binds specifically to PD-L1 and inhibits its interaction with PD-1, thereby increasing immune activity. Any antibodies known in the art which bind to PD-L1 and disrupt the interaction between the PD-1 and PD-L1, and stimulates an anti- tumor immune response, are suitable for use in co-administration methods with TILs produced according to Steps A through F as described herein. For example, antibodies that target PD-L1 and are in clinical trials, include BMS-936559 (Bristol-Myers Squibb) and MPDL3280A (Genentech). Other suitable antibodies that target PD-L1 are disclosed in U.S. Patent No. 7,943,743, herein incorporated by reference. It will be understood by one of ordinary skill that any antibody which binds to PD-1 or PD-L1, disrupts the PD-1/PD-L1 interaction, and stimulates an anti-tumor immune response, are suitable for use in co-administration methods with TILs produced according to Steps A through F as described herein. In some embodiments, the subject administered the combination of TILs produced according to Steps A through F is co administered with a and anti-PD-1 antibody when the patient has a cancer type that is refractory to administration of the anti-PD-1 antibody alone. In some embodiments, the patient is administered TILs in combination with and anti-PD-1 when the patient has refractory melanoma. In some embodiments, the patient

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is administered TILs in combination with and anti-PD-1 when the patient has non-small-cell lung carcinoma (NSCLC).

3. PD-1 and PD-L1 Inhibitors

[001728] In some embodiments, the TILs therapy provided to patients with cancer may include treatment with therapeutic populations of TILs alone or may include a combination treatment including TILs and one or more PD-1 and/or PD-L1 inhibitors.

[001729] Programmed death 1 (PD-1) is a 288-amino acid transmembrane immunocheckpoint receptor protein expressed by T cells, B cells, natural killer (NK) T cells, activated monocytes, and dendritic cells. PD-1, which is also known as CD279, belongs to the CD28 family, and in humans is encoded by the Pdcd1 gene on chromosome 2. PD-1 consists of one immunoglobulin (Ig) superfamily domain, a transmembrane region, and an intracellular domain containing an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). PD-1 and its ligands (PD-L1 and PD-L2) are known to play a key role in immune tolerance, as described in Keir, et al., Annu. Rev. Immunol. 2008, 26, 677-704. PD-1 provides inhibitory signals that negatively regulate T cell immune responses. PD-L1 (also known as B7-H1 or CD274) and PD-L2 (also known as B7-DC or CD273) are expressed on tumor cells and stromal cells, which may be encountered by activated T cells expressing PD-1, leading to immunosuppression of the T cells. PD-L1 is a 290 amino acid transmembrane protein encoded by the Cd274 gene on human chromosome 9. Blocking the interaction between PD-1 and its ligands PD-L1 and PD-L2 by use of a PD-1 inhibitor, a PD-L1 inhibitor, and/or a PD-L2 inhibitor can overcome immune resistance, as demonstrated in recent clinical studies, such as that described in Topalian, et al., N. Eng. J. Med. 2012, 366, 2443-54. PD-L1 is expressed on many tumor cell lines, while PD-L2 is expressed is expressed mostly on dendritic cells and a few tumor lines. In addition to T cells (which inducibly express PD-1 after activation), PD-1 is also expressed on B cells, natural killer cells, macrophages, activated monocytes, and dendritic cells.

[001730] In some embodiments, the PD-1 inhibitor may be any PD-1 inhibitor or PD-1 blocker known in the art. In particular, it is one of the PD-1 inhibitors or blockers described in more detail in the following paragraphs. The terms "inhibitor," "antagonist," and "blocker" are used interchangeably herein in reference to PD-1 inhibitors. For avoidance of doubt, references herein to a PD-1 inhibitor that is an antibody may refer to a compound or antigen-binding fragments, variants, conjugates, or biosimilars thereof. For avoidance of doubt,

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references herein to a PD-1 inhibitor may also refer to a small molecule compound or a pharmaceutically acceptable salt, ester, solvate, hydrate, cocrystal, or prodrug thereof.

[001731] In some embodiments, the PD-1 inhibitor is an antibody (*i.e.*, an anti-PD-1 antibody), a fragment thereof, including Fab fragments, or a single-chain variable fragment (scFv) thereof. In some embodiments the PD-1 inhibitor is a polyclonal antibody. In some embodiments, the PD-1 inhibitor is a monoclonal antibody. In some embodiments, the PD-1 inhibitor competes for binding with PD-1, and/or binds to an epitope on PD-1. In some embodiments, the antibody competes for binding with PD-1, and/or binds to an epitope on PD-1.

[001732] In some embodiments, the PD-1 inhibitor is one that binds human PD-1 with a KD of about 100 pM or lower, binds human PD-1 with a KD of about 90 pM or lower, binds human PD-1 with a KD of about 80 pM or lower, binds human PD-1 with a KD of about 70 pM or lower, binds human PD-1 with a KD of about 60 pM or lower, binds human PD-1 with a KD of about 50 pM or lower, binds human PD-1 with a KD of about 40 pM or lower, binds human PD-1 with a KD of about 30 pM or lower, binds human PD-1 with a KD of about 20 pM or lower, binds human PD-1 with a KD of about 10 pM or lower, or binds human PD-1 with a KD of about 1 pM or lower.

[001733] In some embodiments, the PD-1 inhibitor is one that binds to human PD-1 with a kassoc of about 7.5 × 105 l/M·s or faster, binds to human PD-1 with a kassoc of about 7.5 × 105 1/M·s or faster, binds to human PD-1 with a kassoc of about 8 × 105 1/M·s or faster, binds to human PD-1 with a kassoc of about 8.5 × 105 1/M·s or faster, binds to human PD-1 with a kassoc of about 9 × 105 1/M·s or faster, binds to human PD-1 with a kassoc of about 9.5 × 105 l/M·s or faster, or binds to human PD-1 with a kassoc of about 1 × 106 1/M·s or faster.

[001734] In some embodiments, the PD-1 inhibitor is one that binds to human PD-1 with a kdissoc of about 2 × 10-5 1/s or slower, binds to human PD-1 with a kdissoc of about 2.1 × 10-5 1/s or slower , binds to human PD-1 with a kdissoc of about 2.2 × 10-5 1/s or slower, binds to human PD-1 with a kdissoc of about 2.3 × 10-5 1/s or slower, binds to human PD-1 with a kdissoc of about 2.4 × 10-5 1/s or slower, binds to human PD-1 with a kdissoc of about 2.5 × 10-5 1/s or slower, binds to human PD-1 with a kdissoc of about 2.6 × 10-5 1/s or slower or binds to human PD-1 with a kdissoc of about 2.7 × 10-5 1/s or slower, binds to human PD-1 with a kdissoc of about 2.8 × 10-5 1/s or slower, binds to human PD-1 with a

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kdissoc of about 2.9 × 10-5 1/s or slower, or binds to human PD-1 with a kdissoc of about 3 × 10-5 1/s or slower.

[001735] In some embodiments, the PD-1 inhibitor is one that blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 10 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 9 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 8 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 7 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 6 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 5 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 4 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 3 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 2 nM or lower, or blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 1 nM or lower.

[001736] In some embodiments, the PD-1 inhibitor is nivolumab (commercially available as OPDIVO from Bristol-Myers Squibb Co.), or biosimilars, antigen-binding fragments, conjugates, or variants thereof. Nivolumab is a fully human IgG4 antibody blocking the PD-1 receptor. In some embodiments, the anti-PD-1 antibody is an immunoglobulin G4 kappa, anti-(human CD274) antibody. Nivolumab is assigned Chemical Abstracts Service (CAS) registry number 946414-94-4 and is also known as 5C4, BMS-936558, MDX-1106, and ONO-4538. The preparation and properties of nivolumab are described in U.S. Patent No. 8,008,449 and International Patent Publication No. WO 2006/121168, the disclosures of which are incorporated by reference herein. The clinical safety and efficacy of nivolumab in various forms of cancer has been described in Wang, et al., Cancer Immunol Res. 2014, 2, 846-56; Page, et al., Ann. Rev. Med., 2014, 65, 185-202; and Weber, et al., J. Clin. Oncology, 2013, 31, 4311-4318, the disclosures of which are incorporated by reference herein. The amino acid sequences of nivolumab are set forth in Table 19. Nivolumab has intra-heavy chain disulfide linkages at 22-96,140-196, 254-314, 360-418, 22"-96", 140"-196", 254"-314", and 360"-418"; intra-light chain disulfide linkages at 23'-88', 134'-194', 23'"-88'", and 134'"-194'"; inter-heavy-light chain disulfide linkages at 127-214', 127"-214'", inter-

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heavy-heavy chain disulfide linkages at 219-219" and 222-222"; and N-glycosylation sites (H CH2 84.4) at 290, 290".

[001737] In some embodiments, a PD-1 inhibitor comprises a heavy chain given by SEQ ID NO:463 and a light chain given by SEQ ID NO:464. In some embodiments, a PD-1 inhibitor comprises heavy and light chains having the sequences shown in SEQ ID NO:463 and SEQ ID NO:464, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In some embodiments, a PD-1 inhibitor comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:463 and SEQ ID NO:464, respectively. In some embodiments, a PD-1 inhibitor comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:463 and SEQ ID NO:464, respectively. In some embodiments, a PD-1 inhibitor comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:463 and SEQ ID NO:464, respectively. In some embodiments, a PD-1 inhibitor comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:463 and SEQ ID NO:464, respectively. In some embodiments, a PD-1 inhibitor comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:463 and SEQ ID NO:464, respectively.

[001738] In some embodiments, the PD-1 inhibitor comprises the heavy and light chain CDRs or variable regions (VRs) of nivolumab. In some embodiments, the PD-1 inhibitor heavy chain variable region (VH) comprises the sequence shown in SEQ ID NO:465, and the PD-1 inhibitor light chain variable region (VL) comprises the sequence shown in SEQ ID NO:466, and conservative amino acid substitutions thereof. In some embodiments, a PD-1 inhibitor comprises VH and VL regions that are each at least 99% identical to the sequences shown in SEQ ID NO:465 and SEQ ID NO:466, respectively. In some embodiments, a PD-1 inhibitor comprises VH and VL regions that are each at least 98% identical to the sequences shown in SEQ ID NO:465 and SEQ ID NO:466, respectively. In some embodiments, a PD-1 inhibitor comprises VH and VL regions that are each at least 97% identical to the sequences shown in SEQ ID NO:465 and SEQ ID NO:466, respectively. In some embodiments, a PD-1 inhibitor comprises VH and VL regions that are each at least 96% identical to the sequences shown in SEQ ID NO:465 and SEQ ID NO:466, respectively. In some embodiments, a PD-1 inhibitor comprises VH and VL regions that are each at least 95% identical to the sequences shown in SEQ ID NO:465 and SEQ ID NO:466, respectively.

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[001739] In some embodiments, a PD-1 inhibitor comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:467, SEQ ID NO:468, and SEQ ID NO:469, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:470, SEQ ID NO:471, and SEQ ID NO:472, respectively, and conservative amino acid substitutions thereof. In some embodiments, the antibody competes for binding with, and/or binds to the same epitope on PD-1 as any of the aforementioned antibodies.

[001740] In some embodiments, the PD-1 inhibitor is an anti-PD-1 biosimilar monoclonal antibody approved by drug regulatory authorities with reference to nivolumab. In some embodiments, the biosimilar comprises an anti-PD-1 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is nivolumab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is an anti-PD-1 antibody authorized or submitted for authorization, wherein the anti-PD-1 antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is nivolumab. The anti-PD-1 antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is nivolumab. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is nivolumab.

TABLE 19. Amino acid sequences for PD-1 inhibitors related to nivolumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:463	QVQLVESGGG VVQPGRSLRL DCKASGITFS NSGMHWVRQA PGKGLEWVAV IWYDGSKRYY	60

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Identifier	Sequence (One-Letter Amino Acid Symbols)	
nivolumab heavy chain	ADSVKGRFTI SRDNSKNTLF LQMNSLRAED TAVYYCATND DYWGQGTLVT VSSASTKGPS VFPLAPCSRS TSESTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TKTYTCNVDH KPSNTKVDKR VESKYGPPCP PCPAPEFLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS QEDPEVQFNW YVDGVEVHNA KTKPREEQFN STYRVVSVLT VLHQDWLNGK EYKCKVSNKG LPSSIEKTIS KAKGQPREPQ VYTLPPSQEE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SRLTVDKSRW QEGNVFSCSV MHEALHNHYT QKSLSLSLGK	120 180 240 300 360 420 440
SEQ ID NO:464 nivolumab light chain	EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ SSNWPRTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC	60 120 180 214
SEQ ID NO:465 nivolumab variable heavy chain	QVQLVESGGG VVQPGRSLRL DCKASGITFS NSGMHWVRQA PGKGLEWVAV IWYDGSKRYY ADSVKGRFTI SRDNSKNTLF LQMNSLRAED TAVYYCATND DYWGQGTLVT VSS	60 113
SEQ ID NO:466 nivolumab variable light chain	EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ SSNWPRTFGQ GTKVEIK	60 107
SEQ ID NO:467 nivolumab heavy chain CDR1	NSGMH	5
SEQ ID NO:468 nivolumab heavy chain CDR2	VIWYDGSKRY YADSVKG	17
SEQ ID NO:469 nivolumab heavy chain CDR3	NDDY	4
SEQ ID NO:470 nivolumab light chain CDR1	RASQSVSSYL A	11
SEQ ID NO:471 nivolumab light chain CDR2	DASNRAT	7
SEQ ID NO:472 nivolumab light chain CDR3	QQSSNWPRT	9

[001741] In some embodiments, the PD-1 inhibitor is nivolumab or a biosimilar thereof, and the nivolumab is administered at a dose of about 0.5 mg/kg to about 10 mg/kg. In some embodiments, the PD-1 inhibitor is nivolumab or a biosimilar thereof, and the nivolumab is administered at a dose of about 0.5 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 3.5 mg/kg, about 4 mg/kg, about 4.5 mg/kg, about 5 mg/kg, about 5.5 mg/kg, about 6 mg/kg, about 6.5 mg/kg, about 7 mg/kg, about 7.5 mg/kg, about 8 mg/kg, about 8.5 mg/kg, about 9 mg/kg, about 9.5 mg/kg, or about 10 mg/kg. In some embodiments, the nivolumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the nivolumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the nivolumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or

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patient). In some embodiments, the nivolumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001742] In some embodiments, the PD-1 inhibitor is nivolumab or a biosimilar thereof, and the nivolumab is administered at a dose of about 200 mg to about 500 mg. In some embodiments, the PD-1 inhibitor is nivolumab or a biosimilar thereof, and the nivolumab is administered at a dose of about 200 mg, about 220 mg, about 240 mg, about 260 mg, about 280 mg, about 300 mg, about 320 mg, about 340 mg, about 360 mg, about 380 mg, about 400 mg, about 420 mg, about 440 mg, about 460 mg, about 480 mg, or about 500 mg. In some embodiments, the nivolumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the nivolumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the nivolumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the nivolumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001743] In some embodiments, the PD-1 inhibitor is nivolumab or a biosimilar thereof, and the nivolumab is administered every 2 weeks, every 3 weeks, every 4 weeks, every 5 weeks, or every 6 weeks. In some embodiments, the nivolumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the nivolumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the nivolumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the nivolumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001744] In some embodiments, the nivolumab is administered to treat unresectable or metastatic melanoma. In some embodiments, the nivolumab is administered to treat unresectable or metastatic melanoma and is administered at about 240 mg every 2 weeks. In some embodiments, the nivolumab is administered to treat unresectable or metastatic melanoma and is administered at about 480 mg every 4 weeks. In some embodiments, the nivolumab is administered to treat unresectable or metastatic melanoma and is administered at about 1 mg/kg followed by ipilimumab 3 mg/kg on the same day every 3 weeks for 4 doses, then 240 mg every 2 weeks or 480 mg every 4 weeks. In some embodiments, the nivolumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the nivolumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the nivolumab can also be administered 1, 2, 3, 4 or 5 weeks pre-

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resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the nivolumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001745] In some embodiments, the nivolumab is administered for the adjuvant treatment of melanoma. In some embodiments, the nivolumab is administered for the adjuvant treatment of melanoma at about 240 mg every 2 weeks. In some embodiments, the nivolumab is administered for the adjuvant treatment of melanoma at about 480 mg every 4 weeks. In some embodiments, the nivolumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the nivolumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the nivolumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the nivolumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001746] In some embodiments, the nivolumab is administered to treat metastatic non-small cell lung cancer. In some embodiments, the nivolumab is administered to treat metastatic non-small cell lung cancer at about 3 mg/kg every 2 weeks along with ipilimumab at about 1 mg/kg every 6 weeks. In some embodiments, the nivolumab is administered to treat metastatic non-small cell lung cancer at about 360 mg every 3 weeks with ipilimumab 1 mg/kg every 6 weeks and 2 cycles of platinum-doublet chemotherapy. In some embodiments, the nivolumab is administered to treat metastatic non-small cell lung cancer at about 240 mg every 2 weeks or 480 mg every 4 weeks. In some embodiments, the nivolumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the nivolumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the nivolumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the nivolumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001747] In some embodiments, the nivolumab is administered to treat small cell lung cancer. In some embodiments, the nivolumab is administered to treat small cell lung cancer at about 240 mg every 2 weeks. In some embodiments, the nivolumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the nivolumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the nivolumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining

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a tumor sample from the subject or patient). In some embodiments, the nivolumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001748] In some embodiments, the nivolumab is administered to treat malignant pleural mesothelioma at about 360 mg every 3 weeks with ipilimumab 1 mg/kg every 6 weeks. In some embodiments, the nivolumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the nivolumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the nivolumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the nivolumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001749] In some embodiments, the nivolumab is administered to treat advanced renal cell carcinoma. In some embodiments, the nivolumab is administered to treat advanced renal cell carcinoma at about 240 mg every 2 weeks. In some embodiments, the nivolumab is administered to treat advanced renal cell carcinoma at about 480 mg every 4 weeks. In some embodiments, the nivolumab is administered to treat advanced renal cell carcinoma at about 3 mg/kg followed by ipilimumab at about 1 mg/kg on the same day every 3 weeks for 4 doses, then 240 mg every 2 weeks. In some embodiments, the nivolumab is administered to treat advanced renal cell carcinoma at about 3 mg/kg followed by ipilimumab at about 1 mg/kg on the same day every 3 weeks for 4 doses, then 240 mg every 2 weeks 480 mg every 4 weeks. In some embodiments, the nivolumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the nivolumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the nivolumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the nivolumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001750] In some embodiments, the nivolumab is administered to treat classical Hodgkin lymphoma. In some embodiments, the nivolumab is administered to treat classical Hodgkin lymphoma at about 240 mg every 2 weeks. In some embodiments, the nivolumab is administered to treat classical Hodgkin lymphoma at about 480 mg every 4 weeks. In some embodiments, the nivolumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the nivolumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the nivolumab can also be administered 1, 2,

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3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the nivolumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001751] In some embodiments, the nivolumab is administered to treat Recurrent or metastatic squamous cell carcinoma of the head and neck. In some embodiments, the nivolumab is administered to treat recurrent or metastatic squamous cell carcinoma of the head and neck at about 240 mg every 2 weeks. In some embodiments, the nivolumab is administered to treat recurrent or metastatic squamous cell carcinoma of the head and neck at about 480 mg every 4 weeks. In some embodiments, the nivolumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the nivolumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the nivolumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the nivolumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001752] In some embodiments, the nivolumab is administered to treat locally advanced or metastatic urothelial carcinoma at about 240 mg every 2 weeks. In some embodiments, the nivolumab is administered to treat locally advanced or metastatic urothelial carcinoma at about 480 mg every 4 weeks. In some embodiments, the nivolumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the nivolumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the nivolumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the nivolumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001753] In some embodiments, the nivolumab is administered to treat microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer. In some embodiments, the nivolumab is administered to treat Microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer in adult and pediatric patients. In some embodiments, the nivolumab is administered to treat microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer in adult and pediatric patients ≥40 kg at about 240 mg every 2 weeks. In some embodiments, the nivolumab is administered to treat microsatellite instability-high (MSI-H) or mismatch

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repair deficient (dMMR) metastatic colorectal cancer in adult and pediatric patients ≥40 kg at about 480 mg every 4 weeks. In some embodiments, the nivolumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the nivolumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the nivolumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the nivolumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001754] In some embodiments, the nivolumab is administered to treat microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer in pediatric patients <40 kg at about 3 mg/kg every 2 weeks. In some embodiments, the nivolumab is administered to treat microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer in adult and pediatric patients ≥40 kg at about 3 mg/kg followed by ipilimumab 1 mg/kg on the same day every 3 weeks for 4 doses, then 240 mg every 2 weeks. In some embodiments, the nivolumab is administered to treat microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer in adult and pediatric patients ≥40 kg at about 3 mg/kg followed by ipilimumab 1 mg/kg on the same day every 3 weeks for 4 doses, then 480 mg every 4 weeks. In some embodiments, the nivolumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the nivolumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the nivolumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the nivolumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001755] In some embodiments, the nivolumab is administered to treat hepatocellular carcinoma. In some embodiments, the nivolumab is administered to treat hepatocellular carcinoma at about 240 mg every 2 weeks. In some embodiments, the nivolumab is administered to treat hepatocellular carcinoma at about 480 mg every 4 weeks. In some embodiments, the nivolumab is administered to treat hepatocellular carcinoma at about 1 mg/kg followed by ipilimumab 3 mg/kg on the same day every 3 weeks for 4 doses, then 240 mg every 2 weeks. In some embodiments, the nivolumab is administered to treat hepatocellular carcinoma at about 1 mg/kg followed by ipilimumab 3 mg/kg on the same day every 3 weeks for 4 doses, then 480 mg every 4 weeks. In some embodiments, the nivolumab

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administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the nivolumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the nivolumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the nivolumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001756] In some embodiments, the nivolumab is administered to treat esophageal squamous cell carcinoma. In some embodiments, the nivolumab is administered to treat esophageal squamous cell carcinoma at about 240 mg every 2 weeks. In some embodiments, the nivolumab is administered to treat esophageal squamous cell carcinoma at about 480 mg every 4 weeks. In some embodiments, the nivolumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the nivolumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the nivolumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the nivolumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001757] In other embodiments, the PD-1 inhibitor comprises pembrolizumab (commercially available as KEYTRUDA from Merck & Co., Inc., Kenilworth, NJ, USA), or antigen-binding fragments, conjugates, or variants thereof. Pembrolizumab is assigned CAS registry number 1374853-91-4 and is also known as lambrolizumab, MK-3475, and SCH-900475. Pembrolizumab has an immunoglobulin G4, anti-(human protein PDCD1 (programmed cell death 1)) (human-Mus musculus monoclonal heavy chain), disulfide with human-Mus musculus monoclonal light chain, dimer structure. The structure of pembrolizumab may also be described as immunoglobulin G4, anti-(human programmed cell death 1); humanized mouse monoclonal [228-L-proline(H10-S>P)]γ4 heavy chain (134-218')-disulfide with humanized mouse monoclonal κ light chain dimer (226-226":229-229")-bisdisulfide. The properties, uses, and preparation of pembrolizumab are described in International Patent Publication No. WO 2008/156712 A1, U.S. Patent No. 8,354,509 and U.S. Patent Application Publication Nos. US 2010/0266617 A1, US 2013/0108651 A1, and US 2013/0109843 A2, the disclosures of which are incorporated herein by reference. The clinical safety and efficacy of pembrolizumab in various forms of cancer is described in Fuerst, Oncology Times, 2014, 36, 35-36; Robert, et al., Lancet, 2014, 384, 1109-17; and Thomas, et al., Exp. Opin. Biol. Ther., 2014, 14, 1061-1064. The amino acid sequences of

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pembrolizumab are set forth in Table 20. Pembrolizumab includes the following disulfide bridges: 22-96, 22"-96", 23'-92', 23'"-92'", 134-218', 134"-218'", 138'-198', 138'"-198'", 147-203, 147"-203", 226-226", 229-229", 261-321, 261"-321", 367-425, and 367"-425", and the following glycosylation sites (N): Asn-297 and Asn-297". Pembrolizumab is an IgG4/kappa isotype with a stabilizing S228P mutation in the Fc region; insertion of this mutation in the IgG4 hinge region prevents the formation of half molecules typically observed for IgG4 antibodies. Pembrolizumab is heterogeneously glycosylated at Asn297 within the Fc domain of each heavy chain, yielding a molecular weight of approximately 149 kDa for the intact antibody. The dominant glycoform of pembrolizumab is the fucosylated agalacto diantennary glycan form (G0F).

[001758] In some embodiments, a PD-1 inhibitor comprises a heavy chain given by SEQ ID NO:473 and a light chain given by SEQ ID NO:474. In some embodiments, a PD-1 inhibitor comprises heavy and light chains having the sequences shown in SEQ ID NO:473 and SEQ ID NO:474, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In some embodiments, a PD-1 inhibitor comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:473 and SEQ ID NO:474, respectively. In some embodiments, a PD-1 inhibitor comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:473 and SEQ ID NO:474, respectively. In some embodiments, a PD-1 inhibitor comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:473 and SEQ ID NO:474, respectively. In some embodiments, a PD-1 inhibitor comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:473 and SEQ ID NO:474, respectively. In some embodiments, a PD-1 inhibitor comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:473 and SEQ ID NO:474, respectively.

[001759] In some embodiments, the PD-1 inhibitor comprises the heavy and light chain CDRs or variable regions (VRs) of pembrolizumab. In some embodiments, the PD-1 inhibitor heavy chain variable region (VH) comprises the sequence shown in SEQ ID NO:475, and the PD-1 inhibitor light chain variable region (VL) comprises the sequence shown in SEQ ID NO:476, and conservative amino acid substitutions thereof. In some embodiments, a PD-1 inhibitor comprises VH and VL regions that are each at least 99% identical to the sequences shown in SEQ ID NO:475 and SEQ ID NO:476, respectively. In some embodiments, a PD-1

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inhibitor comprises VH and VL regions that are each at least 98% identical to the sequences shown in SEQ ID NO:475 and SEQ ID NO:476, respectively. In some embodiments, a PD-1 inhibitor comprises VH and VL regions that are each at least 97% identical to the sequences shown in SEQ ID NO:475 and SEQ ID NO:476, respectively. In some embodiments, a PD-1 inhibitor comprises VH and VL regions that are each at least 96% identical to the sequences shown in SEQ ID NO:475 and SEQ ID NO:476, respectively. In some embodiments, a PD-1 inhibitor comprises VH and VL regions that are each at least 95% identical to the sequences shown in SEQ ID NO:475 and SEQ ID NO:476, respectively.

[001760] In some embodiments, a PD-1 inhibitor comprises the heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:477, SEQ ID NO:478, and SEQ ID NO:479, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:480, SEQ ID NO:481, and SEQ ID NO:482, respectively, and conservative amino acid substitutions thereof. In some embodiments, the antibody competes for binding with, and/or binds to the same epitope on PD-1 as any of the aforementioned antibodies.

[001761] In some embodiments, the PD-1 inhibitor is an anti-PD-1 biosimilar monoclonal antibody approved by drug regulatory authorities with reference to pembrolizumab. In some embodiments, the biosimilar comprises an anti-PD-1 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is pembrolizumab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is an anti-PD-1 antibody authorized or submitted for authorization, wherein the anti-PD-1 antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is pembrolizumab. The anti-PD-1 antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product,

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wherein the reference medicinal product or reference biological product is pembrolizumab. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is pembrolizumab.

TABLE 20. Amino acid sequences for PD-1 inhibitors related to pembrolizumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:473 pembrolizumab heavy chain	QVQLVQSGVE VKKPGASVKV SCKASGYTFT NYYMYWVRQA PGQGLEWMGG INPSNGGTNF NEKFKNRVTL TTDSSTTTAY MELKSLQFDD TAVYYCARRD YRFDMGFDYW GQGTTVTVSS ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES KYGPPCPPCP APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE ALHNHYTQKS LSLSLGK	60 120 180 240 300 360 420 447
SEQ ID NO:474 pembrolizumab light chain	EIVLTQSPAT LSLSPGERAT LSCRASKGVS TSGYSYLHWY QQKPGQAPRL LIYLASYLES GVPARFSGSG SGTDFTLTIS SLEPEDFAVY YCQHSRDLPL TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC	60 120 180 218
SEQ ID NO:475 pembrolizumab variable heavy chain	QVQLVQSGVE VKKPGASVKV SCKASGYTFT NYYMYWVRQA PGQGLEWMGG INPSNGGTNF NEKFKNRVTL TTDSSTTTAY MELKSLQFDD TAVYYCARRD YRFDMGFDYW GQGTTVTVSS	60 120
SEQ ID NO:476 pembrolizumab variable light chain	EIVLTQSPAT LSLSPGERAT LSCRASKGVS TSGYSYLHWY QQKPGQAPRL LIYLASYLES GVPARFSGSG SGTDFTLTIS SLEPEDFAVY YCQHSRDLPL TFGGGTKVEI K	60 111
SEQ ID NO:477 pembrolizumab heavy chain CDR1	NYYMY	5
SEQ ID NO:478 pembrolizumab heavy chain CDR2	GINPSNGGTN FNEKFK	16
SEQ ID NO:479 pembrolizumab heavy chain CDR3	RDYRFDMGFD Y	11
SEQ ID NO:480 pembrolizumab light chain CDR1	RASKGVSTSG YSYLH	15
SEQ ID NO:481 pembrolizumab light chain CDR2	LASYLES	7
SEQ ID NO:482 pembrolizumab light chain CDR3	QHSRDLPLT	9

[001762] In some embodiments, the PD-1 inhibitor is pembrolizumab or a biosimilar thereof, and the pembrolizumab is administered at a dose of about 0.5 mg/kg to about 10 mg/kg. In some embodiments, the PD-1 inhibitor is pembrolizumab or a biosimilar thereof, and the pembrolizumab is administered at a dose of about 0.5 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 3.5 mg/kg, about 4 mg/kg, about 4.5 mg/kg, about 5 mg/kg, about 5.5 mg/kg, about 6 mg/kg, about 6.5 mg/kg, about 7

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mg/kg, about 7.5 mg/kg, about 8 mg/kg, about 8.5 mg/kg, about 9 mg/kg, about 9.5 mg/kg, or about 10 mg/kg. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001763] In some embodiments, the PD-1 inhibitor is pembrolizumab or a biosimilar thereof, wherein the pembrolizumab is administered at a dose of about 200 mg to about 500 mg. In some embodiments, the PD-1 inhibitor is pembrolizumab or a biosimilar thereof, and the nivolumab is administered at a dose of about 200 mg, about 220 mg, about 240 mg, about 260 mg, about 280 mg, about 300 mg, about 320 mg, about 340 mg, about 360 mg, about 380 mg, about 400 mg, about 420 mg, about 440 mg, about 460 mg, about 480 mg, or about 500 mg. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001764] In some embodiments, the PD-1 inhibitor is pembrolizumab or a biosimilar thereof, wherein the pembrolizumab is administered every 2 weeks, every 3 weeks, every 4 weeks, every 5 weeks, or every 6 weeks. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001765] In some embodiments, the pembrolizumab is administered to treat melanoma. In some embodiments, the pembrolizumab is administered to treat melanoma at about 200 mg every 3 weeks. In some embodiments, the pembrolizumab is administered to treat melanoma

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at about 400 mg every 6 weeks. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001766] In some embodiments, the pembrolizumab is administered to treat NSCLC. In some embodiments, the pembrolizumab is administered to treat NSCLC at about 200 mg every 3 weeks. In some embodiments, the pembrolizumab is administered to treat NSCLC at about 400 mg every 6 weeks. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001767] In some embodiments, the pembrolizumab is administered to treat small cell lung cancer (SCLC). In some embodiments, the pembrolizumab is administered to treat SCLC at about 200 mg every 3 weeks. In some embodiments, the pembrolizumab is administered to treat SCLC at about 400 mg every 6 weeks. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001768] In some embodiments, the pembrolizumab is administered to treat head and neck squamous cell cancer (HNSCC). In some embodiments, the pembrolizumab is administered to treat HNSCC at about 200 mg every 3 weeks. In some embodiments, the pembrolizumab is administered to treat HNSCCat about 400 mg every 6 weeks. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3

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days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001769] In some embodiments, the pembrolizumab is administered to treat Classical Hodgkin Lymphoma (cHL) or Primary Mediastinal Large B-Cell Lymphoma (PMBCL) at about 200 mg every 3 weeks. In some embodiments, the pembrolizumab is administered to treat Classical Hodgkin Lymphoma (cHL) or Primary Mediastinal Large B-Cell Lymphoma (PMBCL) at about 400 mg every 6 weeks for adults. In some embodiments, the pembrolizumab is administered to treat Classical Hodgkin Lymphoma (cHL) or Primary Mediastinal Large B-Cell Lymphoma (PMBCL) at about 2 mg/kg (up to 200 mg) every 3 weeks for pediatrics. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001770] In some embodiments, the pembrolizumab is administered to treat urothelial carcinoma at about 200 mg every 3 weeks. In some embodiments, the pembrolizumab is administered to treat urothelial carcinoma at about 400 mg every 6 weeks. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001771] In some embodiments, the pembrolizumab is administered to treat microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) cancer at about 200 mg every 3 weeks. In some embodiments, the pembrolizumab is administered to treat MSI-H or dMMR cancer at about 400 mg every 6 weeks for adults. In some embodiments, the pembrolizumab is administered to treat MSI-H or dMMR cancer at about 2 mg/kg (up to 200 mg) every 3 weeks for pediatrics. In some embodiments, the pembrolizumab

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administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001772] In some embodiments, the pembrolizumab is administered to treat microsatellite instability-high (MSI-H) or mismatch repair deficient colorectal cancer (dMMR CRC at about 200 mg every 3 weeks. In some embodiments, the pembrolizumab is administered to treat MSI-H or dMMR CRC at about 400 mg every 6 weeks. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001773] In some embodiments, the pembrolizumab is administered to treat gastric cancer at about 200 mg every 3 weeks. In some embodiments, the pembrolizumab is administered to treat gastric cancer at about 400 mg every 6 weeks. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001774] In some embodiments, the pembrolizumab is administered to treat Esophageal Cancer at about 200 mg every 3 weeks. In some embodiments, the pembrolizumab is

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administered to treat Esophageal Cancer at about 400 mg every 6 weeks. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001775] In some embodiments, the pembrolizumab is administered to treat cervical cancer at about 200 mg every 3 weeks. In some embodiments, the pembrolizumab is administered to treat cervical cancer at about 400 mg every 6 weeks. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001776] In some embodiments, the pembrolizumab is administered to treat hepatocellular carcinoma (HCC) at about 200 mg every 3 weeks. In some embodiments, the pembrolizumab is administered to treat HCC at about 400 mg every 6 weeks. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001777] In some embodiments, the pembrolizumab is administered to treat Merkel cell carcinoma (MCC) at about 200 mg every 3 weeks for adults. In some embodiments, the pembrolizumab is administered to treat MCC at about 400 mg every 6 weeks for adults. In some embodiments, the pembrolizumab is administered to treat MCC at about 2 mg/kg (up to 200 mg) every 3 weeks for pediatrics. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection

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(*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001778] In some embodiments, the pembrolizumab is administered to treat renal cell carcinoma (RCC) at about 200 mg every 3 weeks. In some embodiments, the pembrolizumab is administered to treat RCC at about 400 mg every 6 weeks with axitinib 5 mg orally twice daily. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001779] In some embodiments, the pembrolizumab is administered to treat endometrial carcinoma at about 200 mg every 3 weeks. In some embodiments, the pembrolizumab is administered to treat Endometrial Carcinoma at about 400 mg every 6 weeks with lenvatinib 20 mg orally once daily for tumors that are not MSI-H or dMMR. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001780] In some embodiments, the pembrolizumab is administered to treat tumor mutational burden-high (TMB-H) Cancer at about 200 mg every 3 weeks for adults. In some embodiments, the pembrolizumab is administered to treat TMB-H Cancer at about 400 mg every 6 weeks for adults. In some embodiments, the pembrolizumab is administered to treat

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TMB-H Cancer at about 2 mg/kg (up to 200 mg) every 3 weeks for pediatrics. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001781] In some embodiments, the pembrolizumab is administered to treat cutaneous squamous cell carcinoma (cSCC) at about 200 mg every 3 weeks. In some embodiments, the pembrolizumab is administered to treat cSCC at about 400 mg every 6 weeks. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001782] In some embodiments, the pembrolizumab is administered to treat triple-negative breast cancer (TNBC) at about 200 mg every 3 weeks. In some embodiments, the pembrolizumab is administered to treat TNBC at about 400 mg every 6 weeks. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

[001783] In some embodiments, if the patient or subject is an adult, *i.e.*, treatment of adult indications, and additional dosing regimen of 400 mg every 6 weeks can be employed. In some embodiments, the pembrolizumab administration is begun 1, 2, 3, 4, or 5 days post IL-2 administration. In some embodiments, the pembrolizumab administration is begun 1, 2, or 3 days post IL-2 administration. In some embodiments, the pembrolizumab can also be administered 1, 2, 3, 4 or 5 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient). In some embodiments, the pembrolizumab can also be administered 1, 2, or 3 weeks pre-resection (*i.e.*, before obtaining a tumor sample from the subject or patient).

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[001784] In some embodiments, the PD-1 inhibitor is a commercially-available anti-PD-1 monoclonal antibody, such as anti-m-PD-1 clones J43 (Cat # BE0033-2) and RMP1-14 (Cat # BE0146) (Bio X Cell, Inc., West Lebanon, NH, USA). A number of commercially-available anti-PD-1 antibodies are known to one of ordinary skill in the art.

[001785] In some embodiments, the PD-1 inhibitor is an antibody disclosed in U.S. Patent No. 8,354,509 or U.S. Patent Application Publication Nos. 2010/0266617 A1, 2013/0108651 A1, 2013/0109843 A2, the disclosures of which are incorporated by reference herein. In some embodiments, the PD-1 inhibitor is an anti-PD-1 antibody described in U.S. Patent Nos. 8,287,856, 8,580,247, and 8,168,757 and U.S. Patent Application Publication Nos. 2009/0028857 A1, 2010/0285013 A1, 2013/0022600 A1, and 2011/0008369 A1, the teachings of which are hereby incorporated by reference. In other embodiments, the PD-1 inhibitor is an anti-PD-1 antibody disclosed in U.S. Patent No. 8,735,553 B1, the disclosure of which is incorporated herein by reference. In some embodiments, the PD-1 inhibitor is pidilizumab, also known as CT-011, which is described in U.S. Patent No. 8,686,119, the disclosure of which is incorporated by reference herein.

[001786] In some embodiments, the PD-1 inhibitor may be a small molecule or a peptide, or a peptide derivative, such as those described in U.S. Patent Nos. 8,907,053; 9,096,642; and 9,044,442 and U.S. Patent Application Publication No. US 2015/0087581; 1,2,4-oxadiazole compounds and derivatives such as those described in U.S. Patent Application Publication No. 2015/0073024; cyclic peptidomimetic compounds and derivatives such as those described in U.S. Patent Application Publication No. US 2015/0073042; cyclic compounds and derivatives such as those described in U.S. Patent Application Publication No. US 2015/0125491; 1,3,4-oxadiazole and 1,3,4-thiadiazole compounds and derivatives such as those described in International Patent Application Publication No. WO 2015/033301; peptide-based compounds and derivatives such as those described in International Patent Application Publication Nos. WO 2015/036927 and WO 2015/04490, or a macrocyclic peptide-based compounds and derivatives such as those described in U.S. Patent Application Publication No. US 2014/0294898; the disclosures of each of which are hereby incorporated by reference in their entireties.

[001787] In some embodiments, the PD-L1 or PD-L2 inhibitor may be any PD-L1 or PD-L2 inhibitor, antagonist, or blocker known in the art. In particular, it is one of the PD-L1 or PD-L2 inhibitors, antagonist, or blockers described in more detail in the following paragraphs. The terms "inhibitor," "antagonist," and "blocker" are used interchangeably herein in

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reference to PD-L1 and PD-L2 inhibitors. For avoidance of doubt, references herein to a PD-L1 or PD-L2 inhibitor that is an antibody may refer to a compound or antigen-binding fragments, variants, conjugates, or biosimilars thereof. For avoidance of doubt, references herein to a PD-L1 or PD-L2 inhibitor may refer to a compound or a pharmaceutically acceptable salt, ester, solvate, hydrate, cocrystal, or prodrug thereof.

[001788] In some embodiments, the compositions, processes and methods described herein include a PD-L1 or PD-L2 inhibitor. In some embodiments, the PD-L1 or PD-L2 inhibitor is a small molecule. In some embodiments, the PD-L1 or PD-L2 inhibitor is an antibody (*i.e.*, an anti-PD-1 antibody), a fragment thereof, including Fab fragments, or a single-chain variable fragment (scFv) thereof. In some embodiments the PD-L1 or PD-L2 inhibitor is a polyclonal antibody. In some embodiments, the PD-L1 or PD-L2 inhibitor is a monoclonal antibody. In some embodiments, the PD-L1 or PD-L2 inhibitor competes for binding with PD-L1 or PD-L2, and/or binds to an epitope on PD-L1 or PD-L2. In some embodiments, the antibody competes for binding with PD-L1 or PD-L2, and/or binds to an epitope on PD-L1 or PD-L2.

[001789] In some embodiments, the PD-L1 inhibitors provided herein are selective for PD-L1, in that the compounds bind or interact with PD-L1 at substantially lower concentrations than they bind or interact with other receptors, including the PD-L2 receptor. In certain embodiments, the compounds bind to the PD-L1 receptor at a binding constant that is at least about a 2-fold higher concentration, about a 3-fold higher concentration, about a 5-fold higher concentration, about a 10-fold higher concentration, about a 20-fold higher concentration, about a 30-fold higher concentration, about a 50-fold higher concentration, about a 100-fold higher concentration, about a 200-fold higher concentration, about a 300-fold higher concentration, or about a 500-fold higher concentration than to the PD-L2 receptor.

[001790] In some embodiments, the PD-L2 inhibitors provided herein are selective for PD-L2, in that the compounds bind or interact with PD-L2 at substantially lower concentrations than they bind or interact with other receptors, including the PD-L1 receptor. In certain embodiments, the compounds bind to the PD-L2 receptor at a binding constant that is at least about a 2-fold higher concentration, about a 3-fold higher concentration, about a 5-fold higher concentration, about a 10-fold higher concentration, about a 20-fold higher concentration, about a 30-fold higher concentration, about a 50-fold higher concentration, about a 100-fold higher concentration, about a 200-fold higher concentration, about a 300-

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fold higher concentration, or about a 500-fold higher concentration than to the PD-L1 receptor.

[001791] Without being bound by any theory, it is believed that tumor cells express PD-L1, and that T cells express PD-1. However, PD-L1 expression by tumor cells is not required for efficacy of PD-1 or PD-L1 inhibitors or blockers. In some embodiments, the tumor cells express PD-L1. In other embodiments, the tumor cells do not express PD-L1. In some embodiments, the methods can include a combination of a PD-1 and a PD-L1 antibody, such as those described herein, in combination with a TIL. The administration of a combination of a PD-1 and a PD-L1 antibody and a TIL may be simultaneous or sequential.

[001792] In some embodiments, the PD-L1 and/or PD-L2 inhibitor is one that binds human PD-L1 and/or PD-L2 with a KD of about 100 pM or lower, binds human PD-L1 and/or PD-L2 with a KD of about 90 pM or lower, binds human PD-L1 and/or PD-L2 with a KD of about 80 pM or lower, binds human PD-L1 and/or PD-L2 with a KD of about 70 pM or lower, binds human PD-L1 and/or PD-L2 with a KD of about 60 pM or lower, a KD of about 50 pM or lower, binds human PD-L1 and/or PD-L2 with a KD of about 40 pM or lower, or binds human PD-L1 and/or PD-L2 with a KD of about 30 pM or lower,

[001793] In some embodiments, the PD-L1 and/or PD-L2 inhibitor is one that binds to human PD-L1 and/or PD-L2 with a kassoc of about 7.5 × 105 1/M·s or faster, binds to human PD-L1 and/or PD-L2 with a kassoc of about 8 × 105 1/M·s or faster, binds to human PD-L1 and/ or PD-L2 with a kassoc of about 8.5 × 105 1/M·s or faster, binds to human PD-L1 and/or PD-L2 with a kassoc of about 9 × 105 1/M·s or faster, binds to human PD-L1 and/or PD-L2 with a kassoc of about 9.5 × 105 1/M·s and/or faster, or binds to human PD-L1 and/or PD-L2 with a kassoc of about 1 × 106 1/M·s or faster.

[001794] In some embodiments, the PD-L1 and/or PD-L2 inhibitor is one that binds to human PD-L1 or PD-L2 with a kdissoc of about 2 × 10-5 1/s or slower, binds to human PD-1 with a kdissoc of about 2.1 × 10-5 1/s or slower , binds to human PD-1 with a kdissoc of about 2.2 × 10-5 1/s or slower, binds to human PD-1 with a kdissoc of about 2.3 × 10-5 1/s or slower, binds to human PD-1 with a kdissoc of about 2.4 × 10-5 1/s or slower, binds to human PD-1 with a kdissoc of about 2.5 × 10-5 1/s or slower, binds to human PD-1 with a kdissoc of about 2.6 × 10-5 1/s or slower, binds to human PD-L1 or PD-L2 with a kdissoc of about 2.7 × 10-5 1/s or slower, or binds to human PD-L1 or PD-L2 with a kdissoc of about 3 × 10-5 1/s or slower.

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[001795] In some embodiments, the PD-L1 and/or PD-L2 inhibitor is one that blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 10 nM or lower; blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 9 nM or lower; blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 8 nM or lower; blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 7 nM or lower; blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 6 nM or lower; blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 5 nM or lower; blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 4 nM or lower; blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 3 nM or lower; blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC50 of about 2 nM or lower; or blocks human PD-1, or blocks binding of human PD-L1 or human PD-L2 to human PD-l with an IC50 of about 1 nM or lower.

[001796] In some embodiments, the PD-L1 inhibitor is durvalumab, also known as MEDI4736 (which is commercially available from Medimmune, LLC, Gaithersburg, Maryland, a subsidiary of AstraZeneca plc.), or antigen-binding fragments, conjugates, or variants thereof. In some embodiments, the PD-L1 inhibitor is an antibody disclosed in U.S. Patent No. 8,779,108 or U.S. Patent Application Publication No. 2013/0034559, the disclosures of which are incorporated by reference herein. The clinical efficacy of durvalumab has been described in Page, et al., Ann. Rev. Med., 2014, 65, 185-202; Brahmer, et al., J. Clin. Oncol. 2014, 32, 5s (supplement, abstract 8021); and McDermott, et al., Cancer Treatment Rev., 2014, 40, 1056-64. The preparation and properties of durvalumab are described in U.S. Patent No. 8,779,108, the disclosure of which is incorporated by reference herein. The amino acid sequences of durvalumab are set forth in Table 21. The durvalumab monoclonal antibody includes disulfide linkages at 22-96, 22"-96", 23'-89', 23'"-89'", 135'-195', 135'"-195'", 148-204, 148"-204", 215'-224, 215'"-224", 230-230", 233-233", 265-325, 265"-325", 371-429, and 371"-429'; and N-glycosylation sites at Asn-301 and Asn-301".

[001797] In some embodiments, a PD-L1 inhibitor comprises a heavy chain given by SEQ ID NO:483 and a light chain given by SEQ ID NO:484. In some embodiments, a PD-L1 inhibitor comprises heavy and light chains having the sequences shown in SEQ ID NO:483 and SEQ ID NO:484, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In some embodiments, a PD-L1

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inhibitor comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:483 and SEQ ID NO:484, respectively. In some embodiments, a PD-L1 inhibitor comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:483 and SEQ ID NO:484, respectively. In some embodiments, a PD-L1 inhibitor comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:483 and SEQ ID NO:484, respectively. In some embodiments, a PD-L1 inhibitor comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:483 and SEQ ID NO:484, respectively. In some embodiments, a PD-L1 inhibitor comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:483 and SEQ ID NO:484, respectively.

[001798] In some embodiments, the PD-L1 inhibitor comprises the heavy and light chain CDRs or variable regions (VRs) of durvalumab. In some embodiments, the PD-L1 inhibitor heavy chain variable region (VH) comprises the sequence shown in SEQ ID NO:485, and the PD-L1 inhibitor light chain variable region (VL) comprises the sequence shown in SEQ ID NO:486, and conservative amino acid substitutions thereof. In some embodiments, a PD-L1 inhibitor comprises VH and VL regions that are each at least 99% identical to the sequences shown in SEQ ID NO:485 and SEQ ID NO:486, respectively. In some embodiments, a PD-L1 inhibitor comprises VH and VL regions that are each at least 98% identical to the sequences shown in SEQ ID NO:485 and SEQ ID NO:486, respectively. In some embodiments, a PD-L1 inhibitor comprises VH and VL regions that are each at least 97% identical to the sequences shown in SEQ ID NO:485 and SEQ ID NO:486, respectively. In some embodiments, a PD-L1 inhibitor comprises VH and VL regions that are each at least 96% identical to the sequences shown in SEQ ID NO:485 and SEQ ID NO:486, respectively. In some embodiments, a PD-L1 inhibitor comprises VH and VL regions that are each at least 95% identical to the sequences shown in SEQ ID NO:485 and SEQ ID NO:486, respectively.

[001799] In some embodiments, a PD-L1 inhibitor comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:487, SEQ ID NO:488, and SEQ ID NO:489, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:490, SEQ ID NO:491, and SEQ ID NO:492, respectively, and conservative amino acid substitutions thereof. In some embodiments, the antibody competes for binding with, and/or binds to the same epitope on PD-L1 as any of the aforementioned antibodies.

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[001800] In some embodiments, the PD-L1 inhibitor is an anti-PD-L1 biosimilar monoclonal antibody approved by drug regulatory authorities with reference to durvalumab. In some embodiments, the biosimilar comprises an anti-PD-L1 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is durvalumab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is an anti-PD-L1 antibody authorized or submitted for authorization, wherein the anti-PD-L1 antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is durvalumab. The anti-PD-L1 antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is durvalumab. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is durvalumab.

TABLE 21. Amino acid sequences for PD-L1 inhibitors related to durvalumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:483 durvalumab heavy chain	EVQLVESGGG LVQPGGSLRL SCAASGFTFS RYWMSWVRQA PGKGLEWVAN IKQDGSEKYY VDSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCAREG GWFGELAFDY WGQGTLVTVS SASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKRVE PKSCDKTHTC PPCPAPEFEG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPASIEKTI SKAKGQPREP QVYTLPPSRE EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K	60 120 180 240 300 360 420 451
SEQ ID NO:484 durvalumab light chain	EVQLVESGGG LVQPGGSLRL SCAASGFTFS RYWMSWVRQA PGKGLEWVAN EIVLTQSPGT LSLSPGERAT LSCRASQRVS SSYLAWYQQK PGQAPRLLIY DASSRATGIP DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSLPWTFG QGTKVEIKRT VAAPSVFIFP PSDEQLKSGT ASVVCLLNNF YPREAKVQWK VDNALQSGNS QESVTEQDSK DSTYSLSSTL TLSKADYEKH KVYACEVTHQ GLSSPVTKSF NRGEC	60 120 180 240 265
SEQ ID NO:485 durvalumab	EVQLVESGGG LVQPGGSLRL SCAASGFTFS RYWMSWVRQA PGKGLEWVAN IKQDGSEKYY VDSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCAREG GWFGELAFDY WGQGTLVTVS S	60 120 121

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Identifier	Sequence (One-Letter Amino Acid Symbols)	
variable heavy chain		
SEQ ID NO:486 durvalumab variable light chain	EIVLTQSPGT LSLSPGERAT LSCRASQRVS SSYLAWYQQK PGQAPRLLIY DASSRATGIP DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSLPWTFG QGTKVEIK	60 108
SEQ ID NO:487 durvalumab heavy chain CDR1	RYWMS	5
SEQ ID NO:488 durvalumab heavy chain CDR2	NIKQDGSEKY YVDSVKG	17
SEQ ID NO:489 durvalumab heavy chain CDR3	EGGWFGELAF DY	12
SEQ ID NO:490 durvalumab light chain CDR1	RASQRVSSSY LA	12
SEQ ID NO:491 durvalumab light chain CDR2	DASSRAT	7
SEQ ID NO:492 durvalumab light chain CDR3	QQYGSLPWT	9

[001801] In some embodiments, the PD-L1 inhibitor is avelumab, also known as MSB0010718C (commercially available from Merck KGaA/EMD Serono), or antigen-binding fragments, conjugates, or variants thereof. The preparation and properties of avelumab are described in U.S. Patent Application Publication No. US 2014/0341917 A1, the disclosure of which is specifically incorporated by reference herein. The amino acid sequences of avelumab are set forth in Table 22. Avelumab has intra-heavy chain disulfide linkages (C23-C104) at 22-96, 147-203, 264-324, 370-428, 22"-96", 147"-203", 264"-324", and 370"-428"; intra-light chain disulfide linkages (C23-C104) at 22'-90', 138'-197', 22'"-90'", and 138'"-197'"; intra-heavy-light chain disulfide linkages (h 5-CL 126) at 223-215' and 223"-215'"; intra-heavy-heavy chain disulfide linkages (h 11, h 14) at 229-229" and 232-232"; N-glycosylation sites (H CH2 N84.4) at 300, 300"; fucosylated complex bi-antennary CHO-type glycans; and H CHS K2 C-terminal lysine clipping at 450 and 450'.

[001802] In some embodiments, a PD-L1 inhibitor comprises a heavy chain given by SEQ ID NO:493 and a light chain given by SEQ ID NO:494. In some embodiments, a PD-L1 inhibitor comprises heavy and light chains having the sequences shown in SEQ ID NO:493 and SEQ ID NO:494, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In some embodiments, a PD-L1 inhibitor comprises heavy and light chains that are each at least 99% identical to the

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sequences shown in SEQ ID NO:493 and SEQ ID NO:494, respectively. In some embodiments, a PD-L1 inhibitor comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:493 and SEQ ID NO:494, respectively. In some embodiments, a PD-L1 inhibitor comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:493 and SEQ ID NO:494, respectively. In some embodiments, a PD-L1 inhibitor comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:493 and SEQ ID NO:494, respectively. In some embodiments, a PD-L1 inhibitor comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:493 and SEQ ID NO:494, respectively.

[001803] In some embodiments, the PD-L1 inhibitor comprises the heavy and light chain CDRs or variable regions (VRs) of avelumab. In some embodiments, the PD-L1 inhibitor heavy chain variable region (VH) comprises the sequence shown in SEQ ID NO:495, and the PD-L1 inhibitor light chain variable region (VL) comprises the sequence shown in SEQ ID NO:496, and conservative amino acid substitutions thereof. In some embodiments, a PD-L1 inhibitor comprises VH and VL regions that are each at least 99% identical to the sequences shown in SEQ ID NO:495 and SEQ ID NO:496, respectively. In some embodiments, a PD-L1 inhibitor comprises VH and VL regions that are each at least 98% identical to the sequences shown in SEQ ID NO:496 and SEQ ID NO:496, respectively. In some embodiments, a PD-L1 inhibitor comprises VH and VL regions that are each at least 97% identical to the sequences shown in SEQ ID NO:495 and SEQ ID NO:496, respectively. In some embodiments, a PD-L1 inhibitor comprises VH and VL regions that are each at least 96% identical to the sequences shown in SEQ ID NO:495 and SEQ ID NO:496, respectively. In some embodiments, a PD-L1 inhibitor comprises VH and VL regions that are each at least 95% identical to the sequences shown in SEQ ID NO:495 and SEQ ID NO:496, respectively.

[001804] In some embodiments, a PD-L1 inhibitor comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:497, SEQ ID NO:498, and SEQ ID NO:499, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:500, SEQ ID NO:501, and SEQ ID NO:502, respectively, and conservative amino acid substitutions thereof. In some embodiments, the antibody competes for binding with, and/or binds to the same epitope on PD-L1 as any of the aforementioned antibodies.

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[001805] In some embodiments, the PD-L1 inhibitor is an anti-PD-L1 biosimilar monoclonal antibody approved by drug regulatory authorities with reference to avelumab. In some embodiments, the biosimilar comprises an anti-PD-L1 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is avelumab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is an anti-PD-L1 antibody authorized or submitted for authorization, wherein the anti-PD-L1 antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is avelumab. The anti-PD-L1 antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is avelumab. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is avelumab.

TABLE 22. Amino acid sequences for PD-L1 inhibitors related to avelumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:493 avelumab heavy chain	EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYIMMWVRQA PGKGLEWVSS IYPSGGITFY ADTVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARIK LGTVTTVDYW GQGTLVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK	60 120 180 240 300 360 420 450
SEQ ID NO:494 avelumab light chain	QSALTQPASV SGSPGQSITI SCTGTSSDVG GYNYVSWYQQ HPGKAPKLMI YDVSNRPSGV SNRFSGSKSG NTASLTISGL QAEDEADYYC SSYTSSSTRV FGTGTKVTVL GQPKANPTVT LFPPSSEELQ ANKATLVCLI SDFYPGAVTV AWKADGSPVK AGVETTKPSK QSNNKYAASS YLSLTPEQWK SHRSYSCQVT HEGSTVEKTV APTECS	60 120 180 216
SEQ ID NO:495 avelumab variable heavy chain	EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYIMMWVRQA PGKGLEWVSS IYPSGGITFY ADTVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARIK LGTVTTVDYW GQGTLVTVSS	60 120
SEQ ID NO:496	QSALTQPASV SGSPGQSITI SCTGTSSDVG GYNYVSWYQQ HPGKAPKLMI YDVSNRPSGV	60

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Identifier	Sequence (One-Letter Amino Acid Symbols)	
avelumab variable light chain	SNRFSGSKSG NTASLTISGL QAEDEADYYC SSYTSSSTRV FGTGTKVTVL	110
SEQ ID NO:497 avelumab heavy chain CDR1	SYIMM	5
SEQ ID NO:498 avelumab heavy chain CDR2	SIYPSGGITF YADTVKG	17
SEQ ID NO:499 avelumab heavy chain CDR3	IKLGTVTTVD Y	11
SEQ ID NO:500 avelumab light chain CDR1	TGTSSDVGGY NYVS	14
SEQ ID NO:501 avelumab light chain CDR2	DVSNRPS	7
SEQ ID NO:502 avelumab light chain CDR3	SSYTSSSTRV	10

[001806] In some embodiments, the PD-L1 inhibitor is atezolizumab, also known as MPDL3280A or RG7446 (commercially available as TECENTRIQ from Genentech, Inc., a subsidiary of Roche Holding AG, Basel, Switzerland), or antigen-binding fragments, conjugates, or variants thereof. In some embodiments, the PD-L1 inhibitor is an antibody disclosed in U.S. Patent No. 8,217,149, the disclosure of which is specifically incorporated by reference herein. In some embodiments, the PD-L1 inhibitor is an antibody disclosed in U.S. Patent Application Publication Nos. 2010/0203056 A1, 2013/0045200 A1, 2013/0045201 A1, 2013/0045202 A1, or 2014/0065135 A1, the disclosures of which are specifically incorporated by reference herein. The preparation and properties of atezolizumab are described in U.S. Patent No. 8,217,149, the disclosure of which is incorporated by reference herein. The amino acid sequences of atezolizumab are set forth in Table 23. Atezolizumab has intra-heavy chain disulfide linkages (C23-C104) at 22-96, 145-201, 262-322, 368-426, 22"-96", 145"-201", 262"-322", and 368"-426"; intra-light chain disulfide linkages (C23-C104) at 23'-88', 134'-194', 23'"-88'", and 134'"-194'"; intra-heavy-light chain disulfide linkages (h 5-CL 126) at 221-214' and 221"-214'"; intra-heavy-heavy chain disulfide linkages (h 11, h 14) at 227-227" and 230-230"; and N-glycosylation sites (H CH2 N84.4>A) at 298 and 298'.

[001807] In some embodiments, a PD-L1 inhibitor comprises a heavy chain given by SEQ ID NO:503 and a light chain given by SEQ ID NO:504. In some embodiments, a PD-L1

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inhibitor comprises heavy and light chains having the sequences shown in SEQ ID NO:503 and SEQ ID NO:504, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In some embodiments, a PD-L1 inhibitor comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:503 and SEQ ID NO:504, respectively. In some embodiments, a PD-L1 inhibitor comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:503 and SEQ ID NO:504, respectively. In some embodiments, a PD-L1 inhibitor comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:503 and SEQ ID NO:504, respectively. In some embodiments, a PD-L1 inhibitor comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:503 and SEQ ID NO:504, respectively. In some embodiments, a PD-L1 inhibitor comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:503 and SEQ ID NO:504, respectively.

[001808] In some embodiments, the PD-L1 inhibitor comprises the heavy and light chain CDRs or variable regions (VRs) of atezolizumab. In some embodiments, the PD-L1 inhibitor heavy chain variable region (VH) comprises the sequence shown in SEQ ID NO:505, and the PD-L1 inhibitor light chain variable region (VL) comprises the sequence shown in SEQ ID NO:506, and conservative amino acid substitutions thereof. In some embodiments, a PD-L1 inhibitor comprises VH and VL regions that are each at least 99% identical to the sequences shown in SEQ ID NO:505 and SEQ ID NO:506, respectively. In some embodiments, a PD-L1 inhibitor comprises VH and VL regions that are each at least 98% identical to the sequences shown in SEQ ID NO:505 and SEQ ID NO:506, respectively. In some embodiments, a PD-L1 inhibitor comprises VH and VL regions that are each at least 97% identical to the sequences shown in SEQ ID NO:505 and SEQ ID NO:506, respectively. In some embodiments, a PD-L1 inhibitor comprises VH and VL regions that are each at least 96% identical to the sequences shown in SEQ ID NO:505 and SEQ ID NO:506, respectively. In some embodiments, a PD-L1 inhibitor comprises VH and VL regions that are each at least 95% identical to the sequences shown in SEQ ID NO:505 and SEQ ID NO:506, respectively.

[001809] In some embodiments, a PD-L1 inhibitor comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:507, SEQ ID NO:508, and SEQ ID NO:509, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:510,

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SEQ ID NO:511, and SEQ ID NO:512, respectively, and conservative amino acid substitutions thereof. In some embodiments, the antibody competes for binding with, and/or binds to the same epitope on PD-L1 as any of the aforementioned antibodies.

[001810] In some embodiments, the anti-PD-L1 antibody is an anti-PD-L1 biosimilar monoclonal antibody approved by drug regulatory authorities with reference to atezolizumab. In some embodiments, the biosimilar comprises an anti-PD-L1 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is atezolizumab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is an anti-PD-L1 antibody authorized or submitted for authorization, wherein the anti-PD-L1 antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is atezolizumab. The anti-PD-L1 antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is atezolizumab. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is atezolizumab.

TABLE 23. Amino acid sequences for PD-L1 inhibitors related to atezolizumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:503 atezolizumab heavy chain	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DSWIHWVRQA PGKGLEWVAW ISPYGGSTYY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARRH WPGGFDYWGQ GTLVTVSSAS TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKKVEPKS CDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYAST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ	60 120 180 240 300 360 420

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Identifier	Sequence (One-Letter Amino Acid Symbols)	
	GNVFSCSVMH EALHNHYTQK SLSLSPGK	448
SEQ ID NO:504 atezolizumab light chain	DIQMTQSPSS LSASVGDRVT ITCRASQDVS TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YLYHPATFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC	60 120 180 214
SEQ ID NO:505 atezolizumab variable heavy chain	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DSWIHWVRQA PGKGLEWVAW ISPYGGSTYY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARRH WPGGFDYWGQ GTLVTVSA	60 118
SEQ ID NO:506 atezolizumab variable light chain	DIQMTQSPSS LSASVGDRVT ITCRASQDVS TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YLYHPATFGQ GTKVEIKR	60 108
SEQ ID NO:507 atezolizumab heavy chain CDR1	GFTFSDSWIH	10
SEQ ID NO:508 atezolizumab heavy chain CDR2	AWISPYGGST YYADSVKG	18
SEQ ID NO:509 atezolizumab heavy chain CDR3	RHWPGGFDY	9
SEQ ID NO:510 atezolizumab light chain CDR1	RASQDVSTAV A	11
SEQ ID NO:511 atezolizumab light chain CDR2	SASFLYS	7
SEQ ID NO:512 atezolizumab light chain CDR3	QQYLYHPAT	9

[001811] In some embodiments, PD-L1 inhibitors include those antibodies described in U.S. Patent Application Publication No. US 2014/0341917 A1, the disclosure of which is incorporated by reference herein. In other embodiments, antibodies that compete with any of these antibodies for binding to PD-L1 are also included. In some embodiments, the anti-PD-L1 antibody is MDX-1105, also known as BMS-935559, which is disclosed in U.S. Patent No. US 7,943,743, the disclosures of which are incorporated by reference herein. In some embodiments, the anti-PD-L1 antibody is selected from the anti-PD-L1 antibodies disclosed in U.S. Patent No. US 7,943,743, which are incorporated by reference herein.

[001812] In some embodiments, the PD-L1 inhibitor is a commercially-available monoclonal antibody, such as INVIVOMAB anti-m-PD-L1 clone 10F.9G2 (Catalog # BE0101, Bio X Cell, Inc., West Lebanon, NH, USA). In some embodiments, the anti-PD-L1 antibody is a commercially-available monoclonal antibody, such as AFFYMETRIX EBIOSCIENCE (MIH1). A number of commercially-available anti-PD-L1 antibodies are known to one of ordinary skill in the art.

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[001813] In some embodiments, the PD-L2 inhibitor is a commercially-available monoclonal antibody, such as BIOLEGEND 24F.10C12 Mouse IgG2a, κ isotype (catalog # 329602 Biolegend, Inc., San Diego, CA), SIGMA anti-PD-L2 antibody (catalog # SAB3500395, Sigma-Aldrich Co., St. Louis, MO), or other commercially-available anti-PD-L2 antibodies known to one of ordinary skill in the art.

4. CTLA-4 Inhibitors

[001814] In some embodiments, the TIL therapy provided to patients with cancer may include treatment with therapeutic populations of TILs alone or may include a combination treatment including TILs and one or more CTLA-4 inhibitors.

[001815] Cytotoxic T lymphocyte antigen 4 (CTLA-4) is a member of the immunoglobulin superfamily and is expressed on the surface of helper T cells. CTLA-4 is a negative regulator of CD28-dependent T cell activation and acts as a checkpoint for adaptive immune responses. Similar to the T cell costimulatory protein CD28, the CTLA-4 binding antigen presents CD80 and CD86 on the cells. CTLA-4 delivers a suppressor signal to T cells, while CD28 delivers a stimulus signal. Human antibodies against human CTLA-4 have been described as immunostimulatory modulators in many disease conditions, such as treating or preventing viral and bacterial infections and for treating cancer (WO 01/14424 and WO 00/37504). Various preclinical studies have shown that CTLA-4 blockade by CTLA-4 inhibitors such as monoclonal antibodies enhances host immune responses against immunogenic tumors and can even rule out established tumors. A number of fully human anti-human CTLA-4 monoclonal antibodies (mAbs) have been studied in clinical trials for the treatment of various types of solid tumors, including, but limited to, ipilimumab (MDX-010) and tremelimumab (CP-675,206).

[001816] In some embodiments, a CTLA-4 inhibitor may be any CTLA-4 inhibitor or CTLA-4 blocker known in the art. In particular, it is one of the CTLA-4 inhibitors or blockers described in more detail in the following paragraphs. The terms "inhibitor," "antagonist," and "blocker" are used interchangeably herein in reference to CTLA-4 inhibitors. For avoidance of doubt, references herein to a CTLA-4 inhibitor that is an antibody may refer to a compound or antigen-binding fragments, variants, conjugates, or biosimilars thereof. For avoidance of doubt, references herein to a CTLA-4 inhibitor may also refer to a small molecule compound or a pharmaceutically acceptable salt, ester, solvate, hydrate, cocrystal, or prodrug thereof.

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[001817] Suitable CTLA-4 inhibitors for use in the methods of the invention, include, without limitation, anti-CTLA-4 antibodies, human anti-CTLA-4 antibodies, mouse anti-CTLA-4 antibodies, mammalian anti-CTLA-4 antibodies, humanized anti-CTLA-4 antibodies, monoclonal anti-CTLA-4 antibodies, polyclonal anti-CTLA-4 antibodies, chimeric anti-CTLA-4 antibodies, MDX-010 (ipilimumab), tremelimumab, anti-CD28 antibodies, anti-CTLA-4 adnectins, anti-CTLA-4 domain antibodies, single chain anti-CTLA-4 fragments, heavy chain anti-CTLA-4 fragments, light chain anti-CTLA-4 fragments, inhibitors of CTLA-4 that agonize the co-stimulatory pathway, the antibodies disclosed in PCT Publication No. WO 2001/014424, the antibodies disclosed in PCT Publication No. WO 2004/035607, the antibodies disclosed in U.S. Publication No. 2005/0201994, and the antibodies disclosed in granted European Patent No. EP 1212422 B1, the disclosures of each of which are incorporated herein by reference. Additional CTLA-4 antibodies are described in U.S. Pat. Nos. 5,811,097, 5,855,887, 6,051,227, and 6,984,720; in PCT Publication Nos. WO 01/14424 and WO 00/37504; and in U.S. Publication Nos. 2002/0039581 and 2002/086014, the disclosures of each of which are incorporated herein by reference. Other anti-CTLA-4 antibodies that can be used in a method of the present invention include, for example, those disclosed in: WO 98/42752; U.S. Pat. Nos. 6,682,736 and 6,207,156; Hurwitz et al., Proc. Natl. Acad. Sci. USA, 95(17):10067-10071 (1998); Camacho et al., J. Clin. Oncology, 22(145): Abstract No. 2505 (2004) (antibody CP-675206); Mokyr et al., Cancer Res., 58:5301-5304 (1998), and U.S. Pat. Nos. 5,977,318, 6,682,736, 7,109,003, and 7,132,281, the disclosures of each of which are incorporated herein by reference.

[001818] Additional CTLA-4 inhibitors include, but are not limited to, the following: any inhibitor that is capable of disrupting the ability of CD28 antigen to bind to its cognate ligand, to inhibit the ability of CTLA-4 to bind to its cognate ligand, to augment T cell responses via the co-stimulatory pathway, to disrupt the ability of B7 to bind to CD28 and/or CTLA-4, to disrupt the ability of B7 to activate the co-stimulatory pathway, to disrupt the ability of CD80 to bind to CD28 and/or CTLA-4, to disrupt the ability of CD80 to activate the co-stimulatory pathway, to disrupt the ability of CD86 to bind to CD28 and/or CTLA-4, to disrupt the ability of CD86 to activate the co-stimulatory pathway, and to disrupt the co-stimulatory pathway, in general from being activated. This necessarily includes small molecule inhibitors of CD28, CD80, CD86, CTLA-4, among other members of the co-stimulatory pathway; antibodies directed to CD28, CD80, CD86, CTLA-4, among other members of the co-stimulatory pathway; antisense molecules directed against CD28, CD80,

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CD86, CTLA-4, among other members of the co-stimulatory pathway; adnectins directed against CD28, CD80, CD86, CTLA-4, among other members of the co-stimulatory pathway, RNAi inhibitors (both single and double stranded) of CD28, CD80, CD86, CTLA-4, among other members of the co-stimulatory pathway, among other CTLA-4 inhibitors.

[001819] In some embodiments a CTLA-4 inhibitor binds to CTLA-4 with a Kd of about 10^{-6} M or less, 10^{-7}M or less, 10^{-8} M or less, 10^{-9} M or less, 10^{-10} M or less, 10^{-11} M or less, 10^{-12} M or less, *e.g.*, between 10^{-13} M and 10^{-16} M, or within any range having any two of the afore-mentioned values as endpoints. In some embodiments a CTLA-4 inhibitor binds to CTLA-4 with a Kd of no more than 10-fold that of ipilimumab, when compared using the same assay. In some embodiments a CTLA-4 inhibitor binds to CTLA-4 with a Kd of about the same as, or less (*e.g.*, up to 10-fold lower, or up to 100-fold lower) than that of ipilimumab, when compared using the same assay. In some embodiments, the IC50 values for inhibition by a CTLA-4 inhibitor of CTLA-4 binding to CD80 or CD86 is no more than 10-fold greater than that of ipilimumab-mediated inhibition of CTLA-4 binding to CD80 or CD86, respectively, when compared using the same assay. In some embodiments, the IC50 values for inhibition by a CTLA-4 inhibitor of CTLA-4 binding to CD80 or CD86 is about the same or less (*e.g.*, up to 10-fold lower, or up to 100-fold lower) than that of ipilimumab-mediated inhibition of CTLA-4 binding to CD80 or CD86, respectively, when compared using the same assay.

[001820] In some embodiments a CTLA-4 inhibitor is used in an amount sufficient to inhibit expression and/or decrease biological activity of CTLA-4 by at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% relative to a suitable control, *e.g.*, between 50% and 75%, 75% and 90%, or 90% and 100%. In some embodiments a CTLA-4 pathway inhibitor is used in an amount sufficient to decrease the biological activity of CTLA-4 by reducing binding of CTLA-4 to CD80, CD86, or both by at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% relative to a suitable control, *e.g.*, between 50% and 75%, 75% and 90%, or 90% and 100% relative to a suitable control. A suitable control in the context of assessing or quantifying the effect of an agent of interest is typically a comparable biological system (*e.g.*, cells or a subject) that has not been exposed to or treated with the agent of interest, *e.g.*, CTLA-4 pathway inhibitor (or has been exposed to or treated with a negligible amount). In some embodiments a biological system may serve as its own control (*e.g.*, the biological system may be assessed before exposure to or treatment with the agent

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and compared with the state after exposure or treatment has started or finished. In some embodiments a historical control may be used.

[001821] In some embodiments, the CTLA-4 inhibitor is ipilimumab (commercially available as Yervoy from Bristol-Myers Squibb Co.), or biosimilars, antigen-binding fragments, conjugates, or variants thereof. As is known in the art, ipilimumab refers to an anti-CTLA-4 antibody, a fully human IgG 1κ antibody derived from a transgenic mouse with human genes encoding heavy and light chains to generate a functional human repertoire. is there. Ipilimumab can also be referred to by its CAS Registry Number 477202-00-9, and in PCT Publication Number WO 01/14424, which is incorporated herein by reference in its entirety for all purposes. It is disclosed as antibody 10DI. Specifically, ipilimumab contains a light chain variable region and a heavy chain variable region (having a light chain variable region comprising SEQ ID NO: 516 and having a heavy chain variable region comprising SEQ ID NO: 515). Represents a human monoclonal antibody or its antigen binding site that specifically binds to CTLA-4. A pharmaceutical composition of ipilimumab includes all pharmaceutically acceptable compositions containing ipilimumab and one or more diluents, vehicles and / or excipients. An example of a pharmaceutical composition containing ipilimumab is described in PCT Publication No. WO 2007/67959. Ipilimumab can be administered intravenously (IV).

[001822] In some embodiments, a CTLA-4 inhibitor comprises a heavy chain given by SEQ ID NO:513 and a light chain given by SEQ ID NO:514. In some embodiments, a CTLA-4 inhibitor comprises heavy and light chains having the sequences shown in SEQ ID NO:513 and SEQ ID NO:514, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In some embodiments, a CTLA-4 inhibitor comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:513 and SEQ ID NO:514, respectively. In some embodiments, a CTLA-4 inhibitor comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:513 and SEQ ID NO:514, respectively. In some embodiments, a CTLA-4 inhibitor comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:513 and SEQ ID NO:514, respectively. In some embodiments, a CTLA-4 inhibitor comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:513 and SEQ ID NO:514, respectively. In some embodiments, a CTLA-4 inhibitor comprises heavy and light

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chains that are each at least 95% identical to the sequences shown in SEQ ID NO:513 and SEQ ID NO:514, respectively.

[001823] In some embodiments, the CTLA-4 inhibitor comprises the heavy and light chain CDRs or variable regions (VRs) of ipilimumab. In some embodiments, the CTLA-4 inhibitor heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:515, and the CTLA-4 inhibitor light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:516, and conservative amino acid substitutions thereof. In some embodiments, a CTLA-4 inhibitor comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:515 and SEQ ID NO:516, respectively. In some embodiments, a CTLA-4 inhibitor comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:515 and SEQ ID NO:516, respectively. In some embodiments, a CTLA-4 inhibitor comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:515 and SEQ ID NO:516, respectively. In some embodiments, a CTLA-4 inhibitor comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:515 and SEQ ID NO:516, respectively. In some embodiments, a CTLA-4 inhibitor comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:515 and SEQ ID NO:516, respectively.

[001824] In some embodiments, a CTLA-4 inhibitor comprises the heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:517, SEQ ID NO:518, and SEQ ID NO:519, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:520, SEQ ID NO:521, and SEQ ID NO:522, respectively, and conservative amino acid substitutions thereof. In some embodiments, the antibody competes for binding with, and/or binds to the same epitope on CTLA-4 as any of the aforementioned antibodies.

[001825] In some embodiments, the CTLA-4 inhibitor is a CTLA-4 biosimilar monoclonal antibody approved by drug regulatory authorities with reference to ipilimumab. In some embodiments, the biosimilar comprises an anti-CTLA-4 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is ipilimumab. In some embodiments, the one or more post-translational modifications are selected from one or more

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of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is an anti-CTLA-4 antibody authorized or submitted for authorization, wherein the anti-CTLA-4 antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is ipilimumab. The anti-CTLA-4 antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is ipilimumab. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is ipilimumab.

TABLE 24. Amino acid sequences for ipilimumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:513 ipilimumab heavy chain	1 QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYTMHWVRQA PGKGLEWVTF ISYDGNNKYY 61 ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYYCARTG WLGPFDYWGQ GTLVTVSSAS 121 TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL 181 YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKRVEPKS CDKTH
SEQ ID NO:514 ipilimumab light chain	1 EIVLTQSPGT LSLSPGERAT LSCRASQSVG SSYLAWYQQK PGQAPRLLIY GAFSRATGIP 61 DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSPWTFG QGTKVEIKRT VAAPSVFIFP 121 PSDEQLKSGT ASVVCLLNNF YPREAKVQWK VDNALQSGNS QESVTEQDSK DSTYSLSSTL 181 TLSKADYEKH KVYACEVTHQ GLSSPVTKSF NRGEC
SEQ ID NO:515 ipilimumab variable heavy chain	1 QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYTMHWVRQA PGKGLEWVTF ISYDGNNKYY 61 ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAIYYCARTG WLGPFDYWGQ GTLVTVSS
SEQ ID NO:516 ipilimumab variable light chain	1 EIVLTQSPGT LSLSPGERAT LSCRASQSVG SSYLAWYQQK PGQAPRLLIY GAFSRATGIP 61 DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSPWTFG QGTKVEIK
SEQ ID NO:517 ipilimumab heavy chain CDR1	GFTFSSYT 8
SEQ ID NO:518 ipilimumab heavy chain CDR2	TFISYDGNNK 10
SEQ ID NO:519 ipilimumab heavy chain CDR3	ARTGWLGPFD Y 11
SEQ ID NO:520 ipilimumab light chain CDR1	QSVGSSY 7
SEQ ID NO:521	CAF 3

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Identifier	Sequence (One-Letter Amino Acid Symbols)
ipilimumab light chain CDR2	
SEQ ID NO:522 ipilimumab light chain CDR3	QQYGSSPWT 9

[001826] In some embodiments, the CTLA-4 inhibitor is ipilimumab or a biosimilar thereof, and the ipilimumab is administered at a dose of about 0.5 mg/kg to about 10 mg/kg. In some embodiments, the CTLA-4 inhibitor is ipilimumab or a biosimilar thereof, and the ipilimumab is administered at a dose of about 0.5 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 3.5 mg/kg, about 4 mg/kg, about 4.5 mg/kg, about 5 mg/kg, about 5.5 mg/kg, about 6 mg/kg, about 6.5 mg/kg, about 7 mg/kg, about 7.5 mg/kg, about 8 mg/kg, about 8.5 mg/kg, about 9 mg/kg, about 9.5 mg/kg, or about 10 mg/kg. In some embodiments, the ipilimumab administration is begun 1, 2, 3, 4, or 5 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient). In some embodiments, the ipilimumab administration is begun 1, 2, or 3 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient).

[001827] In some embodiments, the CTLA-4 inhibitor is ipilimumab or a biosimilar thereof, and the ipilimumab is administered at a dose of about 200 mg to about 500 mg. In some embodiments, the CTLA-4 inhibitor is ipilimumab or a biosimilar thereof, and the ipilimumab is administered at a dose of about 200 mg, about 220 mg, about 240 mg, about 260 mg, about 280 mg, about 300 mg, about 320 mg, about 340 mg, about 360 mg, about 380 mg, about 400 mg, about 420 mg, about 440 mg, about 460 mg, about 480 mg, or about 500 mg. In some embodiments, the ipilimumab administration is begun 1, 2, 3, 4, or 5 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient). In some embodiments, the ipilimumab administration is begun 1, 2, or 3 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient).

[001828] In some embodiments, the CTLA-4 inhibitor is ipilimumab or a biosimilar thereof, and the ipilimumab is administered every 2 weeks, every 3 weeks, every 4 weeks, every 5 weeks, or every 6 weeks. In some embodiments, the ipilimumab administration is begun 1, 2, 3, 4, or 5 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the

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subject or patient). In some embodiments, the ipilimumab administration is begun 1, 2, or 3 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient).

[001829] In some embodiments, the ipilimumab is administered to treat unresectable or metastatic melanoma. In some embodiments, the ipilimumab is administered to treat Unresectable or Metastatic Melanoma at about mg/kg every 3 weeks for a maximum of 4 doses. In some embodiments, the ipilimumab administration is begun 1, 2, 3, 4, or 5 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient). In some embodiments, the ipilimumab administration is begun 1, 2, or 3 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient).

[001830] In some embodiments, the ipilimumab is administered for the adjuvant treatment of melanoma. In some embodiments, the ipilimumab is administered to for the adjuvant treatment of melanoma at about 10 mg/kg every 3 weeks for 4 doses, followed by 10 mg/kg every 12 weeks for up to 3 years. In some embodiments, the ipilimumab administration is begun 1, 2, 3, 4, or 5 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient). In some embodiments, the ipilimumab administration is begun 1, 2, or 3 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient).

[001831] In some embodiments, the ipilimumab is administered to treat advanced renal cell carcinoma. In some embodiments, the ipilimumab is administered to treat advanced renal cell carcinoma at about 1 mg/kg immediately following nivolumab 3 mg/kg on the same day, every 3 weeks for 4 doses. In some embodiments, after completing 4 doses of the combination, nivolumab can be administered as a single agent according to standard dosing regimens for advanced renal cell carcinoma and/or renal cell carcinoma. In some embodiments, the ipilimumab administration is begun 1, 2, 3, 4, or 5 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient). In some embodiments, the ipilimumab administration is begun 1, 2, or 3 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient).

[001832] In some embodiments, the ipilimumab is administered to treat microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer. In some embodiments, the ipilimumab is administered to treat microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer at about 1 mg/kg intravenously over 30 minutes immediately following nivolumab 3 mg/kg intravenously over

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30 minutes on the same day, every 3 weeks for 4 doses. In some embodiments, after completing 4 doses of the combination, administer nivolumab as a single agent as recommended according to standard dosing regimens for microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer. In some embodiments, the ipilimumab administration is begun 1, 2, 3, 4, or 5 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient). In some embodiments, the ipilimumab administration is begun 1, 2, or 3 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient).

[001833] In some embodiments, the ipilimumab is administered to treat hepatocellular carcinoma. In some embodiments, the ipilimumab is administered to treat hepatocellular carcinoma at about 3 mg/kg intravenously over 30 minutes immediately following nivolumab 1 mg/kg intravenously over 30 minutes on the same day, every 3 weeks for 4 doses. In some embodiments, after completion 4 doses of the combination, administer nivolumab as a single agent according to standard dosing regimens for hepatocellular carcinoma. In some embodiments, the ipilimumab administration is begun 1, 2, 3, 4, or 5 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient). In some embodiments, the ipilimumab administration is begun 1, 2, or 3 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient).

[001834] In some embodiments, the ipilimumab is administered to treat metastatic non-small cell lung cancer. In some embodiments, the ipilimumab is administered to treat metastatic non-small cell lung cancer at about 1 mg/kg every 6 weeks with nivolumab 3 mg/kg every 2 weeks. In some embodiments, the ipilimumab is administered to treat metastatic non-small cell lung cancer at about 1 mg/kg every 6 weeks with nivolumab 360 mg every 3 weeks and 2 cycles of platinum-doublet chemotherapy. In some embodiments, the ipilimumab administration is begun 1, 2, 3, 4, or 5 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient). In some embodiments, the ipilimumab administration is begun 1, 2, or 3 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient).

[001835] In some embodiments, the ipilimumab is administered to treat malignant pleural mesothelioma. In some embodiments, the ipilimumab is administered to treat malignant pleural mesothelioma at about 1 mg/kg every 6 weeks with nivolumab 360 mg every 3 weeks. In some embodiments, the ipilimumab administration is begun 1, 2, 3, 4, or 5 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient). In

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some embodiments, the ipilimumab administration is begun 1, 2, or 3 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient).

[001836] Tremelimumab (also known as CP-675,206) is a fully human IgG2 monoclonal antibody and has the CAS number 745013-59-6. Tremelimumab is disclosed as antibody 11.2.1 in U.S. Patent No: 6,682,736 (incorporated herein by reference). The amino acid sequences of the heavy chain and light chain of tremelimumab are set forth in SEQ IND NOs:xx and xx, respectively. Tremelimumab has been investigated in clinical trials for the treatment of various tumors, including melanoma and breast cancer; in which Tremelimumab was administered intravenously either as single dose or multiple doses every 4 or 12 weeks at the dose range of 0.01 and 15 mg/kg. In the regimens provided by the present invention, tremelimumab is administered locally, particularly intradermally or subcutaneously. The effective amount of tremelimumab administered intradermally or subcutaneously is typically in the range of 5 - 200 mg/dose per person. In some embodiments, the effective amount of tremelimumab is in the range of 10 -150 mg/dose per person per dose. In some particular embodiments, the effective amount of tremelimumab is about 10, 25, 37.5, 40, 50, 75, 100, 125, 150, 175, or 200 mg/dose per person.

[001837] In some embodiments, a CTLA-4 inhibitor comprises a heavy chain given by SEQ ID NO:523 and a light chain given by SEQ ID NO:524. In some embodiments, a CTLA-4 inhibitor comprises heavy and light chains having the sequences shown in SEQ ID NO:523 and SEQ ID NO:524, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In some embodiments, a CTLA-4 inhibitor comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:523 and SEQ ID NO:524, respectively. In some embodiments, a CTLA-4 inhibitor comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:523 and SEQ ID NO:524, respectively. In some embodiments, a CTLA-4 inhibitor comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:523 and SEQ ID NO:524, respectively. In some embodiments, a CTLA-4 inhibitor comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:523 and SEQ ID NO:524, respectively. In some embodiments, a CTLA-4 inhibitor comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:523 and SEQ ID NO:524, respectively.

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[001838] In some embodiments, the CTLA-4 inhibitor comprises the heavy and light chain CDRs or variable regions (VRs) of tremelimumab. In some embodiments, the CTLA-4 inhibitor heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:525, and the CTLA-4 inhibitor light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:526, and conservative amino acid substitutions thereof. In some embodiments, a CTLA-4 inhibitor comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:525 and SEQ ID NO:526, respectively. In some embodiments, a CTLA-4 inhibitor comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:525 and SEQ ID NO:526, respectively. In some embodiments, a CTLA-4 inhibitor comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:525and SEQ ID NO:526, respectively. In some embodiments, a CTLA-4 inhibitor comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO: 525 and SEQ ID NO:526, respectively. In some embodiments, a CTLA-4 inhibitor comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO: 525and SEQ ID NO:526, respectively.

[001839] In some embodiments, a CTLA-4 inhibitor comprises the heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:527, SEQ ID NO:528, and SEQ ID NO:529, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:530, SEQ ID NO:531, and SEQ ID NO:532, respectively, and conservative amino acid substitutions thereof. In some embodiments, the antibody competes for binding with, and/or binds to the same epitope on CTLA-4 as any of the aforementioned antibodies.

[001840] In some embodiments, the CTLA-4 inhibitor is an anti-CTLA-4 biosimilar monoclonal antibody approved by drug regulatory authorities with reference to tremelimumab. In some embodiments, the biosimilar comprises an anti-CTLA-4 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is tremelimumab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is an anti-CTLA-4 antibody authorized or submitted for authorization, wherein the

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anti-CTLA-4 antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is tremelimumab. The anti-CTLA-4 antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is tremelimumab. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is tremelimumab.

TABLE 25. Amino acid sequences for tremelimumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:523 tremelimumab heavy chain	1 QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYGMHWVRQA PGKGLEWVAV IWYDGSNKYY 61 ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDP RGATLYYYYY GMDVWGQGTT 121 VTVSSASTKG PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA 181 VLQSSGLYSL SSVVTVPSSN FGTQTYTCNV DHKPSNTKVD KTVERKCCVE CPPCPAPPVA 241 GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVQFN WYVDGVEVHN AKTKPREEQF 301 NSTFRVVSVL TVVHQDWLNG KEYKCKVSNK GLPAPIEKTI SKTKGQPREP QVYTLPPSRE 361 EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP MLDSDGSFFL YSKLTVDKSR 421 WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K
SEQ ID NO:524 tremelimumab light chain	1 DIQMTQSPSS LSASVGDRVT ITCRASQSIN SYLDWYQQKP GKAPKLLIYA ASSLQSGVPS 61 RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YYSTPFTFGP GTKVEIKRTV AAPSVFIFPP 121 SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT 181 LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
SEQ ID NO:525 tremelimumab variable heavy chain	1 GVVQPGRSLR LSCAASGFTF SSYGMHWVRQ APGKGLEWVA VIWYDGSNKY YADSVKGRFT 61 ISRDNSKNTL YLQMNSLRAE DTAVYYCARD PRGATLYYYY YGMDVWGQGT TVTVSSASTK 121 GPSVFPLAPC SRSTSESTAA LGCLVKDYFP EPVTVSWNSG ALTSGVH
SEQ ID NO:526 tremelimumab variable light chain	1 PSSLSASVGD RVTITCRASQ SINSYLDWYQ QKPGKAPKLL IYAASSLQSG VPSRFSGSGS 61 GTDFTLTISS LQPEDFATYY CQQYYSTPFT FGPGTKVEIK RTVAAPSVFI FPPSDEQLKS 121 GTASVVCLLN NFYPREAKV
SEQ ID NO:527 tremelimumab heavy chain CDR1	GFTFSSYGMH 10
SEQ ID NO:528 tremelimumab heavy chain CDR2	VIWYDGSNKY YADSV 15
SEQ ID NO:529 tremelimumab heavy chain CDR3	DPRGATLYYY YYGMDV 16
SEQ ID NO:530 tremelimumab light chain CDR1	RASQSINSYL D 11
SEQ ID NO:531 tremelimumab light chain CDR2	AASSLQS 7

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Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:532 tremelimumab light chain CDR3	QQYYSTPFT	9

[001841] In some embodiments, the CTLA-4 inhibitor is tremelimumab or a biosimilar thereof, and the tremelimumab is administered at a dose of about 0.5 mg/kg to about 10 mg/kg. In some embodiments, the CTLA-4 inhibitor is tremelimumab or a biosimilar thereof, and the tremelimumab is administered at a dose of about 0.5 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 3.5 mg/kg, about 4 mg/kg, about 4.5 mg/kg, about 5 mg/kg, about 5.5 mg/kg, about 6 mg/kg, about 6.5 mg/kg, about 7 mg/kg, about 7.5 mg/kg, about 8 mg/kg, about 8.5 mg/kg, about 9 mg/kg, about 9.5 mg/kg, or about 10 mg/kg. In some embodiments, the tremelimumab administration is begun 1, 2, 3, 4, or 5 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient). In some embodiments, the tremelimumab administration is begun 1, 2, or 3 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient).

[001842] In some embodiments, the CTLA-4 inhibitor is tremelimumab or a biosimilar thereof, and the tremelimumab is administered at a dose of about 200 mg to about 500 mg. In some embodiments, the CTLA-4 inhibitor is tremelimumab or a biosimilar thereof, and the tremelimumab is administered at a dose of about 200 mg, about 220 mg, about 240 mg, about 260 mg, about 280 mg, about 300 mg, about 320 mg, about 340 mg, about 360 mg, about 380 mg, about 400 mg, about 420 mg, about 440 mg, about 460 mg, about 480 mg, or about 500 mg. In some embodiments, the tremelimumab administration is begun 1, 2, 3, 4, or 5 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient). In some embodiments, the tremelimumab administration is begun 1, 2, or 3 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient).

[001843] In some embodiments, the CTLA-4 inhibitor is tremelimumab or a biosimilar thereof, and the tremelimumab is administered every 2 weeks, every 3 weeks, every 4 weeks, every 5 weeks, or every 6 weeks. In some embodiments, the tremelimumab administration is begun 1, 2, 3, 4, or 5 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient). In some embodiments, the tremelimumab administration is begun 1, 2, or 3 weeks pre-resection (*i.e.*, prior to obtaining the tumor sample from the subject or patient).

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[001844] In some embodiments, the CTLA-4 inhibitor is zalifrelimab from Adgenus, or biosimilars, antigen-binding fragments, conjugates, or variants thereof. Zalifrelimab is a fully human monoclonal antibody. Zalifrelimab is assigned Chemical Abstracts Service (CAS) registry number 2148321-69-9 and is also known as also known as AGEN1884. The preparation and properties of zalifrelimab are described in U.S. Patent No. 10,144,779 and US Patent Application Publication No. US2020/0024350 A1, the disclosures of which are incorporated by reference herein.

[001845] In some embodiments, a CTLA-4 inhibitor comprises a heavy chain given by SEQ ID NO:533 and a light chain given by SEQ ID NO:534. In some embodiments, a CTLA-4 inhibitor comprises heavy and light chains having the sequences shown in SEQ ID NO: 533 and SEQ ID NO: 534, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In some embodiments, a CTLA-4 inhibitor comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO: 533 and SEQ ID NO: 534, respectively. In some embodiments, a CTLA-4 inhibitor comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO: 533 and SEQ ID NO: 534, respectively. In some embodiments, a CTLA-4 inhibitor comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO: 533 and SEQ ID NO: 534, respectively. In some embodiments, a CTLA-4 inhibitor comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO: 533 and SEQ ID NO: 534, respectively. In some embodiments, a CTLA-4 inhibitor comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO: 533 and SEQ ID NO: 534, respectively.

[001846] In some embodiments, the CTLA-4 inhibitor comprises the heavy and light chain CDRs or variable regions (VRs) of zalifrelimab. In some embodiments, the CTLA-4 inhibitor heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:535, and the CTLA-4 inhibitor light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:536, and conservative amino acid substitutions thereof. In some embodiments, a CTLA-4 inhibitor comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:535 and SEQ ID NO:536, respectively. In some embodiments, a CTLA-4 inhibitor comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:535 and SEQ ID NO:536, respectively. In some embodiments, a CTLA-4 inhibitor comprises V_H and V_L regions that are each at least

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97% identical to the sequences shown in SEQ ID NO:535 and SEQ ID NO:536, respectively. In some embodiments, a CTLA-4 inhibitor comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:535 and SEQ ID NO:536, respectively. In some embodiments, a CTLA-4 inhibitor comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:535 and SEQ ID NO:536, respectively.

[001847] In some embodiments, a CTLA-4 inhibitor comprises the heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:536, SEQ ID NO:538, and SEQ ID NO:539, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:540, SEQ ID NO:541, and SEQ ID NO:542, respectively, and conservative amino acid substitutions thereof. In some embodiments, the antibody competes for binding with, and/or binds to the same epitope on CTLA-4 as any of the aforementioned antibodies.

[001848] In some embodiments, the CTLA-4 inhibitor is a CTLA-4 biosimilar monoclonal antibody approved by drug regulatory authorities with reference to zalifrelimab. In some embodiments, the biosimilar comprises an anti-CTLA-4 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is zalifrelimab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is an anti-CTLA-4 antibody authorized or submitted for authorization, wherein the anti-CTLA-4 antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is zalifrelimab. The anti-CTLA-4 antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is zalifrelimab. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or

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different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is zalifrelimab.

TABLE 26. Amino acid sequences for zalifrelimab.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:533 zalifrelimab heavy chain	1 EVQLVESGGG LVKPGGSLRL SCAASGFTFS SYSMNWVRQA PGKGLEWVSS ISSSSSYIYY 61 ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARVG LMGPFDIWGQ GTMVTVSSAS 121 TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL 181 YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPELLGGPS 241 VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST 301 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSREEMT 361 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ 421 GNVFSCSVMH EALHNHYTQK SLSLSPGK
SEQ ID NO:534 zalifrelimab light chain	1 EIVLTQSPGT LSLSPGERAT LSCRASQSVS RYLGWYQQKP GQAPRLLIYG ASTRATGIPD 61 RFSGSGSGTD FTLTITRLEP EDFAVYYCQQ YGSSPWTFGQ GTKVEIKRTV AAPSVFIFPP 121 SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT 181 LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
SEQ ID NO:535 zalifrelimab variable heavy chain	1 EVQLVESGGG LVKPGGSLRL SCAASGFTFS SYSMNWVRQA PGKGLEWVSS ISSSSSYIYY 61 ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARVG LMGPFDIWGQ GTMVTVSS
SEQ ID NO:536 zalifrelimab variable light chain	1 EIVLTQSPGT LSLSPGERAT LSCRASQSVS RYLGWYQQKP GQAPRLLIYG ASTRATGIPD 61 RFSGSGSGTD FTLTITRLEP EDFAVYYCQQ YGSSPWTFGQ GTKVEIK
SEQ ID NO:537 zalifrelimab heavy chain CDR1	GFTFSSYS 8
SEQ ID NO:538 zalifrelimab heavy chain CDR2	ISSSSSYI 8
SEQ ID NO:539 zalifrelimab heavy chain CDR3	ARVGLMGPFD I 11
SEQ ID NO:540 zalifrelimab light chain CDR1	QSVSRY 6
SEQ ID NO:541 zalifrelimab light chain CDR2	GAS 3
SEQ ID NO:542 zalifrelimab light chain CDR3	QQYGSSPWT 9

[001849] Examples of additional anti-CTLA-4 antibodies includes, but are not limited to: AGEN1181, BMS-986218, BCD-145, ONC-392, CS1002, REGN4659, and ADG116, which are known to one of ordinary skill in the art.

[001850] In some embodiments, the anti-CTLA-4 antibody is an anti-CTLA-4 antibody disclosed in any of the following patent publications (incorporated herein by reference): US2019/0048096A1; US2020/0223907; US2019/0201334; US2019/0201334; US2005/0201994; EP 1212422 B1; WO2018204760; WO2018204760; WO2001014424; WO2004035607; WO2003086459; WO2012120125; WO2000037504; WO2009100140;

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WO200609649; WO2005092380; WO2007123737; WO2006029219; WO20100979597; WO200612168; and WO1997020574. Additional CTLA-4 antibodies are described in U.S. Pat. Nos. 5,811,097, 5,855,887, 6,051,227, and 6,984,720; in PCT Publication Nos. WO 01/14424 and WO 00/37504; and in U.S. Publication Nos. 2002/0039581 and 2002/086014; and/or U.S. Patent Nos. 5,977,318, 6,682,736, 7,109,003, and 7,132,281, incorporated herein by reference). In some embodiments, the anti-CTLA-4 antibody is an, for example, those disclosed in: WO 98/42752; U.S. Pat. Nos. 6,682,736 and 6,207,156; Hurwitz et al., *Proc. Natl. Acad. Sci.* USA, 95(17): 10067-10071 (1998); Camacho et al., *J. Clin. Oncol.*, 22(145): Abstract No. 2505 (2004) (antibody CP-675206); Mokyr et al., *Cancer Res.*, 58:5301-5304 (1998) (incorporated herein by reference).

[001851] In some embodiments, the CTLA-4 inhibitor is a CTLA-4 ligand as disclosed in WO1996040915 (incorporated herein by reference).

[001852] In some embodiments, the CTLA-4 inhibitor is a nucleic acid inhibitor of CTLA-4 expression. For example, anti-CTLA-4 RNAi molecules may take the form of the molecules described by Mello and Fire in PCT Publication Nos. WO 1999/032619 and WO 2001/029058; U.S. Publication Nos. 2003/0051263, 2003/0055020, 2003/0056235, 2004/265839, 2005/0100913, 2006/0024798, 2008/0050342, 2008/0081373, 2008/0248576, and 2008/055443; and/or U.S. Pat. Nos. 6,506,559, 7,282,564, 7,538,095, and 7,560,438 (incorporated herein by reference). In some instances, the anti-CTLA-4 RNAi molecules take the form of double stranded RNAi molecules described by Tuschl in European Patent No. EP 1309726 (incorporated herein by reference). In some instances, the anti-CTLA-4 RNAi molecules take the form of double stranded RNAi molecules described by Tuschl in U.S. Pat. Nos. 7,056,704 and 7,078,196 (incorporated herein by reference). In some embodiments, the CTLA-4 inhibitor is an aptamer described in PCT Publication No. WO2004081021 (incorporated herein by reference).

[001853] In other embodiments, the anti-CTLA-4 RNAi molecules of the present invention are RNA molecules described by Crooke in U.S. Patent Nos. 5,898,031, 6,107,094, 7,432,249, and 7,432,250, and European Application No. EP 0928290 (incorporated herein by reference).

5. Lymphodepletion Preconditioning of Patients

[001854] In some embodiments, the invention includes a method of treating a cancer with a population of TILs, wherein a patient is pre-treated with non-myeloablative chemotherapy

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prior to an infusion of TILs according to the present disclosure. In some embodiments, the invention includes a population of TILs for use in the treatment of cancer in a patient which has been pre-treated with non-myeloablative chemotherapy. In some embodiments, the population of TILs is for administration by infusion. In some embodiments, the non-myeloablative chemotherapy is cyclophosphamide 60 mg/kg/d for 2 days (days 27 and 26 prior to TIL infusion) and fludarabine 25 mg/m^2/d for 5 days (days 27 to 23 prior to TIL infusion). In some embodiments, after non-myeloablative chemotherapy and TIL infusion (at day 0) according to the present disclosure, the patient receives an intravenous infusion of IL-2 (aldesleukin, commercially available as PROLEUKIN) intravenously at 720,000 IU/kg every 8 hours to physiologic tolerance. In certain embodiments, the population of TILs is for use in treating cancer in combination with IL-2, wherein the IL-2 is administered after the population of TILs.

[001855] Experimental findings indicate that lymphodepletion prior to adoptive transfer of tumor-specific T lymphocytes plays a key role in enhancing treatment efficacy by eliminating regulatory T cells and competing elements of the immune system ('cytokine sinks'). Accordingly, some embodiments of the invention utilize a lymphodepletion step (sometimes also referred to as "immunosuppressive conditioning") on the patient prior to the introduction of the TILs of the invention.

[001856] In general, lymphodepletion is achieved using administration of fludarabine or cyclophosphamide (the active form being referred to as mafosfamide) and combinations thereof. Such methods are described in Gassner, *et al., Cancer Immunol. Immunother.* **2011**, *60,* 75–85, Muranski, *et al., Nat. Clin. Pract. Oncol.,* **2006,** *3,* 668–681, Dudley, *et al., J. Clin. Oncol.* **2008**, *26,* 5233-5239, and Dudley, *et al., J. Clin. Oncol.* **2005**, *23,* 2346–2357, all of which are incorporated by reference herein in their entireties.

[001857] In some embodiments, the fludarabine is administered at a concentration of 0.5 μg/mL to 10 μg/mL fludarabine. In some embodiments, the fludarabine is administered at a concentration of 1 μg/mL fludarabine. In some embodiments, the fludarabine treatment is administered for 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days or more. In some embodiments, the fludarabine is administered at a dosage of 10 mg/kg/day, 15 mg/kg/day, 20 mg/kg/day, 25 mg/kg/day, 30 mg/kg/day, 35 mg/kg/day, 40 mg/kg/day, or 45 mg/kg/day. In some embodiments, the fludarabine treatment is administered for 2-7 days at 35 mg/kg/day. In some embodiments, the fludarabine treatment is administered for 4-5 days

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at 35 mg/kg/day. In some embodiments, the fludarabine treatment is administered for 4-5 days at 25 mg/kg/day.

[001858] In some embodiments, the mafosfamide, the active form of cyclophosphamide, is obtained at a concentration of 0.5 μg/mL to 10 μg/mL by administration of cyclophosphamide. In some embodiments, mafosfamide, the active form of cyclophosphamide, is obtained at a concentration of 1 μg/mL by administration of cyclophosphamide. In some embodiments, the cyclophosphamide treatment is administered for 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days or more. In some embodiments, the cyclophosphamide is administered at a dosage of 100 mg/m^2/day, 150 mg/m^2/day, 175 mg/m^2/day, 200 mg/m^2/day, 225 mg/m^2/day, 250 mg/m^2/day, 275 mg/m^2/day, or 300 mg/m^2/day. In some embodiments, the cyclophosphamide is administered intravenously *(i.e.*, i.v.) In some embodiments, the cyclophosphamide treatment is administered for 2-7 days at 35 mg/kg/day. In some embodiments, the cyclophosphamide treatment is administered for 4-5 days at 250 mg/m^2/day i.v. In some embodiments, the cyclophosphamide treatment is administered for 4 days at 250 mg/m^2/day i.v.

[001859] In some embodiments, lymphodepletion is performed by administering the fludarabine and the cyclophosphamide together to a patient. In some embodiments, fludarabine is administered at 25 mg/m^2/day i.v. and cyclophosphamide is administered at 250 mg/m^2/day i.v. over 4 days.

[001860] In some embodiments, the lymphodepletion is performed by 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.

[001861] In some embodiments, the lymphodepletion is performed by administration of cyclophosphamide at a dose of 60 mg/m^2/day for two days and administration of fludarabine at a dose of 25 mg/m^2/day for five days, wherein cyclophosphamide and fludarabine are both administered on the first two days, and wherein the lymphodepletion is performed in five days in total.

[001862] In some embodiments, the lymphodepletion is performed by administration of cyclophosphamide at a dose of about 50 mg/m^2/day for two days and administration of fludarabine at a dose of about 25 mg/m^2/day for five days, wherein cyclophosphamide and fludarabine are both administered on the first two days, and wherein the lymphodepletion is performed in five days in total.

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[001863] In some embodiments, the lymphodepletion is performed by administration of cyclophosphamide at a dose of about 50 mg/m^2/day for two days and administration of fludarabine at a dose of about 20 mg/m^2/day for five days, wherein cyclophosphamide and fludarabine are both administered on the first two days, and wherein the lymphodepletion is performed in five days in total.

[001864] In some embodiments, the lymphodepletion is performed by administration of cyclophosphamide at a dose of about 40 mg/m^2/day for two days and administration of fludarabine at a dose of about 20 mg/m^2/day for five days, wherein cyclophosphamide and fludarabine are both administered on the first two days, and wherein the lymphodepletion is performed in five days in total.

[001865] In some embodiments, the lymphodepletion is performed by administration of cyclophosphamide at a dose of about 40 mg/m^2/day for two days and administration of fludarabine at a dose of about 15 mg/m^2/day for five days, wherein cyclophosphamide and fludarabine are both administered on the first two days, and wherein the lymphodepletion is performed in five days in total.

[001866] In some embodiments, the lymphodepletion is performed by administration of cyclophosphamide at a dose of 60 mg/m^2/day and fludarabine at a dose of 25 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for three days.

[001867] In some embodiments, the cyclophosphamide is administered with mesna. In some embodiments, mesna is administered at 15 mg/kg. In some embodiments where mesna is infused, and if infused continuously, mesna can be infused over approximately 2 hours with cyclophosphamide (on Days -5 and/or -4), then at a rate of 3 mg/kg/hour for the remaining 22 hours over the 24 hours starting concomitantly with each cyclophosphamide dose.

[001868] In some embodiments, the lymphodepletion comprises the step of treating the patient with an IL-2 regimen starting on the day after administration of the third population of TILs to the patient.

[001869] In some embodiments, the lymphodepletion comprises the step of treating the patient with an IL-2 regimen starting on the same day as administration of the third population of TILs to the patient.

[001870] In some embodiments, the lymphodeplete comprises 5 days of preconditioning treatment. In some embodiments, the days are indicated as days -5 through -1, or Day 0

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through Day 4. In some embodiments, the regimen comprises cyclophosphamide on days -5 and -4 (*i.e.*, days 0 and 1). In some embodiments, the regimen comprises intravenous cyclophosphamide on days -5 and -4 (*i.e.*, days 0 and 1). In some embodiments, the regimen comprises 60 mg/kg intravenous cyclophosphamide on days -5 and -4 (*i.e.*, days 0 and 1). In some embodiments, the cyclophosphamide is administered with mesna. In some embodiments, the regimen further comprises fludarabine. In some embodiments, the regimen further comprises intravenous fludarabine. In some embodiments, the regimen further comprises 25 mg/m^2 intravenous fludarabine. In some embodiments, the regimen further comprises 25 mg/m^2 intravenous fludarabine on days -5 and -1 (*i.e.*, days 0 through 4). In some embodiments, the regimen further comprises 25 mg/m^2 intravenous fludarabine on days -5 and -1 (*i.e.*, days 0 through 4).

[001871] In some embodiments, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day and fludarabine at a dose of 25 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for five days.

[001872] In some embodiments, 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.

[001873] In some embodiments, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day and fludarabine at a dose of 25 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for three days.

[001874] In some embodiments, 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 three days.

[001875] In some embodiments, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day and fludarabine at a dose of 25 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for one day.

[001876] In some embodiments, the non-myeloablative lymphodepletion regimen is administered according to Table 27.

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TABLE 27. Exemplary lymphodepletion and treatment regimen.

Day	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Cyclophosphamide 60 mg/kg	X	X								
Mesna (as needed)	X	X								
Fludarabine 25 mg/m^2/day	X	X	X	X	X					
TIL infusion						X				

[001877] In some embodiments, the non-myeloablative lymphodepletion regimen is administered according to Table 28.

TABLE 28. Exemplary lymphodepletion and treatment regimen.

Day	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Cyclophosphamide 60 mg/kg	X	X							
Mesna (as needed)	X	X							
Fludarabine 25 mg/m^2/day	X	X	X	X					
TIL infusion					X				

[001878] In some embodiments, the non-myeloablative lymphodepletion regimen is administered according to Table 29.

TABLE 29. Exemplary lymphodepletion and treatment regimen.

Day	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Cyclophosphamide 60 mg/kg	X	X						
Mesna (as needed)	X	X						
Fludarabine 25 mg/m^2/day	X	X	X					
TIL infusion				X				

[001879] In some embodiments, the non-myeloablative lymphodepletion regimen is administered according to Table 30.

TABLE 30. Exemplary lymphodepletion and treatment regimen.

Day	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Cyclophosphamide 60 mg/kg	X	X								
Mesna (as needed)	X	X								
Fludarabine 25 mg/m^2/day			X	X	X					
TIL infusion						X				

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[001880] In some embodiments, the non-myeloablative lymphodepletion regimen is administered according to Table 31.

TABLE 31. Exemplary lymphodepletion and treatment regimen.

Day	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Cyclophosphamide 300 mg/kg	X	X								
Mesna (as needed)	X	X								
Fludarabine 30 mg/m^2/day	X	X	X	X	X					
TIL infusion						X				

[001881] In some embodiments, the non-myeloablative lymphodepletion regimen is administered according to Table 32.

TABLE 32. Exemplary lymphodepletion and treatment regimen.

Day	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Cyclophosphamide 300 mg/kg	X	X							
Mesna (as needed)	X	X							
Fludarabine 30 mg/m^2/day	X	X	X	X					
TIL infusion					X				

[001882] In some embodiments, the non-myeloablative lymphodepletion regimen is administered according to Table 33.

TABLE 33. Exemplary lymphodepletion and treatment regimen.

Day	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Cyclophosphamide 300 mg/kg	X	X						
Mesna (as needed)	X	X						
Fludarabine 30 mg/m^2/day	X	X	X					
TIL infusion				X				

[001883] In some embodiments, the non-myeloablative lymphodepletion regimen is administered according to Table 34.

TABLE 34. Exemplary lymphodepletion and treatment regimen.

Day	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Cyclophosphamide 300 mg/kg	X	X								
Mesna (as needed)	X	X								
Fludarabine 30 mg/m^2/day			X	X	X					

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Day	-5	-4	-3	-2	-1	0	1	2	3	4
TIL infusion						X				

6. IL-2 Regimens

[001884] In some embodiments, the IL-2 regimen comprises a high-dose IL-2 regimen, wherein the high-dose IL-2 regimen comprises aldesleukin, or a biosimilar or variant thereof, administered intravenously starting on the day after administering a therapeutically effective portion of the therapeutic population of TILs, wherein the aldesleukin or a biosimilar or variant thereof is administered at a dose of 0.037 mg/kg or 0.044 mg/kg IU/kg (patient body mass) using 15-minute bolus intravenous infusions every eight hours until tolerance, for a maximum of 14 doses. Following 9 days of rest, this schedule may be repeated for another 14 doses, for a maximum of 28 doses in total. In some embodiments, IL-2 is administered in 1, 2, 3, 4, 5, or 6 doses.

[001885] In some embodiemtns, IL-2 is administered at a maximum dosage of up to 6 doses. In some embodiments, the high-dose IL-2 regimen is adapted for pediatric use. In some embodiments, a dose of 600,000 international units (IU)/kg of aldesleukin every 8–12 hours for up to a maximum of 6 doses is used. In some embodiments, a dose of 500,000 international units (IU)/kg of aldesleukin every 8–12 hours for up to a maximum of 6 doses is used. In some embodiments, a dose of 400,000 international units (IU)/kg of aldesleukin every 8–12 hours for up to a maximum of 6 doses is used. In some embodiments, a dose of 500,000 international units (IU)/kg of aldesleukin every 8–12 hours for up to a maximum of 6 doses is used. In some embodiments, a dose of 300,000 international units (IU)/kg of aldesleukin every 8–12 hours for up to a maximum of 6 doses is used. In some embodiments, a dose of 200,000 international units (IU)/kg of aldesleukin every 8–12 hours for up to a maximum of 6 doses is used. In some embodiments, a dose of 100,000 international units (IU)/kg of aldesleukin every 8–12 hours for up to a maximum of 6 doses is used.

[001886] In some embodiments, the IL-2 regimen comprises a decrescendo IL-2 regimen. Decrescendo IL-2 regimens have been described in O'Day, *et al.*, *J. Clin. Oncol.* **1999,** *17,* 2752-61 and Eton, *et al.*, *Cancer* **2000,** *88,* 1703-9, the disclosures of which are incorporated herein by reference. In some embodiments, a decrescendo IL-2 regimen comprises 18×10^6 IU/m^2 administered intravenously over 6 hours, followed by 18×10^6 IU/m^2 administered intravenously over 12 hours, followed by 18×10^6 IU/m^2 administered intravenously over 24

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hrs, followed by 4.5×10^6 IU/m^2 administered intravenously over 72 hours. This treatment cycle may be repeated every 28 days for a maximum of four cycles. In some embodiments, a decrescendo IL-2 regimen comprises 18,000,000 IU/m^2 on day 1, 9,000,000 IU/m^2 on day 2, and 4,500,000 IU/m^2 on days 3 and 4.

[001887] In an embodiment, the IL-2 regimen comprises a low-dose IL-2 regimen. Any low-dose IL-2 regimen known in the art may be used, including the low-dose IL-2 regimens described in Dominguez-Villar and Hafler, *Nat. Immunology* **2000,** *19,* 665-673; Hartemann, *et al.*, *Lancet Diabetes Endocrinol.* **2013**, *1*, 295-305; and Rosenzwaig, *et al.*, *Ann. Rheum. Dis.* **2019,** *78,* 209–217, the disclosures of which are incorporated herein by reference. In an embodiment, a low-dose IL-2 regimen comprises 18×10^6 IU per m^2 of aldesleukin, or a biosimilar or variant thereof, per 24 hours, administered as a continuous infusion for 5 days, followed by 2-6 days without IL-2 therapy, optionally followed by an additional 5 days of intravenous aldesleukin or a biosimilar or variant thereof, as a continuous infusion of 18 x 10^6 IU per m^2 per 24 hours, optionally followed by 3 weeks without IL-2 therapy, after which additional cycles may be administered.

[001888] In some embodiments, IL-2 is administered at a maximum dosage of up to 6 doses. In some embodiments, the high-dose IL-2 regimen is adapted for pediatric use. In some embodiments, a dose of 600,000 international units (IU)/kg of aldesleukin every 8–12 hours for up to a maximum of 6 doses is used. In some embodiments, a dose of 500,000 international units (IU)/kg of aldesleukin every 8–12 hours for up to a maximum of 6 doses is used. In some embodiments, a dose of 400,000 international units (IU)/kg of aldesleukin every 8–12 hours for up to a maximum of 6 doses is used. In some embodiments, a dose of 500,000 international units (IU)/kg of aldesleukin every 8–12 hours for up to a maximum of 6 doses is used. In some embodiments, a dose of 300,000 international units (IU)/kg of aldesleukin every 8–12 hours for up to a maximum of 6 doses is used. In some embodiments, a dose of 200,000 international units (IU)/kg of aldesleukin every 8–12 hours for up to a maximum of 6 doses is used. In some embodiments, a dose of 100,000 international units (IU)/kg of aldesleukin every 8–12 hours for up to a maximum of 6 doses is used.

[001889] In some embodiments, the IL-2 regimen comprises administration of pegylated IL-2 every 1, 2, 4, 6, 7, 14 or 21 days at a dose of 0.10 mg/day to 50 mg/day. In some embodiments, the IL-2 regimen comprises administration of bempegaldesleukin, or a fragment, variant, or biosimilar thereof, every 1, 2, 4, 6, 7, 14 or 21 days at a dose of 0.10 mg/day to 50 mg/day.

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[001890] In some embodiments, the IL-2 regimen comprises administration of THOR-707, or a fragment, variant, or biosimilar thereof, every 1, 2, 4, 6, 7, 14 or 21 days at a dose of 0.10 mg/day to 50 mg/day.

[001891] In some embodiments, the IL-2 regimen comprises administration of nemvaleukin alfa, or a fragment, variant, or biosimilar thereof, every 1, 2, 4, 6, 7, 14 or 21 days at a dose of 0.10 mg/day to 50 mg/day.

[001892] In some embodiments, the IL-2 regimen comprises administration of an IL-2 fragment engrafted onto an antibody backbone. In some embodiments, the IL-2 regimen comprises administration of an antibody-cytokine engrafted protein that binds the IL-2 low affinity receptor. In some embodiments, the antibody cytokine engrafted protein comprises a heavy chain variable region (V_H), comprising complementarity determining regions HCDR1, HCDR2, HCDR3; a light chain variable region (V_L), comprising LCDR1, LCDR2, LCDR3; and an IL-2 molecule or a fragment thereof engrafted into a CDR of the V_H or the V_L, wherein the antibody cytokine engrafted protein preferentially expands T effector cells over regulatory T cells. In some embodiments, the antibody cytokine engrafted protein comprises a heavy chain variable region (V_H), comprising complementarity determining regions HCDR1, HCDR2, HCDR3; a light chain variable region (V_L), comprising LCDR1, LCDR2, LCDR3; and an IL-2 molecule or a fragment thereof engrafted into a CDR of the V_H or the V_L, wherein the IL-2 molecule is a mutein, and wherein the antibody cytokine engrafted protein preferentially expands T effector cells over regulatory T cells. In some embodiments, the IL-2 regimen comprises administration of an antibody comprising a heavy chain selected from the group consisting of SEQ ID NO:559 and SEQ ID NO:568 and a light chain selected from the group consisting of SEQ ID NO:567 and SEQ ID NO:569, or a fragment, variant, or biosimilar thereof, every 1, 2, 4, 6, 7, 14 or 21 days at a dose of 0.10 mg/day to 50 mg/day.

[001893] In some embodiments, the antibody cytokine engrafted protein described herein has a longer serum half-life that a wild-type IL-2 molecule such as, but not limited to, aldesleukin (Proleukin®) or a comparable molecule.

[001894] In some embodiments, the TIL infusion used with the foregoing embodiments of myeloablative lymphodepletion regimens may be any TIL composition described herein and may also include infusions of MILs and PBLs in place of the TIL infusion, as well as the addition of IL-2 regimens and administration of co-therapies (such as PD-1 and/or PD-L1 inhibitors and/or CTLA-4 inhibitors) as described herein.

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7. Additional Methods of Treatment

[001895] In other embodiments, the invention provides a method for treating a subject with cancer comprising administering to the subject a therapeutically effective dosage of the therapeutic TIL population described in any one of the preceding paragraphs as applicable above.

[001896] In other embodiments, the invention provides a method for treating a subject with cancer comprising administering to the subject a therapeutically effective dosage of the TIL composition described in any of the preceding paragraph as applicable above.

[001897] In other embodiments, the invention provides the method for treating a subject with cancer described in any of the preceding paragraphs as applicable above modified such that prior to administering the therapeutically effective dosage of the therapeutic TIL population described in any of the preceding paragraphs as applicable above or the therapeutically effective dosage of the TIL composition described in any of the preceding paragraphs as applicable above, a non-myeloablative lymphodepletion regimen has been administered to the subject.

[001898] In other embodiments, the invention provides the method for treating a subject with cancer described in any of the preceding paragraph as applicable above modified such that 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.

[001899] In other embodiments, the invention provides the method for treating a subject with cancer described in any of the preceding paragraphs as applicable above modified to further comprise the step of treating the subject with a high-dose IL-2 regimen starting on the day after administration of the TIL cells to the subject.

[001900] In other embodiments, the invention provides the method for treating a subject with cancer described in any of the preceding paragraphs as applicable above modified such that 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.

[001901] In other embodiments, the invention provides the method for treating a subject with cancer described in any of the preceding paragraphs as applicable above modified such that the cancer is a solid tumor.

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[001902] In other embodiments, the invention provides the method for treating a subject with cancer described in any of the preceding paragraphs as applicable above modified such that the cancer is melanoma, ovarian cancer, endometrial cancer, thyroid cancer, colorectal 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, or renal cell carcinoma.

[001903] In other embodiments, the invention provides the method for treating a subject with cancer described in any of the preceding paragraphs as applicable above modified such that the cancer is melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

[001904] In other embodiments, the invention provides the method for treating a subject with cancer described in any of the preceding paragraphs as applicable above modified such that the cancer is melanoma. In other embodiments, the invention provides the method for treating a subject with cancer described in any of the preceding paragraphs as applicable above modified such that the cancer is refractory or unresecteable melanoma.

[001905] In other embodiments, the invention provides the method for treating a subject with cancer described in any of the preceding paragraphs as applicable above modified such that the cancer is HNSCC.

[001906] In other embodiments, the invention provides the method for treating a subject with cancer described in any of the preceding paragraphs as applicable above modified such that the cancer is a cervical cancer.

[001907] In other embodiments, the invention provides the method for treating a subject with cancer described in any of the preceding paragraphs as applicable above modified such that the cancer is NSCLC.

[001908] In other embodiments, the invention provides the method for treating a subject with cancer described in any of the preceding paragraphs as applicable above modified such that the cancer is glioblastoma (including GBM).

[001909] In other embodiments, the invention provides the method for treating a subject with cancer described in any of the preceding paragraphs as applicable above modified such that the cancer is gastrointestinal cancer.

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[001910] In other embodiments, the invention provides the method for treating a subject with cancer described in any of the preceding paragraphs as applicable above modified such that the cancer is a hypermutated cancer.

[001911] In other embodiments, the invention provides the method for treating a subject with cancer described in any of the preceding paragraphs as applicable above modified such that the cancer is a pediatric hypermutated cancer.

[001912] In other embodiments, the invention provides the therapeutic TIL population described in any one of the preceding paragraphs as applicable above for use in a method for treating a subject with cancer comprising administering to the subject a therapeutically effective dosage of the therapeutic TIL population.

[001913] In other embodiments, the invention provides the TIL composition described in any of the preceding paragraphs as applicable above for use in a method for treating a subject with cancer comprising administering to the subject a therapeutically effective dosage of the TIL composition.

[001914] In other embodiments, the invention provides the therapeutic TIL population described in any of the preceding paragraphs as applicable above or the TIL composition described in any of the preceding paragraphs as applicable above modified such that prior to administering to the subject the therapeutically effective dosage of the therapeutic TIL population described in any of the preceding paragraphs as applicable above or the TIL composition described in any of the preceding paragraphs as applicable above, a non-myeloablative lymphodepletion regimen has been administered to the subject.

[001915] In other embodiments, the invention provides the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that 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.

[001916] In other embodiments, the invention provides the therapeutic TIL population described in any of the preceding paragraphs as applicable above or the TIL composition described in any of the preceding paragraphs as applicable above modified to further comprise the step of treating patient with a high-dose IL-2 regimen starting on the day after administration of the TIL cells to the patient.

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[001917] In other embodiments, the invention provides the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that 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.

[001918] In other embodiments, the invention provides the therapeutic TIL population described in any of the preceding paragraphs as applicable above or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is a solid tumor.

[001919] In other embodiments, the invention provides the therapeutic TIL population described in any of the preceding paragraphs as applicable above or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is melanoma, ovarian cancer, endometrial cancer, thyroid cancer, colorectal cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, triple negative 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, or renal cell carcinoma.

[001920] In other embodiments, the invention provides the therapeutic TIL population described in any of the preceding paragraphs as applicable above or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

[001921] In other embodiments, the invention provides the therapeutic TIL population described in any of the preceding paragraphs as applicable above or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is melanoma.

[001922] In other embodiments, the invention provides the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is HNSCC.

[001923] In other embodiments, the invention provides the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is a cervical cancer.

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[001924] In other embodiments, the invention provides the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is NSCLC.

[001925] In other embodiments, the invention provides the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is glioblastoma (including GBM).

[001926] In other embodiments, the invention provides the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is gastrointestinal cancer.

[001927] In other embodiments, the invention provides the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is a hypermutated cancer.

[001928] In other embodiments, the invention provides the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is a pediatric hypermutated cancer.

[001929] In other embodiments, the invention provides the use of the therapeutic TIL population described in any one of the preceding paragraphs as applicable above in a method of treating cancer in a subject comprising administering to the subject a therapeutically effective dosage of the therapeutic TIL population.

[001930] In other embodiments, the invention provides the use of the TIL composition described in any of the preceding paragraphs as applicable above in a method of treating cancer in a subject comprising administering to the subject a therapeutically effective dosage of the TIL composition.

[001931] In other embodiments, the invention provides the use of the therapeutic TIL population described in any of the preceding paragraphs as applicable above or the TIL composition described in any of the preceding paragraphs as applicable above in a method of treating cancer in a subject comprising administering to the subject a non-myeloablative lymphodepletion regimen and then administering to the subject a therapeutically effective dosage of the therapeutic TIL population described in any of the preceding paragraphs as applicable above or a therapeutically effective dosage of the TIL composition described in any of the preceding paragraphs as applicable above.

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[001932] In other embodiments, the invention provides the use of the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that prior to administering to the subject the therapeutically effective dosage of the therapeutic TIL population or the therapeutically effective dosage of the TIL composition, a non-myeloablative lymphodepletion regimen has been administered to the subject.

[001933] In other embodiments, the invention provides the use of the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that 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.

[001934] In other embodiments, the invention provides the use of the therapeutic TIL population described in any of the preceding paragraphs as applicable above or the use of the TIL composition described in any of the preceding paragraphs as applicable above modified to further comprise the step of treating patient with a high-dose IL-2 regimen starting on the day after administration of the TIL cells to the patient.

[001935] In other embodiments, the invention provides the use of the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that 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.

[001936] In other embodiments, the invention provides the use of the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is a solid tumor.

[001937] In other embodiments, the invention provides the use of the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is melanoma, ovarian cancer, endometrial cancer, thyroid cancer, colorectal cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, triple negative 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, or renal cell carcinoma.

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[001938] In other embodiments, the invention provides the use of the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

[001939] In other embodiments, the invention provides the use of the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is melanoma.

[001940] In other embodiments, the invention provides the use of the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is HNSCC.

[001941] In other embodiments, the invention provides the use of the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is a cervical cancer.

[001942] In other embodiments, the invention provides the use of the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is NSCLC.

[001943] In other embodiments, the invention provides the use of the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is glioblastoma (including GBM).

[001944] In other embodiments, the invention provides the use of the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is gastrointestinal cancer.

[001945] In other embodiments, the invention provides the use of the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is a hypermutated cancer.

[001946] In other embodiments, the invention provides the use of the therapeutic TIL population or the TIL composition described in any of the preceding paragraphs as applicable above modified such that the cancer is a pediatric hypermutated cancer.

[001947] A method of treating unresectable and/or double-refractory melanoma, including metastatic melanoma, in a patient/subject in need thereof, the method comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor resected from a

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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 either antigen presenting cells (APCs) and/or culture supernatant from a first culture of APCs comprising OKT-3 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 or 1 to 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 either antigen presenting cells (APCs) and/or culture supernatant from a second culture of APCs comprising OKT-3, 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 patient/subject.

[001948] A method of treating unresectable and/or double-refractory melanoma, including metastatic melanoma, in a patient/subject in need thereof, the method comprising:

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

obtaining a first population of TILs from a tumor resected from a patient by processing a tumor sample obtained from the patient into a tumor digest;

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

(c) performing a first expansion or priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of

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TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about to about 1 to 5 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 4 to 8 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

(f) harvesting the second plurality of subpopulations of TILs obtained from step (e), wherein the transition from step (e) to step (f) occurs without opening the system when optionally performed in a closed system;

(g) transferring the harvested subpopulations of TILs from step (g) to one or more infusion bags, wherein the transition from step (f) to (g) occurs without opening the system when optionally performed in a closed system; and

(h) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (g) to the patient/subject.

[001949] A method of treating unresectable and/or double-refractory melanoma, including metastatic melanoma, in a patient/subject in need thereof, the method comprising:

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

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fragments or obtaining a first population of TILs from a tumor resected from a patient by processing a tumor sample obtained from the patient into a tumor digest;

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

(c) performing a first expansion or priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the first expansion or priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

(f) harvesting the second plurality of subpopulations of TILs obtained from step (f), wherein the transition from step (e) to step (f) occurs without opening the system when optionally performed in a closed system;

(g) transferring the harvested subpopulations of TILs from step (f) to one or more infusion bags, wherein the transition from step (f) to (g) occurs without opening

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the system when optionally performed in a closed system; and

(h) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (g) to the patient/subject.

[001950] A method of treating unresectable and/or double-refractory melanoma, including metastatic melanoma, in a patient/subject in need thereof, the method comprising:

(a) performing a first expansion or priming first expansion by (i) thawing a cryopreserved tumor digest comprising a first population of TILs from a tumor that was resected from a subject, digested after the resection, and cryopreserved after the digestion, and (ii) culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the first expansion or priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) harvesting the second plurality of subpopulations of TILs obtained from step (c), wherein the transition from step (d) to step (e) occurs without opening the system

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when optionally performed in a closed system;

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system;

(f) administering a therapeutically effective dosage of the harvested population of TILs from the infusion bag in step (e) to the subject.

[001951] In some embodiments, step (a)(i) comprises thawing a cryopreserved tumor comprising a first population of TILs from a tumor that was resected from a subject and cryopreserved after the resection to produce a thawed tumor, and fragmenting the thawed tumor into multiple tumor fragments, and wherein step (a)(ii) comprises culturing the multiple tumor fragments comprising the first population of TILs.

[001952] A method of treating unresectable and/or double-refractory melanoma, including metastatic melanoma, in a patient/subject in need thereof, the method comprising:

(a) performing a first expansion or priming first expansion by culturing a tumor sample comprising a first population of TILs from a tumor resected from a patient in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about to about 1 to 5 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is

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performed for about 4 to 8 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) harvesting the second plurality of subpopulations of TILs obtained from step (c), wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system; and

(f) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (e) to the subject.The method of claim 376-379, wherein before culturing in step (a) the tumor sample is fragmenting into multiple tumor fragments comprising the first population of TILs.

[001953] A method of treating unresectable and/or double-refractory melanoma, including metastatic melanoma, in a patient/subject in need thereof, the method comprising:

(a) performing a first expansion or priming first expansion by culturing a tumor sample comprising a first population of TILs from a tumor resected from a patient in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first

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plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) harvesting the second plurality of subpopulations of TILs obtained from step (f), wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system; and

(f) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (e) to the patient/subject.

[001954] In some embodiments, the patient/subject has been previously treated with a PD-1 inhibitor or a biosimilar thereof. In some embodiments, the PD-1 inhibitor is selected from the group consisting of nivolumab, pembrolizumab, and biosimilars thereof.

[001955] In some embodiments, the patient/subject has been previously treated with a PD-L1 inhibitor or a biosimilar thereof. In some embodiments, the PD-L1 inhibitor is selected from the group consisting of avelumab, atezolizumab, durvalumab, and biosimilars thereof.

[001956] In some embodiments, the PD-1 inhibitor or a biosimilar thereof was co-administered with a CTLA-4 inihbitor or biosimilar thereof. In some embodiments, the PD-L1 inhibitor or a biosimilar thereof was co-administered with a CTLA-4 inihbitor or biosimilar thereof.

[001957] In some embodiments, the patient has been previously treated with one additional prior line of systemic therapy. In some embodiments, the one additional prior line of systemic therapy is a BRAF inhibitor or a pharmaceutically-acceptable salt thereof. In some embodiments, the BRAF inhibitor is selected from the group consisting of vemurafenib, dabrafenib, and pharmaceutically-acceptable salts thereof. In some embodiments, the one additional prior line of systemic therapy is a MEK inhibitor or a pharmaceutically-acceptable

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salt or solvate thereof. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, and pharmaceutically-acceptable salts or solvates thereof. In some embodiments, the one additional prior line of systemic therapy is a combination of a BRAF inhibitor or a pharmaceutically-acceptable salt thereof and a MEK inhibitor or a pharmaceutically-acceptable salt or solvate thereof. In some embodiments, the BRAF inhibitor is selected from the group consisting of vemurafenib, dabrafenib, and pharmaceutically-acceptable salts thereof, and the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, and pharmaceutically-acceptable salts or solvates thereof. In some embodiments, the one additional prior line of systemic therapy is a CTLA-4 inhibitor or a biosimilar thereof. In some embodiments, the CTLA-4 inhibitor is selected from the group consisting of ipilumumab, tremelimumab, and biosimilars thereof. In some embodiments, the one additional prior line of systemic therapy is chemotherapeutic regimen. In some embodiments, the chemotherapeutic regimen comprises dacarbazine or temozolimide.

[001958] In some embodiments, the IL-2 is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL in the cell culture medium in the first expansion or priming first expansion step.

[001959] In some embodiments, the second expansion step (d), the IL-2 is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL and the OKT-3 antibody is present at an initial concentration of about 30 ng/mL.

[001960] In some embodiments, the cell culture medium in the first expansion or priming first expansion step further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

[001961] In some embodiments, the cell culture medium in the rapid second expansion or third expansion further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

[001962] In some embodiments, the methods further comprise the step of treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the TILs to the patient. In some embodiments, 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.

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[001963] In some embodiments, the methods further comprise the step of treating the patient with an IL-2 regimen starting on the day after administration of the TILs to the patient. In some embodiments, the IL-2 regimen is a high-dose IL-2 regimen comprising 600,000 or 720,000 IU/kg of aldesleukin, or a biosimilar or variant thereof, administered as a 15-minute bolus intravenous infusion every eight hours until tolerance.

[001964] In some embodiments, the therapeutically effective population of TILs comprises from about 2.3×10^{10} to about 13.7×10^{10} TILs.

[001965] Adoptive Cell Transfer: Adoptive cell transfer (ACT) is an effective form of immunotherapy and involves the transfer of immune cells with antitumor activity into cancer patients. ACT is a treatment approach that involves the identification, *in vitro*, of lymphocytes with antitumor activity, the in vitro expansion of these cells to large numbers and their infusion into the cancer-bearing host. Lymphocytes used for adoptive transfer can be derived from the stroma of resected tumors (tumor infiltrating lymphocytes or TILs). TILs for ACT can be prepared as described herein. In some embodiments, the TILs are prepared, for example, according to a method as described in Figure 1 (in particular, *e.g.*, Figure 1B and/or Figure 1C and/or Figure 1E and/or Figure 1F and /or Figure 1G). They can also be derived or from blood if they are genetically engineered to express antitumor T-cell receptors (TCRs) or chimeric antigen receptors (CARs), enriched with mixed lymphocyte tumor cell cultures (MLTCs), or cloned using autologous antigen presenting cells and tumor derived peptides. ACT in which the lymphocytes originate from the cancer-bearing host to be infused is termed autologous ACT. U.S. Publication No. 2011/0052530 relates to a method for performing adoptive cell therapy to promote cancer regression, primarily for treatment of patients suffering from metastatic melanoma, which is incorporated by reference in its entirety for these methods. In some embodiments, TILs can be administered as described herein. In some embodiments, TILs can be administered in a single dose. Such administration may be by injection, *e.g.*, intravenous injection. In some embodiments, TILs and/or cytotoxic lymphocytes may be administered in multiple doses. Dosing may be once, twice, three times, four times, five times, six times, or more than six times per year. Dosing may be once a month, once every two weeks, once a week, or once every other day. Administration of TILs and/or cytotoxic lymphocytes may continue as long as necessary.

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EXAMPLES

[001966] The embodiments encompassed herein are now described with reference to the following examples. These examples are provided for the purpose of illustration only and the disclosure encompassed herein should in no way be construed as being limited to these examples, but rather should be construed to encompass any and all variations which become evident as a result of the teachings provided herein.

EXAMPLE 1: PREPARATION OF MEDIA FOR PRE-REP AND REP PROCESSES

[001967] This Example describes the procedure for the preparation of tissue culture media for use in protocols involving the culture of tumor infiltrating lymphocytes (TIL) derived from various tumor types including, but not limited to, metastatic melanoma, head and neck squamous cell carcinoma (HNSCC), ovarian carcinoma, triple-negative breast carcinoma, and lung adenocarcinoma. This media can be used for preparation of any of the TILs described in the present application and Examples.

Preparation of CM1

[001968] Removed the following reagents from cold storage and warmed them in a 37°C water bath: (RPMI1640, Human AB serum, 200mM L-glutamine). Prepared CM1 medium according to Table 35 below by adding each of the ingredients into the top section of a 0.2µm filter unit appropriate to the volume to be filtered. Stored at 4°C.

TABLE 35: Preparation of CM1

Ingredient	Final concentration	Final Volume 500 ml	Final Volume IL
RPMI1640	NA	450 ml	900 ml
Human AB serum, heat-inactivated 10%	50 ml	100 ml	
200mM L-glutamine	2 mM	5 ml	10 ml
55mM BME	55 µM	0.5 ml	1 ml
50mg/ml gentamicin sulfate	50 µg/ml	0.5 ml	1 ml

[001969] On the day of use, prewarmed required amount of CM1 in 37°C water bath and add 6000 IU/ml IL-2.

[001970] Additional supplementation - as needed according to Table 36.

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TABLE 36: Additional supplementation of CM1, as needed.

Supplement	Stock concentration	Dilution	Final concentration
GlutaMAX™	200mM	1:100	2mM
Penicillin/streptomycin	10,000 U/ml penicillin 10,000µg/ml streptomycin	1:100	100 U/ml penicillin 100 µg/ml streptomycin
Amphotericin B	250µg/ml	1:100	2.5µg/ml

Preparation of CM2

[001971] Removed prepared CM1 from refrigerator or prepare fresh CM1 as per Table 35 above. Removed AIM-V® from refrigerator and prepared the amount of CM2 needed by mixing prepared CM1 with an equal volume of AIM-V® in a sterile media bottle. Added 3000 IU/ml IL-2 to CM2 medium on the day of usage. Made sufficient amount of CM2 with 3000 IU/ml IL-2 on the day of usage. Labeled the CM2 media bottle with its name, the initials of the preparer, the date it was filtered/prepared, the two-week expiration date and stored at 4°C until needed for tissue culture.

Preparation of CM3

[001972] Prepared CM3 on the day it was required for use. CM3 was the same as AIM-V® medium, supplemented with 3000 IU/ml IL-2 on the day of use. Prepared an amount of CM3 sufficient to experimental needs by adding IL-2 stock solution directly to the bottle or bag of AIM-V. Mixed well by gentle shaking. Labeled bottle with "3000 IU/ml IL-2" immediately after adding to the AIM-V. If there was excess CM3, stored it in bottles at 4°C labeled with the media name, the initials of the preparer, the date the media was prepared, and its expiration date (7 days after preparation). Discarded media supplemented with IL-2 after 7 days storage at 4°C.

Preparation of CM4

[001973] CM4 was the same as CM3, with the additional supplement of 2mM GlutaMAX™ (final concentration). For every 1L of CM3, added 10ml of 200mM GlutaMAX™. Prepared an amount of CM4 sufficient to experimental needs by adding IL-2 stock solution and GlutaMAX™ stock solution directly to the bottle or bag of AIM-V. Mixed well by gentle shaking. Labeled bottle with "3000 IL/nil IL-2 and GlutaMAX" immediately after adding to the AIM-V. If there was excess CM4, stored it in bottles at 4°C

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labeled with the media name, "G1utaMAX", and its expiration date (7 days after preparation). Discarded media supplemented with IL-2 after 7-days storage at 4°C.

EXAMPLE 2: PREPARATION OF IL-2 STOCK SOLUTION (CELLGENIX)

[001974] This Example describes the process of dissolving purified, lyophilized recombinant human interleukin-2 into stock samples suitable for use in further tissue culture protocols, including all of those described in the present application and Examples, including those that involve using rhIL-2.

Procedure

[001975] Prepared 0.2% Acetic Acid solution (HAc). Transferred 29 mL sterile water to a 50 mL conical tube. Added l mL 1N acetic acid to the 50mL conical tube. Mixed well by inverting tube 2-3 times. Sterilized the HAc solution by filtration using a Steriflip filter

[001976] Prepared 1% HSA in PBS. Added 4 mL of 25% HSA stock solution to 96mL PBS in a 150 mL sterile filter unit. Filtered solution. Stored at 4°C. For each vial of rhIL-2 prepared, fill out forms.

[001977] Prepared rhIL-2 stock solution (6×10^6 IU/mL final concentration). Each lot of rhIL-2 was different and required information found in the manufacturer's Certificate of Analysis (COA), such as: 1) Mass of rhIL-2 per vial (mg), 2) Specific activity of rhIL-2 (IU/mg) and 3) Recommended 0.2% HAc reconstitution volume (mL).

[001978] Calculated the volume of 1% HSA required for rhIL-2 lot by using the equation below:

$$\left(\frac{Vial\ Mass\ (mg)\ x\ Biological\ Activity\ (\frac{IU}{mg})}{6x10^6\ \frac{IU}{mL}}\right) - HAc\ vol\ (mL) = 1\%\ HSA\ vol\ (mL)$$

[001979] For example, according to CellGenix's rhIL-2 lot 10200121 COA, the specific activity for the lmg vial is 25×10^6 IU/mg. It recommends reconstituting the rhIL-2 in 2 mL 0.2% HAc.

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$$\left(\frac{1mg\ x\ 25x10^6\ \frac{IU}{mg}}{6x10^6\ \frac{IU}{mL}}\right) - 2mL = 2.167mL\ HSA$$

[001980] Wiped rubber stopper of IL-2 vial with alcohol wipe. Using a 16G needle attached to a 3mL syringe, injected recommended volume of 0.2% HAc into vial. Took care to not dislodge the stopper as the needle is withdrawn. Inverted vial 3 times and swirled until all powder was dissolved. Carefully removed the stopper and set aside on an alcohol wipe. Added the calculated volume of 1% HSA to the vial.

[001981] Storage of rhIL-2 solution. For short-term storage (<72hrs), stored vial at 4°C. For long-term storage (>72hrs), aliquoted vial into smaller volumes and stored in cryovials at -20°C until ready to use. Avoided freeze/thaw cycles. Recorded expiration date of 6 months after date of preparation. Rh-IL-2 labels included vendor and catalog number, lot number, expiration date, operator initials, concentration and volume of aliquot.

EXAMPLE 3: CRYOPRESERVATION PROCESS

[001982] This example describes the cryopreservation process method for TILs prepared with the abbreviated, closed procedure described in Example 12 using the CryoMed Controlled Rate Freezer, Model 7454 (Thermo Scientific).

[001983] The equipment used was as follows: aluminum cassette holder rack (compatible with CS750 freezer bags), cryostorage cassettes for 750 mL bags, low pressure (22 psi) liquid nitrogen tank, refrigerator, thermocouple sensor (ribbon type for bags), and CryoStore CS750 Freezing bags (OriGen Scientific).

[001984] The freezing process provided for a 0.5 °C rate from nucleation to -20 °C and 1 °C per minute cooling rate to -80 °C end temperature. The program parameters were as follows: Step 1 - wait at 4 °C; Step 2: 1.0 °C/min (sample temperature) to -4 °C; Step 3: 20.0 °C/min (chamber temperature) to -45 °C; Step 4: 10.0 °C/min (chamber temperature) to -10.0 °C; Step 5: 0.5 °C/min (chamber temperature) to -20 °C; and Step 6: 1.0 °C/min (sample temperature) to -80 °C.

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EXAMPLE 4: AN EXEMPLARY EMBODIMENT OF SELECTING AND EXPANDING PBLS FROM PBMCS IN CLL PATIENTS.

[001985] PBMCs are collected from patients and either frozen for later use, or used fresh. Enough volume of peripheral blood is collected to yield at least about 400,000,000 (400×10^6) PBMCs for starting material in the method of the present invention. On Day 0 of the method, IL-2 at 6×10^6 IU/mL is either prepared fresh or thawed, and stored at 4°C or on ice until ready to use. 200 mL of CM2 medium is prepared by combining 100 mL of CM1 medium (containing GlutaMAX®), then diluting it with 100 mL (1:1) with AIM-V to make CM2. The CM2 is protected from light, and sealed tightly when not in use.

[001986] All of the following steps are performed under sterile cell culture conditions. An aliquot of CM2 is warmed in a 50 mL conical tube in a 37°C water bath for use in thawing and/or washing a frozen PBMC sample. If a frozen PBMC sample is used, the sample is removed from freezer storage and kept on dry ice until ready to thaw. When ready to thaw the PBMC cryovial, 5 mL of CM2 medium is placed in a sterile 50 mL conical tube. The PBMC sample cryovial is placed in a 37°C water bath until only a few ice crystals remain. Warmed CM2 medium is added, dropwise, to the sample vial in a 1:1 volume ratio of sample:medium (about 1 mL). The entire contents is removed from the cryovial and transferred to the remaining CM2 medium in the 50 mL conical tube. An additional 1-2 mL of CM2 medium is used to rinse the cryovial and the entire contents of the cryovial is removed and transferred to the 50 mL conical tube. The volume in the conical tube is then adjusted with additional CM2 medium to 15 mL, and swirled gently to rinse the cells. The conical tube is then centrifuged at 400g for 5 minutes at room temperature in order to collect the cell pellet.

[001987] The supernatant is removed from the pellet, the conical tube is capped, and then the cell pellet is disrupted by, for example, scraping the tube along a rough surface. About 1mL of CM2 medium is added to the cell pellet, and the pellet and medium are aspirated up and down 5-10 times with a pipette to break up the cell pellet. An additional 3-5 mL of CM2 medium is added to the tube and mixed via pipette to suspend the cells. At this point, the volume of the cell suspension is recorded. Remove 100 µL of the cell suspension from the tube for cell counting with an automatic cell counter, for example, a Nexcelom Cellometer K2. Determine the number of live cells in the sample and record.

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[001988] Reserve a minimum of 5×10^6 cells for phenotyping and other characterization experiments. Spin the reserved cells at 400g for 5 minutes at room temperature to collect the cell pellet. Resuspend the cell pellet in freezing medium (sterile, heat-inactivated FBS containing 20% DMSO). Freeze one or two aliquots of the reserved cells in freezing medium, and slow-freeze the aliquots in a cell freezer (Mr. Frosty™) in a -80°C freezer. Transfer to liquid nitrogen storage after a minimum of 24 hours at -80°C.

[001989] For the following steps, use pre-cooled solutions, work quickly, and keep the cells cold. The next step is to purify the T-cell fraction of the PBMC sample. This is completed using a Pan T-cell Isolation Kit (Miltenyi, catalog # 130-096-535). Prepare the cells for purification by washing the cells with a sterile-filtered wash buffer containing PBS, 0.5% BSA, and 2 mM EDTA at pH 7.2. The PBMC sample is centrifuged at 400g for 5 minutes to collect the cell pellet. The supernatant is aspirated off and the cell pellet is resuspended in 40 uL of wash buffer for every 10^7 cells. Add 10 µL of Pan T Cell Biotin-Antibody Cocktail for every 10^7 cells. Mix well and incubate for 5 minutes in refrigerator or on ice. Add 30 µL of wash buffer for every 10^7 cells. Add 20 uL of Pan T-cell MicroBead Cocktail for every 10^7 cells. Mix well and incubate for 10 minutes in refrigerator or on ice. Prepare an LS column and magnetically separate cells from the microbeads. The LS column is placed in the QuadroMACS magnetic field. The LS column is washed with 3 mL of cold wash buffer, and the wash is collected and discarded. The cell suspension is applied to the column and the flow-through (unlabeled cells) is collected. This flow-through is the enriched T-cell fraction (PBLs). Wash the column with 3 mL of wash buffer and collect the flow-through in the same tube as the initial flow-through. Cap the tube and place on ice. This is the T-cell fraction, or PBLs. Remove the LS column from the magnetic field, wash the column with 5 mL of wash buffer, and collect the non-T-cell fraction (magnetically labeled cells) into another tube. Centrifuge both fractions at 400g for 5 minutes to collect the cell pellets. Supernatants are aspirated from both samples, disrupt the pellet, and resuspend the cells in 1 mL of CM2 medium supplemented with 3000 IU/mL IL-2 to each pellet, and pipette up and down 5-10 times to break up the pellets. Add 1-2 mL of CM2 to each sample, and mix each sample well, and store in tissue culture incubator for next steps. Remove about a 50 µL aliquot from each sample, count cells, and record count and viability.

[001990] The T-cells (PBLs) are then cultured with Dunabeads™ Human T-Expander CD3/CD28. A stock vial of Dynabeads is vortexed for 30 seconds at medium spead. A required aliquot of beads is removed from the stock vial into a sterile 1.5 mL microtube. The

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beads are washed with bead wash solution by adding 1 mL of bead wash to the 1.5 mL microtube containing the beads. Mix gently. Place the tube onto the DynaMag™-2 magnet and let sit for 30 minutes while beads draw toward the magnet. Aspirate the wash solution off the beads and remove tube from the magnet. 1mL of CM2 medium supplemented with 3000 IU/mL IL-2 is added to the beads. The entire contents of the microtube is transferred to a 15 or 50 mL conical tube. Bring the beads to a final concentration of about 500,000/mL using CM2 medium with IL-2.

[001991] The T-cells (PBLs) and beads are cultured together as follows. On day 0: In a G-Rex 24 well plate, in a total of 7mL per well, add 500,000 T-cells, 500,000 CD3/CD28 Dynabeads, and CM2 supplemented with IL-2. The G-Rex plate is placed into a humidified 37°C, 5% CO_2 incubator until the next step in the process (on Day 4). Remaining cells are frozen in CS10 cryopreservation medium using a Mr. Frosty™ cell freezer. The non-T-cell fraction of cells are frozen in CS10 cryopreservation medium using a Mr. Frosty cell freezer. On day 4, medium is exchanged. Half of the medium (about 3.5 mL) is removed from each well of the G-rex plate. A sufficient volume (about 3.5 mL) of CM4 medium supplemented with 3000 IU/mL IL-2 warmed to 37°C is added to replace the medium removed from each sample well. The G-rex plate is returned to the incubator.

[001992] On day 7, cells are prepared for expansion by REP. The G-rex plate is removed from the incubator and half of medium is removed from each well and discarded. The cells are resuspended in the remaining medium and transferred to a 15 mL conical tube. The wells are washed with 1 mL each of CM4 supplemented with 3000 IU/mL IL-2 warmed to 37°C and the wash medium is transferred to the same 15 mL tube with the cells. A representative sample of cells is removed and counted using an automated cell counter. If there are less than 1×10^6 live cells, the Dynabead expansion process at Day 0 is repeated. The remainder of the cells are frozen for back-up expansion or for phenotyping and other characterization studies. If there are 1×10^6 live cells or more, the REP expansion is set up in replicate according to the protocol from Day 0. Alternatively, with enough cells, the expansion may be set up in a G-rex 10M culture flask using 10-15 $\times 10^6$ PBLs per flask and a 1:1 ratio of Dynabeads:PBLs in a final volume of 100 mL/well of CM4 medium supplemented with 3000 IU/mL IL-2. The plate and/or flask is returned to the incubator. Excess PBLs may be aliquotted and slow-frozen in a Mr. Frosty™ cell freezer in a -80°C freezer, and the transferred to liquid nitrogen storage after a minimum of 24 hours at -

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80°C. These PBLs may be used as back-up samples for expansion or for phenotyping or other characterization studies.

[001993] On Day 11, the medium is exchanged. Half of the medium is removed from either each well of the G-rex plate or the flask and replaced with the same amount of fresh CM4 medium supplemented with 3000 IU/mL IL-2 at 37°C.

[001994] On Day 14, the PBLs are harvested. If the G-rex plate is used, about half of the medium is removed from each well of the plate and discarded. The PBLs and beads are suspended in the remaining medium and transferred to a sterile 15 mL conical tube (Tube 1). The wells are washed with 1-2 mL of fresh AIM-V medium warmed to 37°C, and the wash is transferred to Tube 1. Tube 1 is capped and placed in the DynaMag™-15 Magnet for 1 minute to allow the beads to be drawn to the magnet. The cell suspension is transferred into a new 15 mL tube (Tube 2), and the beads are washed with 2 mL of fresh AIM-V at 37°C. Tube 1 is placed back in the magnet for an additional 1 minute, and the wash medium is then transferred to Tube 2. The wells may be combined if desired, after the final washing step. Remove a representative sample of cells and count, record count and viability. Tubes may be placed in the incubator while counting. Additional AIM-V medium may be added to the Tube 2 if cells appear very dense. If a flask is used, the volume in the flask should be reduced to about 10 mL. The contents of the flask is mixed and transferred to a 15 mL conical tube (Tube A). The flask is washed with 2 mL of the AIM-V medium as described above and the wash medium is also transferred to Tube A. Tube A is capped and placed in the DynaMag™-15 Magnet for 1 minute to allow the beads to be drawn to the magnet. The cell suspension is transferred into a new 15 mL tube (Tube B), and the beads are washed with 2 mL of fresh AIM-V at 37°C. Tube A is placed back in the magnet for an additional 1 minute, and the wash medium is then transferred to Tube B. The wells may be combined if desired, after the final washing step. Remove a representative sample of cells and count, record count and viability. Tubes may be placed in the incubator while counting. Additional AIM-V medium may be added to the Tube B if cells appear very dense. Cells may be used fresh or frozen in CS10 preservation medium at desired concentrations.

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EXAMPLE 5: AN EXEMPLARY EMBODIMENT OF EXPANDING T CELLS FROM HEMATOPOIETIC MALIGNANCES USING GEN 3 EXPANSION PLATFORM.

[001995] At Day 0, a T cell fraction (CD3+,CD45+) is isolated from an apheresis product enriched for lymphocytes, whole blood, or tumor digest (fresh or thawed) using positve or negative selection methods, i.e removing the T-cells using a T-cell marker (CD2, CD3, etc., or removing other cells leaving T-cells), or gradient centrifugation.

[001996] The Gen 3.1 process is commenced by seeding ~1×10^7 cells/ flask according to Gen3 process described herein.

[001997] At Day 7, the cells are reactivated per the Gen 3.1 process.

[001998] At Day 9-11, the cells are scaled up per the Gen 3.1 process.

[001999] At Day 14-16, the cells are harvested per the Gen 3.1 process.

[002000] Figure 21 provides a schematic diagram of an exemplary embodiment for expanding TILs from hematopoietic malignancies using the Gen 3 process.

EXAMPLE 6: TUMOR EXPANSION PROCESSES WITH DEFINED MEDIUM.

[002001] The processes disclosed in Examples 5 through 12 are performed with substituting the CM1 and CM2 media with a defined medium according to the present invention (*e.g.*, CTS™ OpTmizer™ T-Cell Expansion SFM, ThermoFisher, including for example DM1 and DM2).

EXAMPLE 7: EXPANSION OF TUMOR INFILTRATING LYMPHOCYTES FROM TISSUE CORE BIOPSY FROM A SUBJECT WITH PANCREATIC CANCER

[002002] This example describes a study performed to expand TILS from a pancreatic cancer tumor core biopsy obtained from a patient. The example provides an exemplary embodiment for a method for expanding TILS from samples of tissue core biopsies.

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[002003] A pancreatic tumor core biopsy including 3 tumor fragments was obtained and processed according to the Gen 2-like tumor processing method outlined below. Figure 1A depicts the process steps of an exemplary Gen 2-like protocol.

Day 0 – Tumor Processing

[002004] The tumor core biopsy was received and washed as described herein on Day 0 of the method. The length and theoretical mass of the tissue core was measured using a ruler and recorded.

Day 0 – Gen 2-like (Pre-REP)

[002005] A G-Rex 100M was labeled with the "Tumor ID, Gen 2, initials, media formulation, and date". 0.5 L of CM1 + 6000 IU/mL IL-2 that was warmed to 37°C (for at least 24-30 h) was added to the G-Rex 100M. A 6 well plate was used and 5 mL of tumor wash buffer was added to three wells labeled "#1," "#2," and "#3". Briefly, all the contents of the core biopsy container solution were transferred to a petri dish (such as a100mm or 150 mm).

[002006] The sample was washed three times by transferring the core biopsy specimen to well 1 using a pasteur pipette. The sample was incubated for 3 min. Then, the specimens were transferred to well #2 for 3 min and then transferred the specimens to well #3 for 3 min.

[002007] Using a transfer pipette or forceps, the biopsy specimens were added directly from well #3 into the labeled G-Rex 100M containing 0.5 L of CM1 + 6000 IU/mL IL-2 prepared in step above. The G-Rex 100M was placed into 37°C / 5% CO_2 incubator until Day 11.

Day 3 – Gen 2-like (Pre-REP Harvest/Activation)

[002008] Day 3 of the Gen 2-like process was performed as follows. Feeder cells (allogenic PBMC feeder cells) were prepared from a pooled population of PBMCs from 2 or more different donors. Feeder cells were minimally manipulated and frozen until needed. 2 x 1mL vials of feeders (allogenic PBMCfeeder cells) were thawed and pipetted into 48 mL of CM-1 + 6000 IU/mL IL-2 that was warmed to 37°C.

[002009] The PBMC feeders were mixed well using a serological pipette and 4 x 1mL aliquots were removed. The thawed feeders were counted at no dilution according to standard procedures known to those of skill in the art. Next, the volume required for 100e6

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PBMC feeders was calculated according to the equation: 100e6 / average live cell concentration = Volume required for 100e6 PBMC.

[002010] The G-Rex 100M flask(s) containing the Pre-REP culture were removed from the incubator and placed into a Biological Safety Cabinet (BSC).

[002011] The calculated volume from above and 30 µL of stock OKT3 (30 ng/mL) was added to each G-Rex 100M flask containing 500 mL CM1 + 6000 IU/mL IL-2. Quantity sufficient (QS) total volume to 1 L: 500 mL – calculated volume of PPBMCs added to each flask = total volume of CM1 + 6000 IU/mL IL-2 was added to flask.

Day 11 – Gen 2-like (REP)

[002012] Day 11 of the Gen 2-like process was performed as follows. For the Pre-REP TIL Harvest, an open system flask was used. The G-Rex 100M flask containing the culture was removed from the incubator and 2 x 1mL aliquots of supernatant were removed for metabolite analysis and stored in -80°C. A sterile 150 mL bottle was weighed and the weight was recorded. About 900 mL of supernatant was aspirated from G-Rex 100M flask. A serological pipette was used to transfer the Pre-REP TIL culture into the weighed sterile 150 mL flask.

[002013] The Pre-REP TIL culture was mixed well with serological pipette and 4 x 1 mL aliquots were collected for cell counting. Four cell counts were performed at no dilution as per standard procedures known to those skilled in the art. The Pre-REP TIL culture was placed to the incubator while the counts were performed.

[002014] Cells exceeding the maximum amount (200e6 TILS) to be seeded into the REP culture were removed from the Pre-REP TIL culture using a 100 mL syringe with an Ashton pipette. 200e6 TILS were transferred into an EV-1000N bag via the blue NIS port.

[002015] The EV-1000N bag containing the Pre-REP TIL was sterile welded onto the RED line of the G-Rex 500MCS and the TILS were gravity drained into the flask. After draining, the red line was heat sealed off.

[002016] 5e9 PBMC feeder cells were thawed as described above. After performing 4 cell counts, the volume of the feeder culture was adjusted based on the cell count in order to add 5e9 feeder cells into the EV1000N Feeder bag. After the feeder cells were added, 150 uL of OKT3 was added to the Feeder bag. The Feeder bag was sterile welded to the red line of

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the G-Rex 500MCS and the 5e9 feeder cells were gravity drained into the G-Rex. 4.5 L of CM2 and 3,000 IU/mL IL-2 were added to the G-Rex 500MCS.

Day 16 – Gen 2-like (Split)

[002017] Day 16 of the Gen 2-like process was performed according to the Gen 2 Day 16 method .

[002018] Firstly, 2 x 1mL aliquots of supernatant were drawn for metabolite analysis and stored in -80°C. Briefly, the volume was reduced and the REP cell culture was split into up to 5 G-REX 500MCS. In each G-REX 500MCS, the culture volume was 4.5 L CM4 media + IL-2 (3000 IU/mL) and $\geq 1 \times 10^9$ TVC / flask.

Day 22 – Gen 2-like (Harvest)

[002019] Day 22 of the Gen 2-like process was performed according to the Gen 2 Day 22 method.

[002020] The Day 22 cells were harvested, processed through the LOVO (TIL Harvest 2cy), and frozen in 30 x 1 mL cryovials (in 1:1 CS10/PLLA 1% HSA) using the CRF Program # 1. In some cases, only 1 flask was present and any additional daughter flasks were extrapolated for the hypothetical yield.

[002021] 10e6 post-LOVO cells were saved for Identity staining prior to freezing. Before discarding supernatant waste, 2 x 1mL aliquots of supernatant were removed for metabolite analysis and stored in -80°C.

Results

[002022] The TVC count on Day 11 (Activation) yielded 47.3e6 cells . The TVC count on Day 22 (REP) Post LOVO yielded 10.4e9 cells with 93% viability. As such, there were 8.4 cell doublings from Day 11 to Day 22 of the Gen 2-like process.

EXAMPLE 8: GEN 2-LIKE AND GEN 3 PROCESSES FOR EXPANSION OF TUMOR INFILTRATING LYMPHOCYTES FROM TISSUE CORE BIOPSIES

[002023] This example describes a study comparing TIL expansion from pancreatic cancer tumor core biopsy samples using a Gen 2-like process and a Gen 3 process. The study utilizes a tumor core biopsy from a patient with pancreatic cancer (P7057). This tumor

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sample includes 3 cores and TIL products are produced according to the Gen 2-like process described herein. The study also utilizes a tumor core biopsy from a patient with pancreatic cancer (P7058). This tumor sample includes 8 cores and TIL products are produced from 4 cores according to the Gen 2-like process and 4 cores according to the Gen 3 process described herein.

Introduction

[002024] Core needle biopsies are standard preliminary diagnostic procedures used to sample aberrant tissue growths when diagnosing cancer. The procedure utilizes large gauge (18, 16, 14, etc.) needles percutaneously to sample the suspicious area, which are then further analyzed and tested to determine if the tissue is cancerous. As core needle biopsy does not require incision or surgery, it is a far less intrusive means of obtaining tumor samples. It is therefore desirable to see if core biopsies could be used as an alternative to resected tumor for starting material in the TIL manufacturing processes outlined.

Background

[002025] Two manufacturing processes (Gen 2 and Gen 3) have been developed, and are in use in clinical manufacturing, for the ex vivo expansion of autologous tumor infiltrating lymphocytes (TIL) derived from newly resected tumors. The Gen 2 and Gen 3 processes utilize the same bioreactors (G-Rex100 MCS and G-Rex500 MCS). The Gen 2 process includes a Pre-Rapid Expansion Protocol (pre-REP) step, and this step has bee replaced by an Activation step in the Gen 3 process. Rapid expansion (REP) and scale-up of TIL culture in both Gen 2 and Gen 3 cell culture expansion processes are performed in the presence of interleukin-2 (IL-2), the monoclonal antibody OKT3, and irradiated peripheral blood mononuclear cells ("feeders"), all of which promote TIL expansion. The Gen 3 has a shorter process duration than the Gen 2 process. The Gen 2 and Gen 3 processes were used as the design basis for a process for core needle biopsies.

Purpose

[002026] This purpose of this study is to develop a TIL manufacturing process using core biopsy specimen as a starting material. The design of this manufacturing process is based on the designs of the Gen 2-like and Gen 3 clinical manufacturing processes outlined herein.

Scope

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[002027] The full-scale studies are performed using the Gen 2-like process and Gen 3 processes (see, *e.g.*, Examples above). There are two changes in the Gen 2-like process compared to the Gen 2 process including 3-days Pre-REP (vs 11 days for Gen 2), and an Activation step on Day 3 by adding Feeders and OKT- 3 on Day 3.

[002028] The major process design differences between Gen 2-like and Gen 3 are as follows. The presence of a Pre-Rapid Expansion Protocol (Pre-REP) in the Gen 2-like process in which the cells extravasate out from the core tissue or biopsy over a 3-day incubation period in the presence of IL-2, but in the absence of OKT-3 and feeder cells. The corresponding step in the Gen 3 process combines the extravasation of TIL from the tumor in combination with their activation by the addition of OKT-3, and feeder cells at Day 0.

[002029] Rapid Expansion Protocol in the Gen 2 process includes single activation using addition of OKT3, Feeders to the pre-REP TIL over a period of 11 days with the split on Day 16. The corresponding REP in the Gen 3 process also uses OKT3 and feeders added on Day 7/8 with the scale up on Day 11.

[002030] Gen 2 process uses G-Rex 100 MCS for Pre-REP and G-Rex 500 MCS for REP. Gen 3 process uses G-Rex 100 MCS for Activation and Reactivation and G-Rex 500 MCS for Scale up.

[002031] The Gen 3 process duration is 16-17 days versus 22 days for the Gen 2 process (*e.g.*, Gen 3 is 5-6 days shorter than Gen 2).

[002032] The Gen 3 process uses defined medium (*i.e.*, no human AB serum) while the Gen 2 process uses complete medium containing human AB serum.

[002033] Figure 1A provides a comparison of the Gen 2-like and Gen 3 processes described herein.

Procedures

[002034] This section of the example outlines the Gen 2-like process to be utilized to produce TIL product from cores from the pancreatic tumor core biopsy sample P7057. The Gen 2-like process and the Gen 3 process described below are used to produce TIL product from cores from the pancreatic tumor core biopsy sample P7058.

[002035] This study utilizes tissue cores/biopsy tumor samples sourced from commercial vendors, collaborators, or partners. Allogeneic PBMC feeder cells are pooled from 2 or more different donors. Feeder cells are minimally manipulated and added directly

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to the TIL culture in the cryopreservation matrix consisting of mononuclear cells suspended in CS10.

Overview of Gen 2-like Process

Day 0 – Gen 2-like (Tumor Processing)

[002036] Tumor processing of the Gen 2-like method is performed as follows. The tumor core biopsies are received and are washed 3 times. The length and theoretical mass of each tissue core is measured using a ruler and is recorded.

Day 0 – Gen 2-like (Pre-REP)

[002037] On Day 0 of the Gen 2-like process, the Pre-REP phase is initiated as follows. Label G-Rex 100M with "Tumor ID, Gen 2, initials, media formulation, and date". Add 0.5 L of CM1 + 6000 IU/mL IL-2 that is warmed to 37°C (for at least 24-30 h) to a G-Rex 100M. Use a 6 well plate and add 5 mL of tumor wash buffer to 3 wells labeled 1, 2, and 3. Briefly, transfer all the contents of the core biopsy container solution to a petri dish (100mm or 150 mm). Wash the sample 3 times by transferring the core biopsy specimen to well 1 using a pasteur pipette. Incubate for 3 min. Then transfer the specimens to well 2 for 3 min and then transfer the specimens to well 3 for 3 min. Using a transfer pipette or forceps, add the biopsy specimens directly from well 3 into the labeled G-Rex 100M containing 0.5 L of CM1 + 6000 IU/mL IL-2 prepared in step above. Place G-Rex 100M into 37°C / 5% CO_2 incubator until Day 11.

Day 3 – Gen 2-like (Pre-REP Harvest/Activation)

[002038] On Day 3 of the Gen 2-like process, the Pre-REP harvest/activation is performed as follows. Thaw 2 x 1mL vials of PBMC and pipette into 48 mL of CM-1 + 6000 IU/mL IL-2 that is warmed to 37°C. Mix well by serological pipette and remove 4 x 1mL aliquots and count thawed feeders as per standard methods at no dilution. Calculated the volume required for 100e6 PBMC: (100e6 / average live cell concentration = Volume required for 100e6 PBMC). Remove G-Rex 100M flask(s) containing culture from incubator and place into BSC. Add calculated volume from above and 30 µL of stock OKT3 (30 ng/mL) to each flask containing 500 mL CM1 + 6000 IU/mL IL-2 QS total volume to 1 L: 500 mL – volume added in 10.5.6. = total volume of CM1 + 6000 IU/mL IL-2 was added to flask.

Day 11 – Gen 2-like (REP)

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[002039] On Day 11 of the Gen 2-like process, the REP phase is initiated as per the Gen 2 Day 11 process, with the exception of Pre-REP TIL harvest and seeding into the G-Rex 500MCS. For Pre-REP TIL Harvest, an open system flask is used. Remove flask from incubator and remove 2 x 1mL aliquots of supernatant for metabolite analysis and store in -80°C. Weigh a sterile 150 mL bottle and record weight. Aspirate ~900 mL of supernatant. Use a serological pipette to transfer the Pre-REP TIL into the weighed sterile 150 mL flask. Mix well with serological pipette and obtain 4 x 1 mL aliquots for cell counting. Perform 4 cell counts at no dilution on NC-200 as standard methods. Keep Pre-REP TIL in incubator while cell counting is performed.

[002040] If necessary, remove an appropriate volume from the flask to leave 200e6 TIL (the maximum amount to be seeded into the REP) and use a 100 mL syringe with an Ashton pipette to transfer the remaining TIL (200e6 TIL) into an EV-1000N bag via the blue NIS port. Sterile weld EV-1000N bag containing Pre-REP TIL onto the RED line of the G-Rex 500MCS and gravity drain the TIL into the flask. After draining, heat seal red line off.

[002041] Thaw 5e9 feeders according to method described above. After performing 4 cell counts, adjust the volume if necessary to achieve 5e9 cells in the EV1000N Feeder bag. Add 150 uL of OKT3 to the Feeder bag. Sterile weld the Feeder bag to the red line of the G-Rex 500MCS and gravity drain the 5e9 feeders into the G-Rex. Add 4.5 L of CM2 + 3000 IU/mL IL-2 to the G-Rex 500MCS.

Day 16 – Gen 2-like (Split)

[002042] On Day 16 of the Gen 2-like process the split step is performed per the Gen 2 Day 16 step. Draw 2 x 1mL aliquots of supernatant were removed for metabolite analysis and store in -80°C. Day 16 of the Gen 2-like process is performed as per the Gen 2 Day 16 process, with the exception as follows. If only 1 flask is moved forward for the scale up/split, the number of daughter flasks is added in at harvest to extrapolate for the full scale. For example, if 5 flasks is needed, only 1 is moved forward and the final product TVC is multiplied by 5 to extrapolate for the expected full-scale yield.

Day 22 – Gen 2-like (Harvest)

[002043] On Day 22 of the Gen-2 like process the harvest step is performed per the Gen 2 Day 22 process. The Day 22 cells for Gen 2-like is harvested, processed through the LOVO cell processing system, and frozen in 30 x 1 mL cryovials (in 1:1 CS10/PLLA 1% HSA). In some cases, only 1 flask is present and any additional daughter flasks are extrapolated for the

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hypothetical yield. 10e6 post-LOVO cells are saved for identity staining prior to freezing. Before discarding supernatant waste, 2 x 1mL aliquots of supernatant are removed for metabolite analysis and stored in -80°C.

Overview of Gen 3 Process

Day 0 – Gen 3 (Tumor Processing)

[002044] Tumor processing of the Gen 3 method is performed as outlined herein. The tumor core biopsies are received and are washed 3 times. The length and theoretical mass of each tissue core is measured using a ruler and is recorded.

Day 0 – Gen 3 (Activation)

[002045] Day 0 of Gen 3 is performed according to process outlined (see, *e.g.*, Examples 5-8). Allot the remaining biopsy specimens as mentioned above into a G-Rex 100M flask containing 500 mL of DM + 6000 IU/mL IL-2 that is warmed to 37°C. For each flask used, thaw 4 x 1 mL vials of irradiated PBMC in a 37°C water bath. Use a transfer pipette to transfer the PBMCs to a 50 mL conical tube with 46 mL warm DM + 6000 IU/mL IL-2. Mix well by serological pipette and remove 4 x 1mL aliquots and count at a 1:10 dilution on NC-200 as per protocol described herein. Calculate the volume required for 250e6 PBMC: (250e6 / average concentration = Volume required for 250e6 PBMC). Add the calculated volume from previous step to each flask containing 500 mL DM+6000 IU/mL IL-2 and tumor fragments. Add 15 uL of stock OKT-3 (1mg/mL) to each flask containing 500 mL DM+6000 IU/mL IL-2, tumor fragments, and PBMC feeders. Label each flask with "Tumor ID, Gen 3, flask number, initials, date". Place into 37°C / 5% CO2 incubator until Day 8.

Day 7/8 – Gen 3 (Reactivation)

[002046] Day 7/8 of the Gen 3 process is performed as described, *e.g.*, in Examples 5-9. Remove G-Rex 100M flask(s) containing culture from incubator and place into BSC. Remove 2 x 1mL aliquots of supernatant for metabolite analysis and store in -80°C. Add 500 mL of DM + 6000 IU/mL IL-2 that is warmed to 37°C to G-Rex 100M flask. Add 25 mL of DM + 6000 IU/mL IL-2 that is warmed to 37°C to a 50 mL conical tube. Thaw 1 x 25 mL bag of PBMC in a 37°C water bath, spike the bag with a plasma extension set, draw the 25 mL with a syringe, and dispense into the prepared 50 mL conical tube. Perform 4 cell counts at either a 1:10 (100 uL PBMC in 900 uL AIM-V) or a 1:100 (make a 1:10 as described and then transfer 100 uL to another 900 uL of AIM-V) count thawed feeders as per standard

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methods. Calculate the volume required for 500e6 PBMC: (500e6 / average concentration = Volume required for 500e6 PBMC). Add the calculated volume of PBMC the steps above to the G-Rex 100M containing 1L DM + 6000 IU/mL IL-2 and core biopsies. Add 30 uL of stock OKT3 (30 ng/mL) to the flask. Place flask in 37°C / 5% CO2 incubator.

Day 10/11 – Gen 3 (Scale-up)

[002047] Day 10/11 Scale up for the Gen 3 process is performed as described, *e.g.*, in Examples 5-10. Flask(s) from incubator are removed and 2 x 1mL aliquots of supernatant are removed for metabolite analysis and stored in -80°C. Sterile weld a fluid transfer set onto the RED line of a G-Rex 500MCS, thread the fluid transfer set through the Baxter pump, and aseptically connect an Ashton pipette to the other end inside of a BSC. Transfer ~700mL of media from the G-Rex 100MCS to the G-Rex 500MCS, stop pump, swirl to disturb cell layer, and transfer remaining cell culture to the G-Rex 500MCS. After all TIL are transferred, sterile weld the RED line of the G-Rex onto a 5 L or 10 L bag of DM + 3K IU/mL IL-2 that is warmed to 37°C. Gravity drain the media up to the 5 L mark of the G-Rex 500MCS. After draining is complete, return flask to incubator.

Day 16/17 – Gen 3 (Harvest)

[002048] Day 16/17 of the Gen 3 process was performed as described, *e.g.*, in Examples 5-1. The Day 17 cells for Gen 3 are harvested, processed through the LOVO (TIL Harvest 5cy), and frozen in 30 x 1 mL cryovials (in a 1:1 ratio of CS10:Plasmalyte with 1% HSA) using the CRF Program # 1. In some cases, only 1 or 2 flasks are present for harvest, depending on the number of fragments seeded in day 0. 10e6 post-LOVO cells are saved for Purity staining prior to freezing.

Final Product and Starting Material Characterization:

[002049] The starting materials and final TIL products manufactured according to the Gen 2-like and Gen 3 processes can be evaluated. The identity (% CD45+/CD3+) is measured on fresh TIL product before freezing using standard protocols. For instance, TIL function in terms of Interferon-gamma production and Granzyme B release is measured. Stimulation of TIL is measured according to IFN-gamma release. Stimulation of TIL is measured according to Granzyme B release via ELISA. CD107a phenotype, extended phenotype, surface antigen staining of TIL using a differentiation panel and/or activation/exhaustion panel can be assessed. TCRvβ Sequencing can be performed.

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Telomerase activity and telomere length can be performed. Metabolites can be measured in the culture supernatant by CEDEX Bio-analyzer.

Expected Results or Acceptance Criteria

[002050] The expected results of the Pre-REP cells and Final Product from the Gen 2-like process as well as the Final Product of the Gen 3 process are provided in Tables 37 and 38.

[002051] Table 37. Pre-REP testing and expected results for Gen 2-like process

Test Type	Method	Acceptance Criteria
Cell Count/Viability	Fluorescence	> 5 x 10e6 Viable cells

[002052] Table 38. Final Product Testing and Acceptance Criteria (Gen 2-like and Gen 3 Processes)

Test Type	Method	Acceptance Criteria
Release Testing		
Cell viability	Fluorescence	≥ 70%
Total Viable Cell Count	Fluorescence	1e9 to 150e9
Identity (%CD45+/CD3+)	Flow Cytometry	Gen 2-like: ≥ 90% CD45+CD3+ TIL for all Indications Gen 3 : ≥ 90% CD45+CD3+ TIL for Non-Ovarian ≥ 85% CD45+CD3+ TIL for Ovarian
Interferon-gamma production (Stimulated – Unstimulated)	Stimulation and ELISA	≥ 500 pg/mL

[002053] In some embodments, frozen final product testing is performed on the products produced in the method described herein, such as the Gen 2-like and Gen 3 methods. In some embodiments, the testing comprises assessment of one or more of the following: differentiation, activation and exhaustion markers, granzyme B, CD107A, TCR vβ sequencing, telomere length, telomerase activity, and metabolites. In some instances, differentiation is evaluated by flow cytometry, for example by flow cytometry of a TIL 1 panel. In some instances, activation and exhaustion markers is evaluated by flow cytometry, for example by flow cytometry of a TIL 2 panel. In some instances, granzyme B is evaluated by bead stimulation and ELISA. In some instances, CD107A is evaluated by mitogen

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stimulation and intracellular flow cytometry. In some instances, TCR vβ sequencing is performed by deep sequencing. In some instances, telomere length is measured using a TAT assay. In some instances, telomerase activity is determined by Q-TRAP. In some instances, telomere length is measured using a TAT assay. In some instances, metabolite is determined using a CEDEX metabolite analyzer.

EXAMPLE 9: EXEMPLARY GEN 3 PROCESS EXAMPLE

Preparation of Defined media for Day 0, day 7/8, AND DAY 10/11.

Prepared IL-2

[002054] Defined Media Batch Volumes:

- Batch 1 = 3L DM1 @ 6000 IU/mL IL-2 (prepared 14 days in advance for D0 and D7/8);
- Batch 2= 4L DM2 @ 3000 IU/mL IL-2 (prepared 14 days in advance for D10/11)
- DM1 = Defined Media 1; DM2 = Defined Media 2
- Batch 1 DM1 = 6000 IU/mL x 3000mL = $18x10^6$ IU
- Batch 2 DM2 = 3000 IU/mL x 4000mL = $12x10^6$ IU

[002055] Prepared IL-2: Akron prefilled syringe 1mg in 1mL.

Table 39. Batch 1: IL-2 Calculation to prepare 3L of DM 1 at 6000 IU.

A Volume to prepare into each bag	**B** Final concentration of IL-2 per bag	**C** Number of bags to prepare	**D** Total IL-2 needed mg=mL **B(A*C)**	**E** Specific Activity IU/mL (CoA)	**F** IL-2 to transfer per bag **(B*A)/E** (1 decimal)	**G** Total volume needed of IL-2 **F*C**	H Number of syringes needed	**I** **H** rounded at 0 decimal
1000mL	6000 IU/mL	3	$18x10^6$					

[002056] Table 40. Batch 2: IL-2 Calculation to prepare 4L of Defined Media at 3000 IU/mL.

A Volume to prepare into each bag	**B** Final concentration of IL-2 per bag	**C** Number of bags to prepare	**D** Total IL-2 needed mg=mL **B(A*C)**	**E** Specific Activity IU/mL (CoA)	**F** IL-2 to transfer per bag **(B*A)/E**	**G** Total volume needed of IL-2

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					(1 decimal)	**F*C**
1000mL	3000 IU/mL	4	$12x10^6$			

Table 41. Batch 2 options according to # of G-Rex flask to prepare: checked option used.

Option	**II** # GREX flask to process	**I** Defined media needed	**J** # 1L bags to prepare	**K** Volume of IL-2 needed for batch **(G*H)**	**L** # IL-2 syringes needed (**K** rounded up to whole number)
A	1	4L	4		
B	2	8L	8		
C	3	12L	12		
D	4	16L	16		

[002057] Prepared IL-2: Akron lyophilized 1mg.

Table 42. Batch 1: IL-2 Calculation to prepare 3L of DM 1 at 6000 IU

A Volume to prepare into each bag	**B** Final concentration of IL-2 per bag	**C** Number of bags to prepare	**D** Total IL-2 needed mg=mL **B(A*C)**	**E** Specific Activity IU/mL (CoA)	**F** IL-2 to transfer per bag **(B*A)/E** (1 decimal)	**G** Total volume needed of IL-2 **F*C**	H Number of syringes needed	**I** **H** rounded at 0 decimal
1000mL	6000 IU/mL	3	$18x10^6$					

[002058] Table 43. Batch 2: IL-2 Calculation to prepare 4L of Defined Media at 3000 IU/mL

A Volume to prepare into each bag	**B** Final concentration of IL-2 per bag	**C** Number of bags to prepare	**D** Total IL-2 needed mg=mL **B(A*C)**	**E** Specific Activity IU/mL (CoA)	**F** IL-2 to transfer per bag **(B*A)/E**	**G** Total volume needed of IL-2 **F*C**

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					(1 decimal)	
1000mL	3000 IU/mL	4	$12x10^6$			

Table 44. Batch 2 options according to # of G-Rex flask to prepare: checked option used;

Option	**II** # GREX flask to process	**I** Defined media needed	**J** # 1L bags to prepare	**K** Volume of IL-2 needed for batch **(G*H)**	**L** # IL-2 syringes needed (**K** rounded up to whole number)
A	1	4L	4		
B	2	8L	8		
C	3	12L	12		
D	4	16L	16		

[002059] Prepared IL-2: Cellgenix lyophilized 1mg.

Table 45. Batch 1: IL-2 Calculation to prepare 3L of DM 1 at 6000 IU.

A Volume to prepare into each bag	**B** Final concentration of IL-2 per bag	**C** Number of bags to prepare	**D** Total IL-2 needed mg=mL **B(A*C)**	**E** Specific Activity IU/mL (CoA)	**F** IL-2 to transfer per bag (B*A)/E (1 decimal)	**G** Total volume needed of IL-2 **F*C**	H Number of syringes needed	**I** **H** rounded at 0 decimal
1000mL	6000 IU/mL	3	$18x10^6$					

Table 46. Batch 2: IL-2 Calculation to prepare 4L of Defined Media at 3000 IU/mL.

A	**B**	**C**	**D**	**E**	**F**	**G**

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Volume to prepare into each bag	Final concentration of IL-2 per bag	Number of bags to prepare	Total IL-2 needed mg=mL **B(A*C)**	Specific Activity IU/mL (CoA)	IL-2 to transfer per bag (B*A)/E (1 decimal)	Total volume needed of IL-2 **F*C**
1000mL	3000 IU/mL	4	$12x10^6$			

Table 47. Batch 2 options according to # of G-Rex flask to prepare: checked option used.

Option	**H** **# GREX flask to process**	**I** **Defined media needed**	**J** **# 1L bags to prepare**	**K** **Volume of IL-2 needed for batch (G*H)**	**L** **# IL-2 syringes needed (K rounded up to whole number)**
A	1	4L	4		
B	2	8L	8		
C	3	12L	12		
D	4	16L	16		

Preliminary preparation

[002060] Premade IL-2: for Akron IL-2 in prefilled syringe, no reconstitution was required, proceeded to step 2.8; for Akron IL-2 lyophilized and proceed with reconstitution; and for Cellgenix IL-2 lyophilized, went to step 2.5 and proceeded with reconstitution.

[002061] Transferred the following into the Bio Safety Cabinet (BSC): Akron IL-2 powder vials, Mini-spike (1), Bottle of Water for injection(1), 10mL syringe (as needed), and Safety needle 18G (as needed). Spiked Water for Injection (WFI) bottle using a 10mL syringe, drew lmL of WFI. Connected an 18G needle to the syringe and transfer 1mL WFI to vial of 1L-2. Inverted the vials 2-3 times and swirled until all powder was dissolved. Avoided

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foam formation and did not mix vigorously. Repeated this step to reconstitute the needed number of vials (used a new syringe when needed).

[002062] Recorded the number of vials to reconstitute. Transferred the following into the BSC: Cellgenix IL-2 lyophilized vials, Mini-spike (1), 500 mL bottle of 0.25% Acetic acid (HAc) (1), 10mL syringe (as needed), Pumpmatic pipette (1), and Safety needle 18G (as needed).

[002063] Using a 10mL syringe and pumpmatic pipette, drew 2mL of HAc. Connected an 18G needle to the syringe and transfer 2mL HAc into the vial through its septum. Inverted the vials 2-3 times and swirled until all powder was dissolved. Avoided foam formation and did not mix vigorously. Repeated this step to reconstitute the needed number of vials in section 2.5.

[002064] Transferred the number of prefilled syringes needed into the BSC. Fluid dispensers as needed. Syringes as needed. Defined media preparation per 1L bag. Checked the thawing of CTS Immune Cell SR.

[002065] Transferred the following into the BSC per each 1L of media to prepare:

- 50 mL CTS Immune Cell SR (1)
- 10 mL bottle Gentamicin sulfate, 50 mg/ml
- 1L CTS OpTmizer T-Cell Expansion SFM Basal Media 1L bag (1)
- CTS OpTmizer T-Cell Expansion Supplement 26ML bottle (1)
- 100 mL Glutamax bottle (1)
- 10 mL serological pipette (2)
- Container or 50 mL conical tube "CTS Immune Cell SR" (1)
- Container or 50 mL conical tube "Glutamax"
- 2L labtainer bag (if needed) for preparation
- bottles
- 60 mL syringes (as needed)

[002066] Table 48. Prepared the Defined Media 1L bags according to the table below. Marked appropriate volume to prepare:

Option	**Process Day**	**Defined Media (L) needed**	**Total 1L bags to prepare**	**IL-2 Final concentration needed (IU/mL)**

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A	Batch 1 (Day 0 or 7/8)	3 L	3	6000 IU/mL
B	Batch 2 (Day 10/11)	4 L = 1 GREX flask 8 L = 2 GREX flask 12 L = 3 GREX flask 16 L = 4 GREX flask	4 8 12 16	3000 IU/mL

[002067] Table 49. Prepared each 1L of defined media following the below volume table instructions.

Reagents	**Volume needed (mL)**
CTS OpTmizer T-Cell Expansion SFM Basal Media	1000
CTS Immune Cell SR	30
CIS OpTmizer T-Cell Expansion Supplement	26
Glutamax	20
Gentamicin	1

[002068] Labeled the containers needed as Glutamax and CTS Immune Cell SR. Using an appropriate sized pipette, removed 20 mL of Glutamax from the bottle and transferred into a 50 mL container labelled Glutamax. Repeated for number of bags of media being prepared. Transferred 30 mL of CTS SR into a 50 mL conical tube labelled CTS Immune Cell SR. Repeated for number of bags of media being prepared. Added 1 mL of Gentamicin into each CTS Immune Cell SR container and homogenize.

[002069] For Cellgenix or Akron reconstituted vials, used an adequate volume syringe and 18G needle, drew the needed volume of IL-2 for a 1L defined media bag and transferred into each CTS Immune Cell SR container and homogenize. For Akron IL-2 prefilled syringe, transferred the volume calculated in section 1 of IL-2 into each CTS Immune Cell SR container and homogenizes. Attached a fluid dispenser connector to 1 mL syringe, and then attached by luer lock connection to the prefilled Akron IL-2, removed the syringe and dispensed the required volume into each CTS Immune Cell SR container and homogenize.

[002070] Attached the extension set on the Defined Media bag to the fluid transfer set and connected the other side of the fluid transfer set to the pumpmatic pipette. Placed the

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pipette tip inside the first 1L bottle of CTS OpTmizer T-Cell Expansion SFM Basal Media. Placed the fluid transfer set on the Acacia pump. Set the parameters on the pump as indicated below. Program: Volume; Speed: 250 RPM; Volume: 1000mL.

[002071] Pumped the whole volume of the first 1L bottle of CTS OpTimizer T-Cell Expansion SFM Basal Media into the Defined Media bag (2L Labtainer). Transferred the pumpmatic pipette to one tube labeled Glutamax and pumped entire volume into bag. Repeated for one tube labeled Immune Cell SR and one 26mL bottle of supplement. Recorded Total volume transferred. Once the bag was filled invert to mix and ensure the line was clear. Closed the clamp on the tubing and heat sealed the extension set line three times.

[002072] After labelling of Defined Media bag: Spiked the bag with a 4" plasma transfer set. Using a 100 mL syringe attached to a pumpmatic pipette, drew into the syringe the whole volume from the CTS Immune Cell SR container to which gentamicin and IL-2 were added.

[002073] Transferred the syringe with the pumpmatic pipette into the Glutamax container and drew into the syringe the whole volume from the Glutamax. Transferred the syringe with the pumpmatic pipette into the CTS OpTmizer T-cell. Expansion Supplement bottle (26mL) and drew the whole volume into the syringe. Removed the luer lock cap of the defined media bag and inject the whole solution from the syringe into the media bag, flushed three times the syringes with the media to ensure the whole solution containing CTS SR, supplement, glutamax, gentamicin, and IL-2 was added to the bag.

[002074] Removed the syringe and connected an extension set by luer lock connection, clamp it and heat sealed three times. Mixed and repeated. Stored bags at 2-8° C in the dark until use.

PROCESS GEN 3 – DAY 0

Preliminary operations

[002075] Defined Media (CTS OpTmizer) DM1. Recorded start date and time of defined media and warm pack incubation. Media and warm packs were warmed overnight (approximately 18hrs). Preparation of tumor wash medium using Gentamicin (50 mg/mL) (1), 500 mL bottle of HBSS (1), and 5 mL serological pipette (1).

[002076] Added 5 mL of gentamicin (50mg/mL) to the 500 mL bottle of HBSS. Identified the 500mL bottle Tumor Wash Medium with label or manually. Added 5mL of

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Tumor Wash Media to a 15mL conical to be used for OKT3 dilution. Kept the tumor wash medium until use.

[002077] Prepared feeder cell bags using the following:

- 10 mL syringe (4)
- Defined Media bag (1 L)
- Pre-warmed pack (2)
- EV1000N bag (1)
- EV3000N bag (1)

[002078] Closed all clamps and sterile welded the prepared media bag to Feeder Cells Bag #1 using the sterile welder. Transferred by gravity 500 mL ± 10 mL of media into Feeder Cells Bag and recorded the volume added. Assume 1 g = 1 mL. Heat sealed and removed Feeder Cells Bag #1 leaving the same original length of tubing. Transferred Feeder Cells Bag #1 to the SSC.

[002079] Prepare feeder cells

[002080] Thawed the feeder bag for 3-5 min in a 37°C water bath. Recorded start and end time of thawing. Connected Feeder Cells Bag #1 to the CC3 using one of the luer connections. Connected Feeder Cells Bag #2 to the CC3 using another one of the luer connections. Replaced the syringe on the CC3 manifold with a 100mL syringe. Spiked the feeder cell bag with a spike from the CC3 into the single port of the feeder bag. Rotated the stopcock valve so Feeder Cells bag #1 and Feeder Cells Bag #2 are in the OFF position. The valve indicates what is closed.

[002081] Feeder cell count and concentration adjustment. If necessary, prepared a dilution of each of the cell fraction samples with AIM-V (1:10 dilutions are recommended). Optimal range for the NC200 was between $5x10^4$ and $5x10^6$ cells/mL. Prepared four conical tubes with 4.5mL of AIM-V. Added 0.5mL of CF for each cell count.

[002082] Performed a cell count on sample 1. Indicated the dilution factor used in the NC-200. Recorded the viable (live) cell concentration and viability below. Repeated for all samples.

[002083] Feeder cell count and concentration and determine viability. Calculated the average of the four counts using the data recorded: (Feeder 1 + Feeder 2 +Feeder 3 + Feeder 4) / 4. Calculated the number of total viable feeder cells. Volume of feeder cell suspension x average concentration. When total viable feeder cell number was at least $1x10^9$ cells,

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proceeded to the next step to adjust the feeder cell concentration. When total viable feeder cell number <$1x10^9$ cells, contacted management.

[002084] Using the p1000 micropipette, transferred 900µl of Tumor Wash Media to the OKT3 aliquot (100µL). Mixed by pipetting up and down 3 times.

[002085] Calculated the volume of feeder cells to remove from Feeder Cells Bag #1 in order to add 1 x 10^9 cells to Feeder Cells Bag #2. 1 x 10^9 / Average viable cell concentration.

[002086] Determined the volume to transfer from Feeder Cells Bag #1 to Feeder Cells Bag #2 while keeping the feeder bag over warm packs. Drew 50mL of air into a new 100ml syringe and replaced the current syringe with it. Expelled the air into Feeder Cells Bag #2. Ensured Feeder Cells Bag #1 was mixed well. Removed the calculated volume (step 5.9) from Feeder Cells Bag #1 with the syringe. For large volumes, used additional syringes. Opened the clamps leading to Feeder Cells Bag #2 and transferred the volume from the syringe into Feeder Cells Bag #2. Inverted syringe and dispensed air to clear the line.

[002087] Wiped the NIS on Feeder Cells Bag #2. Using a 1ml syringe with an 18G needle attached, drew up 0.6ml of OKT3 prepared in step 5.8. Removed the needle and dispensed the OKT3 into Feeder Cells Bag #2 through the NIS.

[002088] Disconnected Feeder Cells Bag #2 from the manifold. Heat sealed leaving enough tubing for future welding. Sterile welded Feeder Cells Bag #2 to a Defined Media bag. Placed Feeder Cells Bag #2 on the balance and tared the balance.

[002089] Calculated the volume necessary to QS to a total volume of 2L 2000mL — volume of feeder cell suspension transferred. Opened all the clamps and transferred the calculated volume, assuming lg = lmL. Clamped when the required volume had been transferred. Replaced the media bag by sterile welding a new media bag when empty.

[002090] Once the required volume had been transferred into Feeder Cells Bag #2, turned the feeder bag up and clear the line with air. Heat sealed three times and break the middle seal leaving approximately 12 inches of tubing. This tubing was attached to the red line on the G-Rex100 MCS flask. Attached an extension set if necessary.

[002091] Transferred Feeder Cells Bag #2 to the incubator. Recorded time placed in incubator and incubator #. Recorded start date and time of tumor processing. Determined the total hold time of the tumor is shipping media.

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Tissue dissection

[002092] Labeled the 6 well plate 'Excess Tumor Pieces'. Labeled one each of the four 100 mm petri dishes as 'Wash_01', 'Wash_02', 'Wash_03', 'Wash_04', and 'Holding'. Added 5 ml of Tumor Wash Medium into all wells of the 6-well plate labelled Excess Tumor Pieces. Added 50 ml of Tumor Wash Medium to each 100 mm petri dish labelled Wash_01, Wash_02, Wash_03, and Holding.

[002093] Labeled one 50ml conical tube Forceps Wash Medium, one Scalpel Wash Medium, and one Lid Drop Wash Medium. Labeled four 50ml conical tubes Fragments Tube 1 through Fragments Tube 4. Transferred 25ml of Tumor Wash Media to each of the 50ml conical tubes labelled Fragments Tube 1 through Fragments Tube 4. Added 20 mL of Tumor Wash Medium into each of the 50 mL conical tubes labelled Forceps Wash Medium, Scalpel Wash Medium and Lid Drops Wash Medium. Kept the Tumor Wash Medium in the BSC for further use in keeping the tumor hydrated during dissection.

[002094] For dissection only, placeed scalpels and short forceps in appropriate tubes labelled Forceps Wash Medium and Scalpel Wash Medium. Transferred the tumor container into the BSC. Using long forceps transferred the tumor(s) from specimen bottle to the 100 mm petri dish labelled Wash_01.

[002095] Incubated the tumor at ambient temperature in Wash_01 for 3 min. Recorded time tumor is removed from shipping media and placed in Wash_01.

[002096] Recapped specimen bottle and transfer to balance. Recorded weight of specimen bottle and calculate weight difference of tumor tissue.

[002097] Transferred 10 mL of tumor shipping medium into the tube labelled Tumor Shipping Medium. Recorded the volume transferred into the Tumor Shipping Medium tube.

[002098] Drew 10mL of the Tumor Shipping Medium into a syringe with an 18G needle. Inoculated one each anaerobic and aerobic sterility bottle with 5 mL of tumor shipping medium

[002099] Labeled each petri dish as Dissection 1 through Dissection 4. Recorded the stop time of tumor incubation 1. (after an incubation of at least 3 min). Using forceps transferred the tumor to the 100 mm petri dish labelled Wash_02 and incubated tumor at ambient temperature for at least 3 min. Recorded tumor incubation start time. Recorded tumor incubation stop time.

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[002100] Using forceps, transferred tumor to 100 mm petri dish labelled Wash_03 and incubate tumor at ambient for 3 min. Recorded tumor incubation start time. Recorded tumor incubation stop time. After washes were complete tumor should be moved to the 'Holding' dish to ensure tissue stayed hydrated.

[002101] Kept the ruler underneath the petri dish lid for the entirety of the dissection process. Using the long forceps transferred the tumor to the petri dish labeled Dissection 1. Measured and recorded length of the tumor and the number of fragments received. The length of the tumor was measured as the sum of original tumor diameters.

[002102] Performed an initial dissection of the tumor in the dissection dish into four intermediate pieces, or group into four groups of equivalent volume. While cutting, took care to conserve the tumor structure of each intermediate piece. If tumor was very small, entire tumor could be dissected at once. Transferred any intermediate tumor pieces not being actively dissected to the Holding dish to keep the tissue hydrated. In each lid of the petri dishes labeled Dissection 1 through Dissection 4, add 10-20 drops (about 1mL) to form a pool of wash buffer close to the edge of the plate to hold dissected fragments. Repeated for each intermediate fragment or group to be dissected. Fresh scalpel and forceps could be used per discretion of operator. Recorded start time of tumor dissection.

[002103] Gently dissected the tumor into 27mm" fragments (3x3x3mm), using the ruler under the Dissection dish lid as a reference. Worked quickly and dissected the entire tissue into fragments. Worked with one dissection dish lid at a time transferring dissected fragments to buffer pool within each dish to prevent dehydration.

[002104] Using transfer pipette, scalpel, or forceps count total fragments obtained. Recorded value for each dish. When an intermediate fragment did not produce 60 fragments, the next intermediate fragment could be dissected in the same dish until 60 fragments were reached.

[002105] Replaced lid and proceed with the dissection of the second, third, and fourth intermediate fragments as necessary.

[002106] Took care to avoid any cross over of the lid during the manipulation of the process.

[002107] Took care to always keep the tissue hydrated throughout the dissection procedure. Used the transfer pipette if it is necessary to add buffer to the fragments to keep them hydrated.

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[002108] Counted total number of final fragments. Note: Up to 4 G-Rex 100MCS flasks wereprepared according to the number of final fragments generated. A maximum of 240 fragments were seeded.

Table 50: Total number of final fragments

Option	Total # of fragments	Flasks to seed	Fragments pre flask
A	less than 30 fragments	1	Total # of fragments/1
B	from 31 to 60 fragments	2	Total # of fragments/2
C	from 61 to 90 fragments	3	Total # of fragments/3
D	from 91 to 240 fragments	4	Total # of fragments/4

[002109] Using the table above, determined the number of flasks to seed and fragments to seed per flask. Using forceps, transfer pipette, or a scalpel transfer the determined number of fragments to the 50 mL conical tubes labelled Fragments Tube 1 through Fragments Tube 4 according to the table and recorded fragments transferred into each 50 mL tube in the following step.

[002110] Added the number of fragments to be added to each Fragment tube (*i.e.*, total number of fragments generated/number of flasks to seed from above table in section 7.24.) to each fragment tube applicable. Recorded the number of floating fragments per tube below. Added additional fragments equal to the number of floating fragments if available from the "Excess Tumor Pieces" dish. Recorded dissection stop time of tumor processing.

[002111] Removed all unnecessary items from the BSC, retaining the favorable tissue fragments in the conical tubes Fragments Tube 1 through Fragments Tube 4. Discarded any unused tumor.

[002112] Prepared G-REX100MCS flask with feeder cell suspension. Calculated the number of G-Rex 100MCS flasks to seed with feeder cell suspension according to the number of fragment tubes. Removed Feeder Cells Bag #2 from the incubator. Seeding G-Rex 100MCS #1: Opened exterior packaging and place G-Rex 100MCS in the BSC; closed all

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clamps of the G-Rex100MCS except the clamp to the filter line; ensured all luer locks are secure. Worked with one GREX 100MCS at the time.

[002113] Sterile welded Feeder Cells Bag #2 to the red line on the G-Rex 100MCS flask and ensured regular mixing of the bag. Placed the G-Rex flask on the analytical balance and tare. Labeled G-Rex100MCS flask #1 Tumor Fragments Culture (DAY 0) and transferred the flask to the BSC.

[002114] Seeding G-Rex 100MCS #2 -#4: For an additional G-Rex 100MCS flask, closed all clamps except the large filter line. Welded the feeder bag to the red line of the flask. Repeated the same operation for seeding each flask as needed. Recorded the cell suspension added volume. Labeled G-Rex100MCS flask #2 Tumor Fragments Culture (DAY 0) and transferred the flasks to the BSC. Labeled G-Rex100MCS flask #1-#4 Tumor Fragments Culture (DAY 0).

Identification of G-Rex100MCS Tumor Fragment Culture D0 (up to 4) GREX100MCS flask.

[002115] Start tumor fragment addition to G-REX100MCS. Worked with one G-Rex 100 MCS at the time. Transferred the G-Rex 100 MCS with feeder cell suspension to the BSC. Inside the BSC, unscrewed the cap of the G-Rex 100MCS labelled Tumor Fragments Culture (D0) 1 and the 50 mL conical tube labelled Fragments Tube. Swirled the opened Fragments Tube 1 and at the same time, slightly lifted the cap of the G-Rex100MCS. Added the medium with the fragments to the G-Rex100MCS while being swirled. Be careful to have good alignment of the G-Rex and lid and close the cap tightly. Recorded the number of fragments transferred into the GRex100MCS. Recorded the number of observed floating fragments in the GRex100MCS.

[002116] Once the fragments were located at the bottom of the GREX flask, connected one 10 mL syringe to the blue capped NIS and drew 7 mL of media. Created seven 1 mL aliquots ~5 mL for extended characterization and 2 mL for sterility samples. Stored the 5 aliquots (final fragment culture supernatant) for extended characterization at 5 -20°C until requested by sponsor. Inoculated one anaerobic BacT/Alert bottle and one aerobic BacT/Alert bottle each with 1mL of final fragment culture supernatant. Repeated for each flask sampled.

[002117] Repeated for each G-Rex 100MCS.

[002118] Recorded time the GREX 100MCS flasks were placed in the incubator. Recorded temperature and CO_2 reading of the incubator. Recoreded ending time of Day 0 processing.

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PROCESS GEN 3 – DAY 7

[002119] Recorded start date and time of defined media 1 and warm pack incubation. Media and warm packs were warmed overnight (approximately 18hrs). Recorded end time and date of media and warm packs incubation. Were media and warm packs warmed overnight? If no, contacted management.

[002120] Prepared feeder cell bag and recorded start of processing. Labeled the EV1000N bag as Feeder Cells Bag #1 and Bag #2. Closed all clamps of Feeder Cells Bag #1 & #2. Sterile welded the prepared media bag to Feeder Cells Bag #1 using the sterile welder. Hemostated the unused tubing on Feeder Cells Bag #1 & #2 during the process. Placed Feeder Cells Bag #1 on the analytical balance and tare. Clamped the line and hang the media bag on the IV pole. Transferred by gravity 500 mL ± 10 mL of media into Feeder Cells Bag #1 and recorded the volume added. Assume 1 g = 1 mL.

[002121] Heat sealed and removed Feeder Cells Bag #1 leaving the same original length of tubing. Transferred Feeder Cells Bag #1 to the BSC.

[002122] Prepared feeder cells. Documented the lot number of the cryopreserved feeder cell bags to be thawed. Ensured two different lots were used. Thawed the feeder cell bags for 3-5 min in a 37°C water bath. Recorded start time of thawing. Recorded end time of thawing. Removed the feeder cell bags from the water bath and verified that the bags are dry.

[002123] Connected Feeder Cells Bag #1 to the CC1 using one of the luer connectors on the manifold. Connected Feeder Cells bag #2 bag to the CC1 using another of the luer connector side of the manifold. Drew 20mL of air into a 100mL syringe. Moved the stopcock so that the syringe was in the off position. Replaced the syringe on the CC1 manifold with the 100 mL syringe. Spiked each of the feeder cell bags with a spike from the CC3 into the single port of the feeder bags.

[002124] Rotated the stopcock valve so the Feeder Cells Bags #1 and 2 were in the OFF position. The valve indicated what was closed.

[002125] Opened the clamps of the feeder bag lines. Drew up the contents of both feeder cell bags into the syringe with a single draw. Recorded the total volume recovered. Keep Feeder Cells Bag #1 and Feeder Cells Bag #2 over the pre-warmed packs.

[002126] Rotated the stopcock valve so the feeder bags were in the OFF position and opened all clamps in direction of Feeder Cells Bag #1.

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[002127] Dispensed the contents of the syringe into Feeder Cells Bag #1 while gently mixing. Rotated the stopcock so Feeder Cells Bag #1 was in the OFF position. Mixed the cells in Feeder Cells Bag #1 well. Attached a 10 mL syringe to the NIS of Feeder Cells Bag #1, mixed the bag and flush the syringe three times with at least 6mL of cells then removed a 1mL sample. Transferred the sample to cryovial 1. Repeated for sample 2, 3 and 4 using a new 10 mL syringe for each sample.

[002128] Calculated the volume of feeder cell suspension.

[002129] Feeder cell count and concentration adjustment. When necessary, prepared a dilution of each of the cell fraction samples with AIM-V (1:10 dilutions are recommended). Optimal concentration range for the NC200 is between $5x10^4$ and $5x10^6$ cells/mL. Prepared four conical tubes with 4.5mL of AIM-V. Add 0.5mL of cell fraction for each cell count. Mixed the sample and from each dilution tube transfer 500pL into a new cryovial tube. Mixed the samples well and proceed with the cell count. Recorded the viable (live) cell concentration and viability. Repeated for samples 2, 3. and 4.

[002130] Calculated the average of the four counts using the data recorded in step 4 .3: (Feeder 1 + Feeder 2 +Feeder 3 + Feeder 4) / 4. Calculated the number of total viable feeder cells. Volume of feeder cell suspension (step 3.15) x average concentration. When total viable feeder cell number was at least $2x10^9$ cells, proceeded to the next step to adjust the feeder cell concentration.

[002131] Calculated the volume of feeder cells to remove from Feeder Cells Bag #1 in order to add 2 x 10^9 cells to Feeder Cells Bag #2. 2 x 10^9 / Average viable cell concentration.

[002132] Using the p1000 micropipette, transferred 900uL of HBSS to a 100uL OKT3 aliquot. Mix by pipetting up and down 3 times. Prepared two aliquots. Determined the volume to transfer from Feeder Cells Bag #1 to Feeder Cells Bag #2 while keeping the feeder bag over warm packs. Drew 50mL of air into a new 100ml syringe and replaced the current syringe with it. Expel the air into Feeder Cells Bag #2. Ensured Feeder Cells Bag #1 was mixed well. Removed the calculated volume from Feeder Cells Bag #1 with the syringe. Opened the clamps leading to Feeder Cells Bag #2 and transferred the volume from the syringe into Feeder Cells Bag #2. Inverted syringe and dispense air to clear the line. When necessary, removed syringe and drew air into the syringe and dispensed into Feeder Cells Bag #2. Ensured enough air was available to facilitate gravity filling of the flasks.

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[002133] Wiped NIS on Feeder Cells Bag #2 Using a 1 mL syringe with an 18G needle attached, drew up 0.6 mL of OKT3 from one of the aliquots prepared in step 14.9. Removed needle and dispensed OKT3 into Feeder Cells Bag #2 through the NIS. Inverted Feeder Cells Bag #2, ensuring the media is close to the ports and all flush the syringe with the 0.5mL of feeder cells to ensure all OKT3 was added to the bag. Repeated with the second aliquot for a total of 1.2ml OKT3 dispensed into Feeder Cells Bag #2. Flushed the syringe with 0.5mL of feeder cells product to ensure all OKT3 was added to the bag.

[002134] Heat sealed Feeder Cells Bag #2 from the manifold leaving enough tubing to use for future welding. Sterile welded Feeder Cells Bag #2 to a media bag. Placed Feeder Cells Bag #2 on a balance and tare. Two media bags could be welded at once if available. Calculated the volume necessary to QS to a total volume of 2L. 2000 mL --volume of feeder cell suspension transferred.

[002135]

[002136] Opened all the clamps and transfer the calculated volume (assuming 1g =1mL). Clamped when the required volume had been transferred. When the media bag was empty, replaced by sterile welding a new media bag, if necessary.

[002137] Once the required volume had been transferred into Feeder Cells Bag #2, turned the feeder bag up and clear the line with air. Closed the clamps and heat sealed three times and break the middle seal leaving approximately 12 inches of tubing. This tubing was attached to the red line on the G-Rex 100 MCS flask.

[002138] Transferred Feeder Cells Bag #2 to the incubator. Recorded time placed in incubator. Prepared G-REX100MCS flask with feeder cell suspension. Recorded the number of G-Rex 100MCS flasks to process according to the number of G-Rex flasks generated on Day 0. Removed G-Rex flasks from incubator and recorded date and time of flask removal each time a flask was removed. Removed Feeder Cells bag #2 from incubator.

[002139] Removal of supernatant prior to feeder cell suspension addition. Transferred the G-Rex100 MCS to the BSC, and connected one 10 mL syringe to the blue capped NIS. Drew up 5 mL of media. Created five 1 mL aliquots. Stored the 5 aliquots (pre-process culture supernatant) for extended characterization at -20°C until requested by sponsor. Labeled vials with appropriate flask number. Continued seeding feeder cells into the G-Rex100 MCS. Repeated step for each G-Rex100 MCS flask.

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[002140] Seeding G-Rex 1: Verified all clamps on the G-Rex100MCS were closed except the clamp to the filter line. Worked with one GREX 100MCS at the time. Sterile welded Feeder Cells Bag #2 to the red line on the G-Rex 100MCS flask. Hung Feeder Cells Bag #2 on the IV pole and ensured regular mixing of the bag. Placed the G-Rex flask on the analytical balance and tare. Unclamped all lines and gravity transfer 500 mL of Feeder Cells Bag #2 by weight into the G-Rex 100MCS flask 1. Assume 1g = 1mL. Recorded the amount of feeder cell fraction added to each flask. Once the required volume was transferred to the G-Rex flask, closed the clamps on the tubing closer to the G-Rex to stop addition of feeder into the flask. Inverted the feeder bag and allow the feeder suspension in the tubing flow back to the feeder bag.

[002141] Labeled G-Rex100MCS (#1) Day 7-TIL Culture + Feeder Cells. Transferred the G-Rex100 MCS to the incubator and recorded date and time. Seeding G-Rex 2 -4: For an additional G-Rex 100MCS flask, verified all clamps are closed except the large filter line. Welded Feeder Cells Bag #2 to the red line. Repeated for seeding each flask as needed. Recorded the cell suspension volume added. Labeled G-Rex100MCS as Day 7-TIL Culture + Feeder Cells and up to 4 GREX100MCS flask. Recorded time.

PROCESS GEN 3 – DAY 11

Preparation for TIL processing

[002142] Recorded start date time of defined media (DM2) incubation. Media was warmed overnight (approximately 18hrs). Recorded end time and date of media incubation. Transfer TIL suspension from G-REX 100MCS to G-REX 500 MCS. Recorded processing initiation time. Removed the first G-Rex 100MCS flask from the incubator and transferred to the BSC. Checked that all clamps were closed except the large filter line. Ensured all luer locks were secure. Connected one 10mL syringe to the blue capped NIS and drew 7mL of preprocess culture supernatant

[002143]

Pre-process culture supernatant

[002144] Created seven 1 mL aliquots: 5mL for extended characterization and 2mL for sterility samples. This sample must be taken from each flask before the flask was mixed. Repeated for each flask.

Mycoplasma supernatant collection:

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[002145] Using a new syringe, removed the appropriate volume of supernatant from each flask using the blue capped NIS. See below for volumes to be removed according to the number of flasks. Transferred the supernatant to a 15mL conical tube labeled as D10/11 Mycoplasma Supernatant. A total of 10mL was removed. This sample must be taken from each flask before the flask was mixed. Retained 15mL conical tube in BSC until needed in section 4.

- 1 flask = 10mL
- 2 flasks = 5mL/flask
- 3 flasks = 3.3mL/flask
- 4 flasks = 2.5mL/flask

QC sample collection:

[002146] Mixed the flask carefully by swirling gently to bring the cells into suspension. Using a new syringe, removed the volume below according to the number of flasks to be processed and added to a 50mL conical tube. The samples drawn from each flask was kept separate and not pooled.

- 1 flask = 40mL
- 2 flasks = 20mL/flask
- 3 flasks = 13.3mL/flask
- 4 flasks = 10mL/flask

[002147] Labeled each conical tube Day 10/11 QC Sample Flask #. Stored in the incubator until needed in section 4. Repeated for each flask.

[002148] Stored the 5 aliquots (pre-process culture supernatant) from each flask for extended characterization at below 20°C until requested by sponsor.

[002149] Inoculated one anaerobic BacT/Alert bottle and one aerobic BacT/Alert bottle each with 1mL of pre-process culture supernatant previously collected for sterility testing for each flask sampled. Continued with the cell suspension transference into the G-Rex 500MCS.

[002150] Note: Subsequent steps could be performed in parallel for multiple G-Rex 100MCS flasks outside the BSC. Each G-Rex 100MCS was transferred into its own G-Rex500MCS with corresponding numbers (*e.g.*, G-Rex 100MCS #1 was transferred into G-Rex 500MCS #1).

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[002151] Sterile welded the clear cell harvest line on the G-Rex100MCS containing the IIL suspension to one of the input lines of a Y-type blood filter. Opened the G-Rex 500MCS in the BSC. Ensured all clamps except the large filter line are closed, then removed GREX 500MCS from the BSC and welded the terminal end of the filter to the red line on the G-Rex 500MCS. Heat sealed off the unused input line of the blood filter to prevent leaking. Inserted the clear line from the G-Rex 100MCS into the blue collection clamp on the GatheRex.Transferred cell suspension to the G-Rex 500MCS: Released all clamps leading to the G-Rex 500MCS. Transferred the cell suspension to the G-Rex500 MCS flask. When fluid transfer begun, elevated terminal end of blood filter (hold the blood filter vertically upside down) until chamber completely filled with fluid. After filter was completely primed it may be set horizontally on the bench. *Avoided tumor fragments being transferred out of the G-Rex100MCS as they had the potential to clog the line. Using the graduations on the flask, stopped the collection when the volume of the G-Rex 100MCS was reduced to 500ml by pressing the blue X. Gently swirled the flask to resuspend the cells in the media. If any tissue fragments were observed tilt the flask at a 45° angle and allowed them to settle opposite the collection straw. Resumed transfer of the cell suspension to the GRex 500MCS flask. Using the graduations on the flask stopped the collection when the volume of the G-Rex100MCS was reduced to between 300ml to 200 ml. Swirled the flask to resuspend the cells in the media. If any tissue fragments were observed tilt the flask at a 45° angle and allowed them to settle opposite the collection straw. Resumed transfer of the cell suspension to the G- Rex500 MCS flask. Using the graduations on the flask stopped the collection when the volume of the 100MCS was reduced to -100ml by pressing the blue X. Swirled the flask to resuspend the cells in the media. If any tissue fragments were observed tilt the flask at a 45° angle and allowed them to settle opposite the collection straw. Once settled slowly tilted the flask back toward the collection straw leaving the fragments opposite and allowing the media to pool around the straw. Maintained the tilt and resumed transfer of the cell suspension to the G-Rex500 MCS flask. GatheRex stopped when air entered the line. Inverted the blood filter (Y-portion up). Repeated until all fluid had been transferred from the filter and tubing to the 500MCS flask. When completed, closed all clamps and heat sealed and removed the G-Rex100MCS. G-Rex100MCS and filter assembly may be discarded.

[002152] Labeled the flask G-Rex500MCS #1 to #4: Repeated labeling step for each G-Rex 500 MCS. Transferred the G-Rex500MCS #1 to the incubator until use and continued with the next G-Rex100MCS collection. G-Rex 2-4: For the additional G-Rex 100MCS flask,

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repeated the same for transfer of TIL suspension from G-Rex100MCS to the G-Rex500MCS flask. Recorded incubator ID and time G-Rex 500MCS was placed in incubator.

Media addition

[002153] Note: The following steps could be performed in parallel for each G-Rex 500MCS flask. For each G-Rex 500MCS to be processed removed 4L of media from incubator. Welded each media bag to a leg of a 4S4M60 manifold. Ensured clamp on terminal end is closed. Removed the 500MCS flask from the incubator and marked at the 5L graduation. Welded the terminal end of the manifold to the red line of the 500MCS flask. Hung the 4 media bags and allowed media to transfer by gravity. Did not allow fill volume to exceed 5L.

[002154] Transferred GREX 500MCS to the incubator. Recorded time flask is placed in incubator in appropriate step below. Repeated step per each G-Rex500 MCS flask. Placed G-Rex 1 – 4 (and any additional) in the incubator at 37°C and 5% CO2. Recorded the Incubator ID and time. Recorded temperature and CO2 reading of the incubator after all flasks had been placed.

Prepared QC samples

[002155] Removed the conical tube(s) labeled D10/11 QC Sample Flask # from the incubator. Recorded the time removed. Using a 5mL pipette, mixed the sample well and transferred four 0.5mL aliquots to cryovials labelled 1-4 for counting. Repeated for each conical. After all samples for counting had been aliquoted from the conical tubes for each flask, pooled the volume into one new 50mL conical tube labeled 'D10/11 QC Sample pooled'. Using a pipette, mixed the pooled sample well and transferred four 0.5mL aliquots of the pooled sample to cryovials labeled 1-4 for counting. Used the Viability and Cell Count_lovance protocol on the NC-200. Using the NC-200, performed a cell count on sample 1. Be sure to indicate the dilution factor used in the NC-200. Recorded the viable (live) cell concentration and viability below. Repeated for samples 2, 3, and 4.

[002156] Flask 1 (if only 1 flask, then no pooled sample will be counted) Calculated the average of the four counts using the data recorded: (Flask 1 TIL 1 + Flask 1 TIL 2 + Flask 1 TIL 3 + Flask 1 TIL 4) / 4. Flask 2 Calculated the average of the four counts using the data recorded in step 4.7: (Flask 2 TIL i + Flask 2 TIL 2 + Flask 2 TIL 3 + Flask 2 TIL 4)/ 4. Flask 3 Calculated the average of the four counts using the data recorded in step 4.9: (Flask 3 TO_ 1 + Flask 3 TIL 2 + Flask 3 TIL 3 + Flask 3 TIL 4)/4. Flask 4 Calculated the average of

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the four counts using the data recorded in step 4.11: (Flask 3 TIL 1 + Flask 3 TIL 2 + Flask 3 TIL 3 + Flask 3 TIL 4) / 4.

[002157] Pooled Calculated the average of the four counts using the data recorded in step 4.13: (Pooled TIL 1 + Pooled TIL 2 + Pooled TIL 3 + Pooled TIL 4) / 4. Calculated the number of total viable cells from the pooled sample count. If only one flask, then use the Flask 1 count. Volume of cell suspension (40mL - total volume removed for counting) x average viable concentration. Calculated the volume needed for 1 x 10^6 cells to be removed for Mycoplasma testing. 1 x 10^6 cells/ average viable concentration (step 4.14 or 4.6). Removed the calculated volume and place in the tube labelled D10/11 Mycoplasma Supernatant. Marked tube to indicate 1 x 10^6 cells have been added. Calculated the total viable cells remaining in D10/11 QC Sample tube. TVC: 1 x 10^6 cells. Calculated the volume needed of the cell fraction (CF) for cryopreservation at a concentration 10x10^6 cells/mL. Calculated the number of vials to prepare. Volume of CS10 was equal to the number of vials to formulate. The final volume was 1mL of CS10 in each vial. Centrifuged conical tube labeled D10/11 QC Sample pooled tube at 350 G for 5 min at 20°C.

[002158] Discarded supernatant. Added an appropriate amount of CS10 as calculated. Each tube final concentration 10 x 10^6 cells/mL. Aliquoted the volume into 1.8ml cryovials; 1mL per cryovial. Labeled vials as D10/11 Retain. Once aliquoted, placed in the -80°C freezer in a Mr. Frosty or equivalent. Recorded ending time of processing.

IL-2 PROLEUKIN ALIQUOTS PREPARATION

[002159] Preparation of 1% HAS in PlasmaLyte A. Added 16mL of 25% HSA stock solution to 384 mL of PlasmaLyte A into in a sterile filter unit. Recorded volumes below.

Table 51: Volumes

Reagents	Volume Required
25% HAS	**16 mL**
PlasmaLyte A	**384 mL**
Total Volume	**400 mL**

[002160] Note: The above volume was enough to prepare one IL-2 vial at final concentration of 6x10^4 IU/mL. Filtered the media through a 0.22 µm filter unit. Labeled as 1% HSA in PlasmaLyte A.

Preparation of rhIL-2 stock solution

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[002161] Prepared rhIL-2 stock solution (6 x 10^2 IU/mL final concentration) in 1% HAS in PlasmaLyte A. Attached an 18G needle to a 3mL syringe and drew up 1.2mL of WFI. Injected into vial of IL-2. Did not remove syringe from vial.

[002162] Inverted the vial 2-3 times and swirled until all powder is dissolved. Did not shake or vortex to prevent foaming. Without removing the syringe from the vial, drew out and measured the solution from the vial and place in a 500mL sterile bottle.

[002163] Calculated volume of 1% HSA diluent required. Note: Per manufacturer instructions after reconstituting with 1.2 mL of WFI, each vial contained 18 x 10^4 IU/mL.

[002164] Labeled the 500mL sterile bottle IL-2 working stock 6x10^4 IU/mL. Transferred the calculated amount of 1% HSA into the 500mL sterile bottle to which the reconstituted IL-2 was already added. Mixed well. Transferred an appropriate amount to a sterile specimen cup if necessary for ease of aliquoting. Labeled as IL-2 working stock 6x10^4 IU/mL.

[002165] Aliquoted the reconstituted IL-2 from IL-2 working stock 6 x 10^4 IU/mL in 1mL aliquots into labeled tubes. Labeled the tubes as Proleukin IL-2, 6 x 10^4 IU/mL and stored at -80°C. After aliquoting is complete, recorded the number of 1 mL aliquots prepared.

PROCESS GEN 3 – DAY 16/17

[002166] Verified Day 10/11 preliminary sterility results. Wash buffer preparation (1% HAS Plasmalyte A). Recorded processing start time. Identified a 5L labtainer: "Plasmalyte 1% HSA wash buffer".

[002167] Transferred HSA and Plasmalyte to 5L bag to make LOVO wash buffer: Using luer connections, aseptically attached an extension set to the 5L labtainer. Spiked each HSA bottle with a mini-spike. Using appropriate sized syringes transferred a total volume of 125 mL of 25% HSA to the 5L bag via the extension line. Closed all clamps on a 4S-4M60 Connector set. Spiked each of the PlasmaLyte bags. Welded one of the male ends of the 4S-4M60 to the inlet line of the Acacia pump boot. Welded one of the sides of the pump boot to a 5L Labtainer. Closed all clamps on 5L bag except the line to pump. Hung the PlasmaLyte bags and pumped entire volume into 5L Labtainer.

[002168] Heat sealed the 4S4M60 manifold line attached to the bag retaining the luer connection to the 5L Labtainer bag. Mixed the bag and keep the LOVO wash buffer bag at

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Room Temperature. Labeled as LOVO Wash buffer with date. Expired within 24 hours at ambient temperature.

[002169] Preparation of a blank bag for cryopreservation: Connected a syringe to the LOVO wash buffer and removed 50 mL of LOVO wash buffer. Transferred into a CS750 bag and used the syringe to remove any air in the bag. Attached red cap to line. Labeled CS750 bag with "blank containing LOVO wash buffer", batch record lot number and initial/date.

IL-2 preparation (Proleukin)

[002170] When IL-2 was prepared in advance: Mixed the LOVO wash buffer bag and using an appropriately sized syringe, removed and transferred 40mL of wash buffer in the "IL-2 6 x 10^4 IU/mL" tube. Calculated for Volume of reconstituted IL-2 to add to Plasmalyte+ 1% HSA: volume of reconstituted IL-2 = (Final concentration of IL-2 x Final volume)/ specific activity. Specific Activity recorded. Final Concentration of 1L-2: 6)(10^4 IU/mL. Final Volume: 40mL Removed quantity of WFI recorded as "Recommended Reconstitution Volume" and fixed an 18G needle on the syringe and re-suspended the IL-2 in the vial. Using a 1 mL syringe connected to a 18G needle removed calculated initial volume of IL-2 needed from reconstituted IL-2 and transferred to the "IL-2 6x10^4 IU/mL" tube.

Cell collection

[002171] Recorded the number of G-Rex 500 flasks that will be processed. Recorded how many flasks were harvested. One 5L Labtainer was needed per flask to be harvested. When more than two flasks are to be harvested, two EV3000N bags were used to harvest cells. Indicated the number of primary EV3000N bags used to collect the cells. Prepared number of EV3000N bags as needed and labeled as Cell Collection Pool #. Closed all clamps of 5L bags and attached extension set to each labtainer bag. Heat sealed the extension set at the end. Labeled the bag as Supernatant bag Flask #. Repeat for each flask to be harvested.

[002172]

[002173] Removed the G-Rex 500MCS flask from the incubator and place it on the benchtop next to the GatheRex. Checked that all clamps are closed, except large filter line. Ensured all luer locks are secure. If 2 or more 5L waste Labtainers bags were to be used a second GatheRex pump can be used concurrently. While the first G-Rex 500M-CS was being volume reduced, the next flask could be prepared for volume reduction. Sterile welded the red media line from the G-Rex 500M-CS flask to the appropriate Supernatant Bag previously prepared. Placed red line into the GatheRex pump in the red slots and connected the

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GatheRex line to the filter line on the G-Rex. Sterile welded the clear collection line of the first G-Rex 500M-CS flask to the Cell Collection Pool EV3000 bag then placed the clear line in the blue slots on the GatheRex. Removed 4500 mL of supernatant from the first G-Rex 500M-CS. When supernatant was removed, placed 'Cell Collection Pool' bag on top of the supernatant bag. Swirled the G-Rex flask to detach the cells from the membrane. Maintained the edge tilted during the next step.

[002174]

[002175] Released the clamps leading to the Cell Collection Pool bag. Started the GatheRex to collect the Cell Fraction. Pushed the Blue button on the GatheRex Gently agitated the G-Rex while collecting the cell suspension to keep cells in suspension. When the cell collection stoped, closed the clamps. Released all the clamps and heat sealed: cleared line of the G-Rex flask; red line of the G-Rex flask. Repeated as needed until all G-Rex 500MCS flasks were volume reduced and harvested.

[002176] QC sampling (including fro mycoplasma testing): labeled one appropriately sized container as mycoplasma pool sample. Sterile welded an extension set to the each "Supernatant" bag. Using 60mL syringes, removed maximum 60mL of supernatant from the total "Supernatant bags" following the table below to have the most representative sample. If more than one flask was harvested collect every supernatant in the mycoplasma pool container. Dispensed 10mL from supernatant pool in each Supernatant 15mL tube. Kept at 2-8°C (Mycoplasma and Mycoplasma reference). Transferred "Supernatant-Mycoplasma" and "Supernatant-Mycoplasma Reference" tubes to QC.

[002177] Supernatant collection for characterization. From each supernatant bag, removed 5 mL and aliquot in lmL for extended characterization. Stored the 5 aliquots for extended characterization at 5-20°C until requested by sponsor. Characterization samples were mixed between supernatant flask collected. After all samples were collected, supernatant bags were discarded.

[002178] Labeled an EV3000N bag "LOVO source bag" and ceat sealed closed the LOVO source bag (new EV3000 bag) and remove the connection. Opened a y-type blood filter in the BSC and closed all clamps. Sterile welded the outlet tubing of the filter to the LOVO source bag. Sterile welded each inlet tubing of the filter to each Cell Fraction (CF) Pool bag(s). Hung the CF Pool bag(s) for filtration. Opened all clamps and allowed the cells

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to drain by gravity through the blood filter to the LOVO source bag. Primed the filter by holding it vertically. Prevent cells from clotting at the bottom of the bag.

[002179] Once all cells were transferred to the LOVO source bag closed all clamps. Heat sealed to keep the same length of tubing as previously (use the mark for guide). Weighed the full LOVO source bag containing the cell suspension. Calculated the cell fraction volume (CF): (Considered lg was equal to 1 Ml) Weight of LOVO source bag - Dry weight. Mixed the LOVO source bag well. Using a 10mL syringe, homogenized and removed 1 mL of cell fraction via the NIS and transfer to the first cryovial. Repeated this step three times using a new 10 mL syringe for the three remaining cryovials. Placed the LOVO Source Bag in the incubator. Calculated the remaining volume of the cell fraction in the LOVO source bag.

[002180] Performed the cell counts with automated cell counter. Prepared four 15mL conical tubes with 4.5mL of AIM-V. These could be prepared in advance. Optimal range for NC200 was between $5x10^4$ and $5x10^6$ cells/mL. (1:10 dilution is recommended). For 1:10 dilution, to 4500pL of AIM V prepared previously, added 500pL of CF. If different dilution was required to achieve optimal range, note dilution used. Dilution Factor used = 10. Using the NC-200, performed a cell count on sample 1. Be sure to indicate the dilution factor used in the NC-200. Recorded the viable (live) cell concentration and viability below. Repeated for samples 2, 3, and 4.

[002181] Calculated the average of the four counts: (TIL01+ TIL02 + TIL03 + TIL04) / 4; Average Total Viable Cell concentration (live); Average Total Cells concentration (live + dead); Average % Viability. Calculated the TC (Total Cells) pre-LOVO (live + dead) = Average Total Cell Concentration (TC conc pre LOVO) (live +dead) (step 4.44) x Volume of LOVO Source Bag. Calculated the TVC (Total Viable Cells) pre-LOVO (live) = Average Total Viable Cell Concentration (pre LOVO) (live)(step 4.44) x Volume of LOVO Source Bag.

[002182] In some embodiments: If the TC was > 5×10^9, removed 5×10^8 cells to be cryopreserved as MDA retention samples. 5×10^8 / avg TC concentration (step 4.44) = volume to remove. If the TC was below 5×10^9, removed 4×10^6 cells to be cryopreserved as MDA retention samples. 4×10^6 / avg TC concentration = volume to remove. Retained in incubator until cryopreservation steps.

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[002183] Determined if cell removal was necessary before loading the LOVO. Is TVC more than $150x10^9$ cells? (YES/NO). If YES, continued. If NO, cell removal would not be required. Calculated the number of cells to remove to retain $150x10^9$ viable cells: TVC pre-LOVO (see step 4.45) -5 x 10^8 or 4 x 10^6 or N/A (see step 4.46)-$150x10^9$ cells. Calculated the volume of cells to remove: Number of cells to remove (see step 4.48) ÷ Average Viable Cell concentration. Removed the calculated volume of cell suspension and discarded the cells in the waste container. Calculated the remaining volume of the cell fraction contained in the Lovo Source Bag. Calculated the remaining Total Cells Remaining in Bag. Calculated the TC (Total Cells) pre-LOVO. Avg. Total cell concentration (See step 14.44) X Remaining Volume (See Step 4.52) = TC pre-LOVO Remaining. Placed the LOVO Source Bag in the incubator, if required. Recorded the incubator number and the time placed in the Incubator. Proceeded to LOVO harvest and LOVO calibration, if necessary, following instructions in LOVO manual. LOVO end of run. Followed instructions until the end of LOVO run. Determined the amount of CS10 bags needed based on the chosen profile. Note: An additional 50mL of CS10 is required to prepare the blank bag.

[002184] Determined the amount of CS750 freezing bags needed. Closed all clamps of the CS750bags near the junctions. Identified each CS750 cryobags: Labeled the final product bags as instructed in the following step.Inserted the bag label of each DP bag into the "pouch" on the top of the bag. Seal three times on the open part of the pouch to keep the label in place. For each DP bag, sealed one of the tubing just under the luer and remove the clamp. Sterile welded the "spike" extremities of the CC3 to the CS10 bags (keeping the claims of the CC3 manifold). Kept the unused "spike" v of the CC3. Sterile welded the "luer" extremities of the CC3 to the CS750 bags (keeping the claims of the CC3 manifold).

[002185] Once all the assembly was done, sealed the unused tubing on each CS750 cryobag, near the junctions. If necessary, the assembly could be put into a ziplock bag and then into the fridge until FCF post LOVO bag was disconnected from LOVO kit. All the manifold assembly with CS10 and CS750 bags previously labeled can be done in advance and stored at the fridge until use. Drew up 50 mL of CS10 using appropriate size syringe. Connected the syringe to the CS750 bag blank containing LOVO wash buffer (previously prepared in section 8) and injected 50mL of CS10. Triple heat sealed tubing below spike port level. Cut on the second sealing point. Relabeled CS750 bag with "blank containing LOVO wash buffer and CS10 lot#, initial/date. Held the blank bag on cold pack until placed in the CRF.

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IL-2 addition

[002186] Chose the volume of IL-2 to add corresponding to the used process. Volume calculated as: Retentate Volume x 2 x 300 IU/mL – IU of 1L-2 required. IU of 1L-2 required/ $6x10^4$ IU/mL= Volume of 1L-2 to add to FCF Post LOVO bag. If using IL-2 tube prepared in steps 3.2 to 3.6: Connected an 18G needle on a 3mL syringe. Drew up the volume of IL-2 from the IL-2 $6x10^4$ IU/mL conical tube prepared. If using previously prepared IL-2 aliquots: Using a proper syringe and needle drew the calculated volume from the aliquots tubes labeled as IL-2 $6x10^4$ IU/mL. Removed the needle and transfer the IL-2 into the FCF Post LOVO bag via the NIS on the post LOVO bag.

[002187] Cleared the line with air. Ensure all IL-2 was added and none remains in the line. Once the 1L-2 had been added to the post LOVO bag, attached the FCF Post LOVO bag to the CC3 by welding to one the remaining spike tubing connector in diagram. Kept the clamp of the CC3 manifold.

Final formulation.

[002188] Labeled a 50mL tube "FCF Retain". Prepared labels for Endotoxin reference, D16 or 17 IFN-γ Retain, D16 or 17 QC testing, and MDA retention, if applicable. Placed the DP bags, Post Lovo bag+IL-2 and the CS10 bags on cold packs.

[002189] Connected a 100mL syringe to the CC3 manifold. Opened the clamps leading to the CS10 bags. Drew up the volume of CS10 determined. Double checked the volume to be added. Dispensed the volume of CS10 to the FCF Post LOVO bag. Recorded the volume added. Cleared the line. Mixed gently. Recorded the CS10 time addition.

[002190] Selected the FCF volume that would be added per DP bag, according to the used profile, as described. Marked the retain volume to remove per DP bag corresponding to the used profile, as described.

[002191] DP bag #1: manipulated a single bag at a time. Be careful to measure all volumes correctly by orienting the syringe with stopcock facing down towards the bench.

[002192] FCF volume: removed the syringe and replaced it by a new one. Mixed the cell product well. Drew the appropriate volume of FCF product according to the used process. Injected it in DP bag #1. Recorded the FCF volume added.

[002193] Retain volume: mixed the cell product well. From DP bag #1, drew the air present in the DP bag #1. Drew the appropriate volume of retain according to used process.

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Inverted the syringe and clear the line with air. Closed DP bag clamp. Injected the retain volume in the retain 50 mL tube Recorded the retain volume added. If necessary, used an additional syringe to remove air clear the line.

[002194] DP bag #2 - #4: Repeated for DP bag #2. FCF volume: Recorded the FCF volume added. Retain volume: Recorded the retain volume removed. Drew all remaining volume of FCF Post LOVO bag with a 10mL syringe and transferred into tube labelled FCF Post LOVO Bag.

[002195] Using hemostats, clamped the lines of all DP bags. Took all the assembly out of the BSC. Placed the cryobag in horizontal plane. Triple heat sealed tubing below spike port level. Cut on the second sealing point. Disconnected tubing from cryobag. Repeated these steps for each cryobag to seal.

[002196] Proceeded to a visual inspection of each DP bag. Checked if: batch number on labels matched with ID batch; if the bag was free of visible leaks; if the bag was free of unusual big cell clumps; and if the bag was free of unusual observations. After inspection, put each DP bag into a cassette and on cold packs or at 2-8°C. Ensured gauze had been placed to cover ports prior to closing cassette.

Preparation of MDA retention vials.

[002197] Centrifuged the tube labelled 'MDA Retention' at 400 x g for 5 min at 20°C with full brake and full acceleration. In the BSC, aspirated supernatant. Gently tapped the bottom of the tube to resuspend cells in the remaining fluid. Set aside the tube for later addition of CS10 media.

Cell cryopreservation

[002198] Disinfected the controlled rate freezer (CRF) chamber with 70% IPA. Prepared and filled Cryovials per Table 52 below:

Sample ID	# and volume
Reference Vials*	8 x 0.5mL
Endotoxin Retain*	2 x 0.12mL
IFN Assay Retain*	7 x 0.5mL
Satellite Vials**	≤20 x 0.5mL

*Samples were taken from tube labelled FCF Retain. **Satellite vials were prepared from the product in the tube labelled FCF Post LOVO Bag.

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[002199] After samples were aliquoted as specified in previous step, the tube labelled FCF Retain was saved for further sampling. DID NOT DISCARD FCF Retain.

[002200] Aliquoted MDA Retention Vials: If applicable, added appropriate volume of 0810 media to the MDA retention tube and aliquot into 5 labeled MDA retention cryovials. Recorded Time. Recorded chamber temperature and record sample temperature. Waited for the sample temperature to reach 8±1°C AND waited the chamber temperature to reach 4±1°C to press on Run again (a light appears beside RUN and the light beside WAIT turns off). Recorded starting time. After the start of the run, operator had to check that the profile continues to step 2 (20°C/min C to -45°C). Time of CS10 addition. Calculated elapsed time. When the freezing profile was completed (freezing run takes about 1h30), transferred immediately the DP and cryovials to the LN2 tank. Recorded ending time of CRF run.

[002201] Fixed an 18G needle on a 10 mL syringe and drew 5.4mL of Retain. Injected 2.5mL first into the anaerobic BacTAlert bottle and then injected 2.5mL into the aerobic BacTAlert bottle. Transferred to QC for Microbiology ordering. One anaerobic and one aerobic BacTAlert bottles was inoculated. Recorded inoculation time of BacTAlert bottles.

[002202] Aliquoted the following samples for QC Testing and transfer to QC: Cell count (4x 0.5 mL vials); FCF Retain tube (remainder saved for applicable QC testing). With a 5mL pipette, drew 2mL of Retain and dispense 0.5mL into 4 cryovials for cell count and viability testing. Note: Retain samples were kept at 2-8°C. Prepared dilutions for the FCF samples to be counted. (1:100 dilution is recommended) Optimal range for NC200 was between $5x10^4$ and $5x10^6$ cells/mL. Indicated the dilution factor used in the NC-200. Recorded the viable (live) cell concentration and viability below. Repeated for all samples. Calculated the average of the four counts: (TIL01+ TIL02 + TIL03 + TIL04) ÷ 4. Average Total Viable Cell concentration (live). Average Total Cell concentration (live + dead). Average % Viability. Calculated the TVC (Total Viable Cells) FCF (live) = Average Total Viable Cell Concentration (FCF) (live) x Volume of FCF (# of bags x volume of bags).

MEASUREMENT OF IFN-γ SECRETION BY TIL POPULATION DURING CURRENT PROCESS GEN 3

[002203] IFN- γ secretion by TILs during current Process Gen 3 at Day 11 and Day 17. The following table shows the measurement results of IFN- γ secretion by TIL populations at various days during current Process Gen 3.

Table 53: Measurements

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Lot Number	Process Day	Result
W329020208240	D17	18051.61 pg/mL
	D11	20377.25 pg/mL

EXAMPLE 10: UPDATED PROCESS GEN 3 FROM EXAMPLE 9

[002204] This example herein describes an updated Process Gen 3 (Updated Process Gen 3) that contains the same steps as in Example 9, subject to the exceptions/changes as follows:

[002205] Example 9: The media formula of (Defined Media type 1 (DM1) did not contain additional antioxidant or reducing reagents.

- Updated Process Gen 3: Addition of B-Mercaptoethanol to Defined Media type 1 (DM1) to a final concentration of 55uM, for example, by adding 1 mL of B-ME (stock 55mM) to 1 L of DM1 media.

[002206] Example 9: Only 3 bags for DM1 and up to 16 bags for DM2 was made for a single cycle of process Gen 3.

- Updated Process: Any number of bags can be made for DM1 and/or DM2 for one cycle of Updated Process Gen 3.

[002207] Example 9: Created aliquot in 15mL conical tube.

- Updated Process Gen 3: Create aliquot in 50mL conical tube.

[002208] Example 9: Used only EV3000 bags for feeder preparation.

- Updated Process Gen 3: Charter Medical bags can also be used for feeder cell preparation.

[002209] Example 9: Used a CC3 for feeder cell harness.

- Updated Process Gen 3: Alternative supplies (CC1, CC2, charter pooling harness set etc.) can also be used for feeder cell harness.

[002210] Example 9: Total viable feeder cells needed $1x10^9$.

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- Updated Process Gen 3: Total viable feeder cells needed $1.25x10^9$.

[002211] Example 9: Final volume of OKT-3 needed was 0.6mL.

- Updated Process Gen 3: Final volume of OKT3 needed is 0.75 mL.

[002212] Example 9: Final volume on the Feeder cell bag #2 was 2000 mL.

- Updated Process Gen 3: Final volume on the Feeder cell bag #2 is 2500 mL ± 25mL.

[002213] Example 9: Incoming tumor was washed three times to remove potential contaminants. The washes were performed in a series of three 100 mm Petri dishes, each containing 50 mL of wash buffer. The tumor was transferred from one wash dish to the next with a 4.5" forceps. Each wash was for ⩾3 minutes.

- Updated Process Gen 3: Perform tumor washes in three individual 100-150 mL bottles, each containing 50-100 mL of wash buffer. The tumor is removed from the tumor shipping container using a sterile 8" forceps and transferred to the first wash bottle. The bottle will be closed and gently swirled to agitate the tumor. The tumor will remain in the wash bottle for 3-5 minutes. Following the first wash, the tumor is moved with new sterile 8" forceps to the second wash bottle and the wash steps are repeated. A third wash is completed in the same manner as described above.

[002214] Example 9: Addition of 20mL to Lid Drops wash medium tube.

- Updated Process Gen 3: Addition of 30mL to Lid Drops wash medium tube.

[002215] Example 9: The maximum number of final fragments allowed was 240.

- Updated Process Gen 3: Reduce the maximum total number of final fragments to 200.

[002216] Example 9: The maximum number of flasks allowed was 4 on Day 0, and a total number of 240 final fragments was divided into 4 flasks.

- Updated Process Gen 3: A maximum of 5 flasks on Day 0, and a total number of 200 final fragment will be divided into 5 flasks.

[002217] Example 9: The maximum number of fragments per flask was 60: Option A = Fragments ⩽30 = Flask to seed 1; Option B = 31⩽ fragments ⩾60 = Flask to seed 2; Option C = 61⩽ fragments ⩾90 = Flask to seed 3; Option D = 91⩽ fragments ⩾240 = Flask to seed 4.

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- Updated Process Gen 3: The maximum number of fragments per flask is 40: Option A = Fragments ≤30 = Flask to seed 1; Option B = 31≤ fragments ≥50 = Flask to seed 2; Option C = 51≤ fragments ≥75 = Flask to seed 3; Option D = 76≤ fragments ≥ 100 = Flask to seed 4; Option E = 101≤ fragments ≥200 = Flask to seed 5.

[002218] Example 9: Four 50mL conical tubes were labeled for Fragments tubes.

- Updated Process Gen 3: Label five 50mL conical tubes for Fragments tubes.

[002219] Example 9: 4 petri dish were labeled as Dissection 1 through Dissection 4.

- Updated Process Gen 3: Label 5 petri dish as Dissection 1 through Dissection 5.

[002220] Example 9: in each lid of the petri dishes labeled Dissection 1 through Dissection 4, 10-20 drops (about 1mL) were added to form a pool of wash buffer close to the edge of the plate to hold dissected fragments. The process was repeated for each intermediate fragment or group to be dissected.

- Updated Process Gen 3: In each lid of the petri dishes labeled Dissection 1 through Dissection 5, add about 20-30 drops to form 4 equal sized pools around edge of plate so operator can count 10 fragments in each group up to 40 pieces.

[002221] Example 9: Up to 4 check boxes per total number of fragments in Dissection 1 through Dissection 4.

- Updated Process Gen 3: Up to 5 check boxes per total number of fragments in Dissection 1 through Dissection 5.

[002222] Example 9: No record of final fragments count after additional fragments were added per tube.

- Updated Process Gen 3: Add a row on the second table to capture the total number of fragments per tube without counting the floaters, and then the Total number of fragments counting all of the flasks, without including the floaters.

[002223] Example 9: Addition of 500 ± 2mL to each flask.

- Updated Process Gen 3: Addition of 500 ± 10mL to each flask.

[002224] Example 9: Record end time and date of media and warm packs incubation.

- Updated Process Gen 3: Record start date and time of defined media 1 and warm pack incubation.

[002225] Example 9: Up to 4 GREX 100 flask can be used.

- Updated Process Gen 3: Up to 5 GREX 100 flask can be used.

[002226] Example 9: 5 mL for extended characterization needed per flask and aliquoted on 5 individual tubes.

- Updated Process Gen 3: 3 mL for extended characterization needed per flask and aliquoted on 3 individual tubes.

[002227] Example 9: Up to 4 flask sterility sample check box.

- Updated Process Gen 3: Up to 5 flask sterility sample check box.

[002228] Example 9: Transfer Cell suspension from GREX100 to GREX500 (up to 4 flasks).

- Updated Process Gen 3: Transfer Cell suspension from GREX100 to GREX500 (up to 5 flasks).

[002229] Example 9: Cell count sample was taken individually from each flask at the beginning of the Process using the NIS on the GREX100. Once the cell count sample have been taken, the entire cell suspension is transferred from the GREX100 to the GREX 500 flask using the Gatherex.

- Updated Process Gen 3: 1) Volume reduce 100MCS using the c to ~500 mL without disturbing the cells transferring the supernatant to an appropriate Labtainer bag. 2) Disconnect flask from Gatherex and transfer to the BSC. 3) Inside the BSC, mix the flask carefully by swirling the residual ensuring homogeneous suspension and take cell count samples using NIP (4x1 mL). 4) Weigh the G-Rex using the balance and record. 5) Transfer the cell suspension to the final GREX500 flask using GatheRex. 6) Backwash the GREX100 flask with the spent media collected and then transfer the spent media the GREX500 flask. 7) add the DM2 media into the GREX500 up to 5 L. 8) Weigh empty G-Rex and calculate volume of transferred suspension by weight difference.

[002230] Example 9: Samples taken up to 4 flasks:
1 flask = 10mL

  - 2 flasks = 5mL/flask
  - 3 flasks = 3.3mL/flask

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- 4 flasks = 2.5mL/flask

- Updated Process Gen 3: "Samples taken up to 5 flasks:
  1 flask = 12mL
  - 2 flasks = 6mL/flask
  - 3 flasks = 4mL/flask
  - 4 flasks = 3mL/flask
    5 flasks = 2.4mL/flask

[002231] Example 9: 5 mL for extended characterization needed per flask and aliquoted on 5 individual tubes.

- Updated Process Gen 3: 3 mL for extended characterization needed per flask and aliquoted on 3 individual tubes.

[002232] Example 9: BacT test up to 4 flasks.

- Updated Process Gen 3: BacT test up to 5 flasks.

[002233] Example 9: Media Addition up to 4 flasks.

- Updated Process Gen 3: Media Addition up to 5 flasks.

[002234] Example 9: Welded each media bag to a leg of a 4S4M60 manifold.

- Updated Process Gen 3: Alternative supplies to 4S4M60 can also be used.

[002235] Example 9: N/A.

- Updated Process Gen 3: Remove date from when flasks are placed into incubators, it is unnecessary as the processing date is recorded on the front page. SM 090919.

[002236] Example 9: No instruction provided for excess cells.

- Updated Process Gen 3: 1) Change required samples to 1 x 10^6 cells for myco, 5 x 10^6 cells for flow and 1 x 10^6 cells for restimulation. 2) contact management if the TVC is less than the necessary 16 x 10^6 cells. 3) Add instructions to freeze down the excess cells, allowing creating aliquots at a concentration of approximately 10^6 cells/mL for excess. Divide TVC by 10^6 for volume of CS10 to use. Round down to the nearest mL so the cells per vial will be always more than 10^6 cells.

[002237] Example 9: Used only EV3000 bags for feeder cell preparation.

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- Updated Process Gen 3: Charter Medical bags can also be used for feeder cell preparation.

[002238] Example 9: No instruction for reporting the final TVC value.

- Update Process Gen 3: On Day 10/11 the final TVC value reported on the COA will be the pooled cell count sample.

[002239] Example 9: Total viable feeder cells needed was 2 x 10^9.

- Updated Process Gen 3: Total viable feeder cells needed is 2.5 x 10^9.

[002240] Example 9: The number of feeder cell to remove from the Feeder cell bag #1 to feeder cell bag #2 was 2 x 10^9cells.

- Updated Process Gen 3: The number of feeder cell to remove from the Feeder cell bag #1 to feeder cell bag #2 is 2.5 x 10^9.

[002241] Example 9: Volume of OKT-3 needed was 1.2 mL total of 30ng/mL.

- Updated Process Gen 3: Volume of OKT-3 needed is 1.5 mL total of 30ng/mL.

[002242] Example 9: Total volume to QS was 2.0L

- Updated Process Gen 3: Total volume to QS is 2.5L.

[002243] Example 9: Maximum 4 GREX 100 flask allowed.

- Updated Process Gen 3: Maximum 5 GREX 100 flask allowed.

[002244] Example 9: 5 mL for extended characterization needed per flask and aliquoted on 5 individual tubes.

- Updated Process Gen 3: 3 mL for extended characterization needed per flask and aliquoted on 3 individual tubes.

[002245] Example 9: No alternative supplies were used for 4S4M69 and CC3.

- Updated Process Gen 3: Alternative supplies to 4S4M69 and CC3 can be used.

[002246] Example 9: EV3000 was used as source bag.

- Updated Process Gen 3: Add as optional Charter medical EXpak5L bag if a large volume of cell suspension is collected.

[002247] Example 9: 5L Labtainer was used for spent media collection.

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- Updated Process Gen 3: Add as optional a 10L labtainer bag for spent media collection if 4 or more flasks are harvested.

[002248] Example 9: Up to 4 flasks were harvested.

- Updated Process Gen 3: Up to 5 flasks are harvested.

[002249] Example 9: Up to 4 flasks were harvested for mycoplasma sample.

- Updated Process Gen 3: Up to 5 flasks are harvested for mycoplasma sample.

[002250] Example 9: 5 mL for extended characterization needed per flask and aliquoted on 5 individual tubes.

- Updated Process Gen 3: 3 mL for extended characterization needed per flask and aliquoted on 3 individual tubes.

[002251] Example 9: There is no instruction to record time when the pre LOVO bag was placed and removed from the incubator.

- Updated Process Gen 3: Record time when the pre LOVO bag is placed and removed from the incubator.

[002252] Example 9: No instructions to record time for LOVO processing.

- Updated Process Gen 3: Record time of LOVO initiation and finalization.

[002253] Example 9: No instructions on the temperature for placing the post LOVO bag when final formulation could not be initiated immediately.

- Updated Process Gen 3: If final formulation cannot be initiated immediately upon LOVO completion, post LOVO bag should be keep at 2-8 degrees and contact Iovance team immediately if the time exceeds 15 min.

[002254] Example 9: Recorded Washout % (1 decimal).

- Updated Process Gen 3: Record Washout % (4 decimal).

[002255] Example 9: Retained QC sample was taken individually from final DP bag and then pooled into a 50mL canonical tube.

- Updated Process Gen 3: Retain QC sample will be taken from the formulation bag (post-LOVO bag) directly after each DP bag is filled with the required volume. The retain volume can be taken with an appropriate sized syringe and then is transferred to a 50 mL conical tube.

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[002256] Example 9: 1:10 dilution was prepared using 500uL sample and 4500uL AIM-V.

- Updated Process Gen 3: Remove specification for 500uL sample and 4500uL AIM-V.

EXAMPLE 11: SECOND GENERATION PROCESS GEN 3

A PROPOSED STUDY TO USE FEEDER CELLS SOUP TO ACTIVATE TIL-GEN 3 – 1ST PHASE AND 2ND PHASE.

[002257] The example provides an embodiment to test a process employing culturing feeders culture in a separate container, then using the culture media (cell culture supernatant) of feeder culture to activate the TILs.

[002258] This method will allow for a more definitive measurement of TIL production on the scale-up day and at harvest, without the feeder background at cell collection. Such a method is described herein and show in for example, Figure 1E-1G.

[002259] This method will also allow for estimating in advance if the lot will be successful at harvest. The final cell count will be only from TIL without the feeders background.

[002260] This method will also allow for a potential increase in viability on Scale-up day and potentially reduce the risk of scheduling lymphodepletion.

[002261] Overall this embodiment of the process can allow for reduced and/or no feeder background.

GEN 3 REP DIAGRAM

[002262] Figure 21 illustrates embodiments of a second generation Gen 3 process.

[002263] Figures 1E-1G provide flowchart overviews of the second generation Gen 3 process.

EXAMPLE 12: EXEMPLARY PRODUCTION OF A CRYOPRESERVED TIL CELL THERAPY

[002264] This example describes an exemplarty cGMP manufacture of TIL Cell Therapy Process in G-Rex Flasks according to current Good Tissue Practices and current Good Manufacturing Practices.

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Table 54. Process Expansion Examplary Plan

Estimated Day (post-seed)	Activity	Target Criteria	Anticipated Vessels	Estimated Total Volume (mL)
0	Tumor Dissection	≤ 50 desirable tumor fragments per G-Rex100MCS	G-Rex100MCS 1 flask	≤1000
11	REP Seed	5 – 200 x 10^6 viable cells per G-Rex500MCS	G-Rex500MCS 1 flasks	≤5000
16	REP Split	1 x 10^9 viable cells per G-Rex500MCS	G-Rex500MCS ≤5 flasks	≤25000
22	Harvest	Total available cells	3-4 CS-750 bags	≤530

Table 55. Flask Volumes

Flask Type	Working Volume/Flask (mL)
G-Rex100MCS	1000
G-Rex500MCS	5000

PROCESS INFORMATION PRIMARY

Day 0 CM1 Media Preparation

[002265] In the BSC added reagents to RPMI 1640 Media bottle. Added the following reagents t Added per bottle: Heat Inactivated Human AB Serum (100.0 mL); GlutaMax (10.0 mL); Gentamicin sulfate, 50 mg/mL (1.0 mL); 2-mercaptoethanol (1.0 mL). Removed unnecessary materials from BSC. Passed out media reagents from BSC, left Gentamicin Sulfate and HBSS in BSC for Formulated Wash Media preparation. Thawed IL-2 aliquot. Thawed one 1.1 mL IL-2 aliquot (6x10^6 IU/mL) (BR71424) until all ice had melted.

[002266] Transferred IL-2 stock solution to media. In the BSC, transferred 1.0 mL of IL-2 stock solution to the CM1 Day 0 Media Bottle prepared. Added CM1 Day 0 Media 1 bottle and IL-2 (6x10^6 IU/mL) 1.0 mL. Passed G-Rex100MCS into BSC. Aseptically passed G-Rex100MCS (W3013130) into the BSC. Pumped all Complete CM1 Day 0 Media into G-Rex100MCS flask. Tissue Fragments Conical or GRex100MCS.

Day 0 Tumor Wash Media Preparation

[002267] In the BSC, added 5.0 mL Gentamicin (W3009832 or W3012735) to 1 x 500 mL HBSS Media (W3013128) bottle. Added per bottle: HBSS (500.0 mL); Gentamicin sulfate, 50 mg/ml (5.0 mL). Filtered HBSS containing gentamicin prepared through a 1L 0.22-micron filter unit (W1218810).

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Day 0 Tumor Processing

[002268] Obtained Tumor. Obtained tumor specimen from QAR and transferred into suite at 2-8°C immediately for processing. Aliquoted Tumor Wash Media. Tumor Wash 1 Using 8" forceps (W3009771), removed the tumor from the specimen bottle and transferred to the "Wash 1" dish prepared. Followed by Tumor Wash 2 and Tumor Wash 3.

[002269] Measured Tumor. Assessed Tumor. Assessed whether > 30% of entire tumor area observed to be necrotic and/or fatty tissue. If applicable: Clean-Up Dissection. If tumor was large and >30% of tissue exterior was observed to be necrotic/fatty, performed "clean up dissection" by removing necrotic/fatty tissue while preserving tumor inner structure using a combination of scalpel and/or forceps.

[002270] Dissect TumorUsing a combination of scalpel and/or forceps, cut the tumor specimen into even, appropriately sized fragments (up to 6 intermediate fragments). Transferred intermediate tumor fragments. Dissected Tumor Fragmentsinto pieces approximately 3x3x3mm in size. Stored Intermediate Fragments to Prevent Drying.

[002271] Repeated Intermediate Fragment Dissection. Determined number of pieces collected. If desirable tissue remains, selected additional Favorable Tumor Pieces from the "favorable intermediate fragments" 6-well plate to fill the drops for a maximum of 50 pieces.

[002272] Prepared Conical Tube. Transferred Tumor Pieces to 50mL Conical Tube. Prepared BSC for G- REX100MCS. Removed G-REX100MCS from Incubator. Aseptically passed G-Rex100MCS flask into the BSC. Added tumor fragments to G-Rex100MCS flask. Evenly distributed pieces.

[002273] Incubated G-Rex100MCS at the following parameters: Incubated G-Rex flask: Temperature LED Display: 37.0±2.0 °C; CO2 Percentage: 5.0±1.5 %CO2. Calculations: Time of incubation; lower limite = time of incubation + 252 hours; upper limit = time of incubation + 276 hours.

Day 11 – Media Preparation

[002274] Monitored Incubator. Monitored Incubator. Incubator parameters: Temperature LED Display: 37.0±2.0 °C; CO2 Percentage: 5.0±1.5 %CO2. Warmed 3x 1000 mL RPMI 1640 Media (W3013112) bottles and 3x 1000 mL AIM-V (W3009501) bottles in an incubator for ≥ 30 minutes. Removed RPMI 1640 Media from incubator. Prepared RPMI 1640 Media. Filter Media. Thawed 3 x 1.1mL aliquots of IL-2 (6x106 IU/mL) (BR71424). Removed AIM-V Media from the incubator. Added IL-2 to AIM-V. Aseptically transferred a

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10L Labtainer Bag and a repeater pump transferr set into the BSC. Prepared 10L Labtainer media bag. Prepared Baxa pump. Prepared 10L Labtainer media bag. Pumped media into 10L Labtainer. Removed pumpmatic from Labtainer bag.

[002275] Mixed media. Gently massaged the bag to mix. Sample media per sample plan. Removed 20.0mL of media and place in a 50mL conical tube. Prepared Cell Count Dilution Tubes In the BSC, added 4.5mL of AIM-V Media that had been labelled with "For Cell Count Dilutions" and lot number to four 15mL conical tubes. Transferred reagents from the BSC to 2-8°C. Prepared 1L Transfer Pack. Outside of the BSC weld (a 1L Transfer Pack to the transfer set attached to the "Complete CM2 Day 11 Media" bag prepared. Prepared feeder cell transfer pack. Incubated Complete CM2 Day 11 Media.

Day 11 - TIL Harvest

[002276] Preprocessing table. Incubator parameters: Temperature LED Display: 37.0±2.0 °C; CO2 Percentage: 5.0±1.5 %CO2. Removed G-Rex100MCS from incubator. Prepared 300mL Transfer Pack. Welded transfer packs to G-Rex100MCS. Prepared flask for TIL Harvest and nitiation of TIL Harvest. TIL Harvested. Using the GatheRex, transferred the cell suspension through the blood filter into the 300mL transfer pack. Inspect membrane for adherent cells. Rinsed flask membrane. Closed clamps on G-Rex100MCS. Ensured all clamps are closed. Heat sealed the TIL and the "Supernatant" transfer pack. Calculated volume of TIL suspension. Prepared Supernatant Transfer Pack for Sampling. Pulled Bac-T Sample. In the BSC, draw up approximately 20.0 mL of supernatant from the 1L "Supernatant" transfer pack and dispense into a sterile 50mL conical tube. Inoculated BacT per Sample Plan. Removed a 1.0 mL sample from the 50mL conical labeled BacT prepared using an appropriately sized syringe and inoculated the anaerobic bottle.

[002277] Incubated TIL. Placed TIL Transfer Pack in incubator until needed. Performed cell counts and calculations. Determined the Average of Viable Cell Concentration and Viability of the cell counts performed. Viability ÷ 2. Viable Cell Concentration ÷ 2. Determined Upper and Lower Limit for counts. Lower Limit: Average of Viable Cell Concentration x 0.9. Upper Limit: Average of Viable Cell Concentration x 1.1. Confirmed both counts within acceptable limits. Determined an average Viable Cell Concentration from all four counts performed.

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[002278] Adjusted volume of TIL Suspension calculated the adjusted volume of TIL suspension after removal of cell count samples. Total TIL Cell Volume (A). Volume of Cell Count Sample Removed (4.0 ml) (B) Adjusted Total TIL Cell Volume C=A-B.

[002279] Calculated Total Viable TIL Cells. Average Viable Cell Concentraion*: Total Volume; Total Viable Cells: C = A x B. Calculation for flow cytometry: if the Total Viable TIL Cell count from was $\geq 4.0x10^7$, calculated the volume to obtain $1.0x10^7$ cells for the flow cytometry sample. Total viable cells required for flow cytometry: $1.0x10^7$ cells. Volume of cells required for flow cytometry: Viable cell concentration divived by $1.0x10^7$ cells A. Calculated the volume of TIL suspension equal to $2.0x10^8$ viable cells. As needed, calculated the excess volume of TIL cells to remove and removed excess TIL and placed TIL in incubator as needed. Calculated total excess TIL removed, as needed. Calculated amount of CS-10 media to add to excess TIL cells with the target cell concentration for freezing is 1.0 x 10^8 cells/ml. Centrifuged excess TILs, as needed. Observed conical tube and added CS-10. Filled Vials. Aliquoted 1.0mL cell suspension, into appropriately sized cryovials. Aliquoted residual volume into appropriately sized cryovial per SOP-00242. If volume is $\leq$0.5mL, add CS10 to vial until volume is 0.5mL.

TIL Cryopreservation of Sample

[002280] Calculated the volume of cells required to obtain $1x10^7$ cells for cryopreservation. Removed sample for Cryopreservation. Placed TIL in Incubator.

Cryopreservation of sample.

[002281] Observed conical tube and added CS-10 slowly and record volume of 0.5mL of CS10 added.

Day 11 - Feeder Cells

[002282] Obtained feeder cells. Obtained 3 bags of feeder cells with at least two different lot numbers from LN2 freezer. Kept cells on dry ice until ready to thaw. Prepared waterbath or Cryotherm. Thawed Feeder Cells at 37.0 ± 2.0°C water bath or cytotherm for ~3-5 minutes or until ice has just disappeared. Removed media from incubator. Pooled thawed feeder cells. Added feeder cells to transfer pack. Dispensed the feeder cells from the syringe into the transfer pack. Mixed pooled feeder cells and labeled transfer pack. Calculated total volume of feeder cell suspension in transfer pack.

[002283] Removed cell count samples. Using a separate 3mL syringe for each sample, pulled 4x1.0mL cell count samples from Feeder Cell Suspension Transfer Pack using the

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needless injection port. Aliquoted each sample into the cryovials labeled. Performed Cell Counts and Determine Multiplication FactorSelected protocols and entered multiplication factors. Determined the Average of Viable Cell Concentration and Viability of the cell counts performed. Determined Upper and Lower Limit for counts and confirm within limits.

[002284] Adjusted Volume of Feeder Cell Suspension. Calculated the adjusted volume of Feeder Cell suspension after removal of cell count samples. Calculated Total Viable Feeder Cells. Obtained additional Feeder Cells as needed. Thawed Additional Feeder Cells as needed. Placed the 4th Feeder Cell bag into a zip top bag and thaw in a 37.0 ± 2.0°C water bath or cytotherm for ~3-5 minutes and pooled additional feeder cells. Measured Volume. Measured the volume of the feeder cells in the syringe and recorded below (B). Calculated the new total volume of feeder cells. Added Feeder Cells to Transfer Pack.

[002285] Prepared dilutions as needed, adding 4.5mL of AIM-V Media to four 15mL conical tubes. Prepared cell counts. Using a separate 3mLsyringe for each sample, removed 4 x 1.0mL cell count samples from Feeder Cell Suspension transfer pack, using the needless injection port. Performed cell counts and calculations. Determined an average Viable Cell Concentration from all four counts performed. Adjusted Volume of Feeder Cell suspension and calculated the adjusted volume of Feeder Cell suspension after removal of cell count samples. Total Feeder Cell Volume minues 4.0 mL removed. Calculated the volume of Feeder Cell Suspension that was required to obtain $5x10^9$ viable feeder cells. Calculated excess feeder cell volume. Calculated the volume of excess feeder cells to remove. Removed excess feeder cells. Using a 1.0mL syringe and 16G needle, drew up 0.15mL of OKT3 and added OKT3. Heat sealed the Feeder Cell Suspension transfer pack.

Day 11 G-Rex Fill and Seed

[002286] Set up G-Rex500MCS. Removed "Complete CM2 Day 11 Media", from incubator and pumped media into G-Rex500MCS. Pumped 4.5L of media into the G-Rex500MCS, filling to the line marked on the flask. Heat sealed and incubated flask as needed. Welded the Feeder Cell suspension transfer pack to the G-Rex500MCS. Added Feeder Cells to G-Rex500MCS. Heat sealed. Welded the TIL Suspension transfer pack to the flask. Added TIL to G-Rex500MCS. Heat sealed and incubated G-Rex500MCS at 37.0±2.0 °C, CO2 Percentage: 5.0±1.5 %CO2.

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[002287] Calculated incubation window. Performed calculations to determine the proper time to remove G-Rex500MCS from incubator on Day 16. Lower limit: Time of incubation + 108 hours. Upper limit: Time of incubation + 132 hours.

Day 11 Excess TIL Cryopreservation

[002288] Applicable: Froze Excess TIL Vials. Verified the CRF has been set up prior to freeze. Perform Cryopreservation. Transferred vials from Controlled Rate Freezer to the appropriate storage. Upon completion of freeze, transfer vials from CRF to the appropriate storage container. Transferred vials to appropriate storage. Recorded storage location in LN2.

Day 16 Media Preparation

[002289] Pre-warmed AIM-V Media. Calculated time Media was warmed for media bags 1, 2, and 3. Ensured all bags have been warmed for a duration between 12 and 24 hours. Setup 10L Labtainer for Supernatant. Attached the larger diameter end of a fluid pump transfer set to one of the female ports of a 10L Labtainer bag using the Luer connectors. Setup 10L Labtainer for Supernatant and label. Setup 10L Labtainer for Supernatant. Ensure all clamps were closed prior to removing from the BSC. NOTE: Supernatant bag was used during TIL Harvest, which may be performed concurrently with media preparation.

[002290] Thawed IL-2. Thawed 5x1.1mL aliquots of IL-2 ($6x10^6$ IU/mL) (BR71424) per bag of CTS AIM V media until all ice had melted. Aliquoted 100.0mL GlutaMax. Added IL-2 to GlutaMax. Prepared CTS AIM V media bag for formulation. Prepared CTS AIM V media bag for formulation. Stage Baxa Pump. Prepared to formulate media. Pumped GlutaMax +IL-2 into bag. Monitored parameters: Temperature LED Display: 37.0±2.0 °C, CO2 Percentage: 5.0±1.5 %CO2. Warmed Complete CM4 Day 16 Media. Prepared Dilutions.

Day 16 REP Spilt

[002291] Monitored Incubator parameters: Temperature LED Display: 37.0±2.0 °C, CO2 Percentage: 5.0±1.5 %CO2. Removed G-Rex500MCS from the incubator. Prepared and labeled 1L Transfer Pack as TIL Suspension and weighed 1L. Volume Reduction of G-Rex500MCS. Transferred ~4.5L of culture supernatant from the G-Rex500MCS to the 10L. Prepared flask for TIL Harvest. After removal of the supernatant, closed all clamps to the red line. Initiation of TIL Harvest. Vigorously tap flask and swirl media to release cellsensure all cells have detached.

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[002292] TIL Harvest. Released all clamps leading to the TIL suspension transfer pack. Using the GatheRex transferred the cell suspension into the TIL Suspension transfer pack. NOTE: Be sure to maintain the tilted edge until all cells and media are collected. Inspected membrane for adherent cells. Rinsed flask membrane. Closed clamps on G-Rex500MCS. Heat sealed the Transfer Pack containing the TIL. Heat sealed the 10L Labtainer containing the supernatant. Recorded weight of Transfer Pack with cell suspension and calculate the volume suspension. Prepared transfer pack for sample removal. Removed testing samples from cell supernatant.

[002293] Sterility & BacT Testing Sampling: removed a 1.0mL sample from the 15 mL conical labeled BacT prepared. Removed Cell Count Samples. In the BSC, using separate 3mL syringes for each sample, removed 4x1.0 mL cell count samples from "TIL Suspension" transfer pack. Removed Mycoplasma Samples. Using a 3mL syringe, removed 1.0 mL from TIL Suspension transfer pack and place into 15 mL conical labeled "Mycoplasma diluent" prepared.

[002294] Prepared Transfer Pack for Seeding. Placed TIL in Incubator. Removed cell suspension from the BSC and place in incubator until needed. Performed cell counts and calculations. Diluted cell count samples initially by adding 0.5mL of cell suspension into 4.5mL of AIM-V media prepared which gave a 1:10 dilution. Determined the Average of Viable Cell Concentration and Viability of the cell counts performed. Determined Upper and Lower Limit for counts. NOTE: Dilution may be adjusted according based off the expected concentration of cells. Determined an average Viable Cell Concentration from all four counts performed. Adjusted Volume of TIL Suspension. Calculated the adjusted volume of TIL suspension after removal of cell count samples. Total TIL Cell Volume minus 5.0 mL removed for testing.

[002295] Calculated Total Viable TIL Cells. Calculated the total number of flasks to seed. NOTE: The maximum number of G-Rex500MCS flasks to seed was five. If the calculated number of flasks to seed exceeded five, only five were seeded USING THE ENTIRE VOLUME OF CELL SUSPENSION AVAILABLE.

[002296] Calculate number of flasks for subculture. Calculated the number of media bags required in addition to the bag prepared. Prepared one 10L bag of "CM4 Day 16 Media" for every two G-Rex-500M flask needed as calculated. Proceeded to seed the first GREX-500M flask(s) while additional media is prepared and warmed. Prepared and warmed the

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calculated number of additional media bags determined. Filled G-Rex500MCS. Prepared to pump media and pumped 4.5L of media into G-Rex500MCS. Heat Sealed. Repeated Fill. Incubated flask. Calculated the target volume of TIL suspension to add to the new G-Rex500MCS flasks. If the calculated number of flasks exceeds five only five will be seeded, USING THE ENTIRE VOLUME OF CELL SUSPENSION. Prepared Flasks for Seeding. Removed G-Rex500MCS from the incubator. Prepared G-Rex500MCS for pumping. Closed all clamps on except large filter line. Removed TIL from incubator. Prepared cell suspension for seeding. Sterile welded "TIL Suspension" transfer pack to pump inlet line. Placed TIL suspension bag on a scale.

[002297] Seeded flask with TIL Suspension. Pump the volume of TIL suspension calculated into flask. Heat sealed. Filled remaining flasks. Monitored Incubator. Incubator parameters: Temperature LED Display: 37.0±2.0 °C, CO2 Percentage: 5.0±1.5 %CO2. Incubated Flasks. Determined the time range to remove G-Rex500MCS from incubator on Day 22.

Day 22 Wash Buffer Preparation

[002298] Prepared 10 L Labtainer Bag. In BSC, attach a 4" plasma transfer set to a 10L Labtainer Bag via luer connection. Prepared 10 L Labtainer Bag. Closed all clamps before transferring out of the BSC. NOTE: Prepared one 10L Labtainer Bag for every two G-Rex500MCS flasks to be harvested. Pumped Plasmalyte into 3000mL bag and removed air from 3000mL Origen bag by reversing the pump and manipulating the position of the bag. Added Human Albumin 25% to 3000mL Bag. Obtain a final volumeof 120.0 mL of Human Albumin 25%.

[002299] Prepared IL-2 Diluent. Using a 10mL syringe, removed 5.0 mL of LOVO Wash Buffer using the needleless injection port on the LOVO Wash Buffer bag. Dispensed LOVO wash buffer into a 50mL conical tube. CRF Blank Bag LOVO Wash Buffer Aliquotted. Using a 100mL syringe, drew up 70.0 mL of LOVO Wash Buffer from the needleless injection port. Thawed one 1.1mL of IL-2 ($6x10^6$ IU/mL), until all ice has melted. IL-2 Preparation. Added 50µL IL-2 stock ($6x10^6$ IU/mL) to the 50mL conical tube labeled "IL-2 Diluent." Cryopreservation Prep. Placed 5 cryo-cassettes at 2-8°C to precondition them for final product cryopreservation.

[002300] Prepared Cell Count Dilutions. In the BSC, added 4.5mL of AIM-V Media that has been labelled with lot number and "For Cell Count Dilutions" to 4 separate 15mL

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conical tubes. Prepared Cell Counts. Labeled 4 cryovials with vial number (1-4). Kept vials under BSC to be used.

Day 22 TIL Harvest

[002301] Monitored Incubator. Incubator Parameters Temperature LED display: 37 ± 2.0°C, CO2 Percentage: 5%±1.5%. Removed G-Rex500MCS Flasks from Incubator. Prepared TIL collection bag and labeled. Sealed off extra connections. Volume Reduction: Transfered ~4.5L of supernatant from the G-Rex500MCS to the Supernatant bag.

[002302] Prepared flask for TIL Harvest. Initiated collection of TIL. Vigorously tap flask and swirl media to release cells. Eusure all cells have detached. Initiated collection of TIL. Released all clamps leading to the TIL suspension collection bag. TIL Harvest. Using the GatheRex, transferred the TIL suspension into the 3000mL collection bag. Inspect membrane for adherent cells. Rinsed flask membrane. Closed clamps on G- Rex500MCS and ensured all clamps are closed. Transferred cell suspension into LOVO source bag. Closed all clamps. Heat Sealed. Removed 4x1.0mL Cell Counts Samples

[002303] Performed Cell Counts. Performed cell counts and calculations utilizing NC-200 and Process Note 5.14. Diluted cell count samples initially by adding 0.5mL of cell suspension into 4.5mL of AIM-V media prepared. This gave a 1:10 dilution. Determined the Average Viability, Viable Cell Concentration, and Total Nucleated Cell concentration of the cell counts performed. Determined Upper and Lower Limit for counts. Determined the Average Viability, Viable Cell Concentration, and Total Nucleated Cell concentration of the cell counts performed. Weighed LOVO Source Bag. Calculated Total Viable TIL Cells. Calculated Total Nucleated Cells.

[002304] Prepared Mycoplasma Diluent. Removed 10.0 mL from one supernatant bag via luer sample port and placed in a 15mL conical.

LOVO

[002305] Performed "TIL G-Rex Harvest" protocoland determined the final product target volume. Loaded disposable kit. Removed filtrate bag. Entered Filtrate capacity. Placed Filtrate container on benchtop. Attached PlasmaLyte. Verified that the PlasmaLyte was attached and observed that the PlasmaLyte is moving. Attached Source container to tubing and verified Source container was attached. Confirmed PlasmaLyte was moving.

Final Formulation and Fill

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[002306] Target volume/bag calculation. Calculated volume of CS-10 and LOVO wash buffer to formulate blank bag. Prepared CRF Blank.

[002307] Calculated the volume of IL-2 to add to the Final Product. Final IL-2 Concentration desired (IU/mL) – 300IU/mL. IL-2 working stock: 6 x 10^4 IU/mL. Assembled Connect apparatus. Sterile welded a 4S-4M60 to a CC2 Cell Connection. Sterile welded (per Process Note 5.11) the CS750 Cryobags to the harness prepared. Welded CS-10 bags to spikes of the 4S-4M60.Prepared TIL with IL-2. Using an appropriately sized syringe, removed amount of IL-2 determined from the "IL-2 6x10^4" aliquot. Labeled Forumlated TIL Bag. Added the Formulated TIL bag to the apparatus. Added CS10. Switched Syringes. Drew ~10mL of air into a 100mL syringe and replaced the 60mL syringe on the apparatus. Added CS10. Prepared CS-750 bags. Dispensed cells.

[002308] Removed air from final product bags and take retain. Once the last final product bag was filled, closed all clamps. Drew 10mL of air into a new 100mL syringe and replace the syringe on the apparatus. Dispensed retain into a 50mL conical tube and label tube as "Retain" and lot number. Repeat air removal step for each bag.

[002309] Prepared final product for cryopreservation, incuding visual inspection. Held the cryobags on cold pack or at 2-8°C until cryopreservation.

[002310] Removed Cell Count Sample. Using an appropriately sized pipette, remove 2.0 mL of retain and place in a 15mL conical tube to be used for cell counts. Performed cell counts and calculations. NOTE: Diluted only one sample to appropriate dilution to verify dilution is sufficient. Diluted additional samples to appropriate dilution factor and proceed with counts. Determined the Average of Viable Cell Concentration and Viability of the cell counts performed. Determined Upper and Lower Limit for counts. NOTE: Dilution may be adjusted according based off the expected concentration of cells. Determined the Average of Viable Cell Concentration and Viability. Determined Upper and Lower Limit for counts. Calculated IFN-γ. Heat Sealed Final Product Bags.

[002311] Labeled and Collected Samples per exemplary Sample Plan below.

Table 56: Sample Plan

Sample	Number of Containers	Sample Volume to Add to Each	Container Type

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*Mycoplasma	1	1.0 mL	15 mL Conical
Endotoxin	2	1.0 mL	2 mL Cryovial
Gram Stain	1	1.0 mL	2 mL Cryovial
IFN-g	1	1.0 mL	2 mL Cryovial
Flow Cytometry	1	1.0 mL	2 mL Cryovial
**Bac-T Sterility	2	1.0 mL	Bac-T Bottle
QC Retain	4	1.0 mL	2 mL Cryovial
Satellite Vials	10	0.5 mL	2 mL Cryovial

[002312] Sterility & BacT. Testing Sampling. In the BSC, remove a 1.0mL sample from the retained cell suspension collected using an appropriately sized syringe and inoculate the anaerobic bottle. Repeat the above for the aerobic bottle

Final Product Cryopreservation

[002313] Prepared Controlled Rate Freezer. Verified the CRF had been set up. Set up CRF probes. Placed final product and samples in CRF. Determined the time needed to reach 4 °C ± 1.5 °C and proceed with the CRF run. CRF Completed and Stored. Stopped the CRF after the completion of the run. Remove cassettes and vials from CRF. Transferred cassettes and vials to vapor phase LN2 for storage.

POST PROCESSING SUMMARY

Post-Processing: Final Drug Product

[002314] (Day 22) Determination of CD3+ Cells on Day 22 REP by Flow Cytometry.

[002315] (Day 22) Gram Staining Method (GMP).

[002316] (Day 22) Bacterial Endotoxin Test by Gel Clot LAL Assay (GMP).

[002317] (Day 16) BacT Sterility Assay (GMP).

[002318] (Day 16) Mycoplasma DNA Detection by TD-PCR (GMP).

[002319] Acceptable Appearance Attributes.

[002320] (Day 22) BacT Sterility Assay (GMP) (Day 22).

[002321] (Day 22) IFN-gamma Assay.

[002322] The examples set forth above are provided to give those of ordinary skill in the art a complete disclosure and description of how to make and use the embodiments of the compositions, systems and methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Modifications of the above-described modes for carrying out the invention that are obvious to persons of skill in the art are intended to be within the scope of the following claims. All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains.

[002323] All headings and section designations are used for clarity and reference purposes only and are not to be considered limiting in any way. For example, those of skill in the art will appreciate the usefulness of combining various aspects from different headings and sections as appropriate according to the spirit and scope of the invention described herein.

[002324] All references cited herein are hereby incorporated by reference herein in their entireties and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[002325] Many modifications and variations of this application can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments and examples described herein are offered by way of example only, and the application is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which the claims are entitled.

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WHAT IS CLAIMED IS:

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

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

(b) performing a priming first expansion by culturing the first population of TILs in a first TIL cell culture comprising a first cell culture medium, IL-2, and either:

i) a first culture supernatant obtained from a first culture of antigen-presenting feeder cells (APCs), wherein the first culture supernatant comprises OKT-3, or

ii) APCs and OKT-3,

wherein the priming first expansion is performed by culturing the first TIL cell culture in a first container comprising a first gas-permeable surface area for a first period of about 1 to 7 or 1 to 8 days to obtain a second population of TILs, and 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 first TIL cell culture with additional first cell culture medium, IL-2, and either:

i) a second culture supernatant obtained from a second culture of APCs, wherein the second culture supernatant comprises OKT-3, or

ii) APCs and OKT-3;

to form a second TIL cell culture, wherein the rapid second expansion is performed by culturing the second TIL cell culture for a second period of about 1 to 11 days to obtain a third population of TILs, and wherein the third population of TILs is a therapeutic population of TILs; wherein the first TIL cell culture does not comprise both the first culture supernatant and APCs; wherein the second TIL cell culture does not comprise both the second culture supernatant and supplemental APCs; and wherein either the first TIL cell culture does not comprise APCs and/or the second TIL cell culture does not comprise supplemental APCs;

(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. The method for expanding TILs according to claim 1, wherein in the priming first expansion of step (b) the first TIL cell culture comprises the first culture supernatant,

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and wherein in the rapid second expansion of step (c) the first TIL cell culture is supplemented with OKT-3 and APCs to form the second TIL cell culture.

3. The method for expanding TILs according to claim 1, wherein in the priming first expansion of step (b) the first TIL cell culture comprises OKT-3 and APCs, and wherein in the rapid second expansion of step (c) the first TIL cell culture is supplemented with the second culture supernatant to form the second TIL cell culture.

4. The method for expanding TILs according to claim 1, wherein in the priming first expansion of step (b) the first TIL cell culture comprises the first culture supernatant, and wherein in the rapid second expansion of step (c) the first TIL cell culture is supplemented with the second culture supernatant to form the second TIL cell culture.

5. The method for expanding TILs according to claim 1, wherein obtaining the first culture supernatant for use in step (b) comprises:

1) providing an APC cell culture medium comprising IL-2 and OKT-3;

2) culturing at least about $5x10^8$ APCs in the APC cell culture medium from 1) for about 3-4 days to generate the first culture supernatant; and

3) collecting the first culture supernatant from the cell culture in 2).

6. The method for expanding TILs according to claim 1, wherein obtaining the second culture supernatant for use in step (c) comprises:

1) providing an APC cell culture medium comprising IL-2 and OKT-3;

2) culturing at least about $1x10^7$ APCs in the APC cell culture medium from 1) for about 3-4 days to generate the second culture supernatant; and

3) collecting the second culture supernatant from the cell culture in 2).

7. The method of claim 1, wherein the rapid second expansion of step (c) further comprises the step of:

i) supplementing the second TIL cell culture with additional IL-2 about 3 or 4 days after the initiation of the second period in step (c).

8. The method of claim 1, wherein the APCs are exogenous to the subject.

9. The method of claim 1, wherein the APCs are peripheral blood mononuclear cells (PBMCs).

10. The method of claim 1, wherein the rapid second expansion of step (c) further comprises the steps of:

i) on or about 3 or 4 days after the initiation of the second period, transferring the

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second TIL cell culture from the first container into a plurality of second containers to form a subculture of the second TIL cell culture in each of the plurality of second containers; and

ii) culturing the subculture of the second TIL cell culture in each of the plurality of second containers for the remainder of the second period.

11. The method of claim 10, wherein in step i) equal volumes of the second TIL cell culture are transferred into the plurality of second containers.
12. The method of claim 10, wherein each of the second containers is equal in size to the first container.
13. The method of claim 10, wherein each of the second containers is larger than the first container.
14. The method of claim 10, wherein the second containers are equal in size.
15. The method of claim 14, wherein the second containers are larger than the first container.
16. The method of claim 14, wherein the second containers are smaller than the first container.
17. The method of claim 1, wherein the first container is a G-Rex 100 flask.
18. The method of claim 10, wherein the first container is a G-Rex 100 flask and each of the plurality of second containers is a G-Rex 100 flask.
19. The method of claim 10, wherein the plurality of second containers is selected from the group consisting of: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20 second containers.
20. The method of claim 10, wherein the plurality of second containers is 5 second containers.
21. The method of claim 10, wherein before step ii) the method further comprises supplementing each subculture of the second TIL cell culture with additional IL-2.
22. The method of claim 10, wherein before step ii) the method further comprises supplementing each subculture of the second TIL cell culture with a second cell culture medium and IL-2.
23. The method of claim 22, wherein the first cell culture medium and the second cell culture medium are the same.
24. The method of claim 22, wherein the first cell culture medium and the second cell culture medium are different.
25. The method of claim 22, wherein the first cell culture medium is DM1 and the second

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cell culture medium is DM2.

26. 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 either antigen-presenting cells (APCs) and/or culture supernatant from a first culture of APCs comprising OKT-3 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 to 1 to 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, optionally OKT-3, and either APCs and/or culture supernatant from a second culture of APCs comprising OKT-3, 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.

27. 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;

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(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 either antigen presenting cells (APCs) and/or culture supernatant from a first culture of APCs comprising OKT-3, to produce a second population of TILs, wherein the priming first expansion is performed for a first period of about 1 to 7 or 1 to 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, optionally OKT-3, and either APCs and/or culture supernatant from a second culture of APCs comprising OKT-3, 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).

28. The method of claim 27, 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).

29. 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 either antigen presenting cells (APCs) and/or culture supernatant from a first culture of APCs comprising OKT-3 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,

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optionally OKT-3, and either APCs and/or culture supernatant from a second culture of APCs comprising OKT-3, 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).

30. 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 either antigen presenting cells (APCs) and/or culture supernatant from a first culture of APCs comprising OKT-3, to produce a second population of TILs, wherein the priming first expansion is performed for a first period of about 1 to 7 or 1 to 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, optionally OKT-3, and either APCs and/or culture supernatant from a second culture of APCs comprising OKT-3, 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).

31. The method of claim 30, 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 (c) is greater than the number of APCs in the culture medium in step (b).

32. The method of claim 26 or 28 or 31, 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.

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33. The method of claim 32, 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 10:1.

34. The method of claim 26 or 28 or 31, 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 2:1 to about 5:1.

35. The method of claim 26 or 28 or 31, 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 2:1 to about 3:1.

36. The method of claim 26 or 28 or 31, 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.

37. The method of claim 26 or 28 or 31, wherein the number of APCs in the priming first expansion is in a range of about 1.0×10^6 APCs/cm^2 to about 4.5×10^6 APCs/cm^2, and wherein the number of APCs in the rapid second expansion is in a range of about 2.5×10^6 APCs/cm^2 to about 7.5×10^6 APCs/cm^2.

38. The method of claim 26 or 28 or 31, wherein the number of APCs in the priming first expansion is in a range of about 1.5×10^6 APCs/cm^2 to about 3.5×10^6 APCs/cm^2, and wherein the number of APCs in the rapid second expansion is in a range of about 3.5×10^6 APCs/cm^2 to about 6.0×10^6 APCs/cm^2.

39. The method of claim 26 or 28 or 31, wherein the number of APCs in the priming first expansion is in a range of about 2.0×10^6 APCs/cm^2 to about 3.0×10^6 APCs/cm^2, and wherein the number of APCs in the rapid second expansion is in a range of about 4.0×10^6 APCs/cm^2 to about 5.5×10^6 APCs/cm^2.

40. The method of claim 26 or 28 or 31, wherein the number of APCs in the priming first expansion is in a range of about 1×10^8 APCs to about 3.5×10^8 APCs, and wherein the number of APCs in the rapid second expansion is in a range of about 3.5×10^8 APCs to about 1×10^9 APCs.

41. The method of claim 26 or 28 or 31, wherein the number of APCs in the priming first expansion is in a range of about 1.5×10^8 APCs to about 3×10^8 APCs, and wherein the number of APCs in the rapid second expansion is in a the range of about 4×10^8 APCs to about 7.5×10^8 APCs.

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42. The method of claim 26 or 28 or 31, wherein the number of APCs in the priming first expansion is in a range of about 2×10^8 APCs to about 2.5×10^8 APCs, and wherein the number of APCs in the rapid second expansion is in a range of about 4.5×10^8 APCs to about 5.5×10^8 APCs.

43. The method of claim 26 or 28 or 31, wherein about 2.5×10^8 APCs are added to the priming first expansion and 5×10^8 APCs are added to the rapid second expansion.

44. The method of any of claims 26-43, 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.

45. The method of any of claims 26-43, 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 50:1.

46. The method of any of claims 26-43, 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 25:1.

47. The method of any of claims 26-40, 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 20:1.

48. The method of any of claims 26-40, 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 10:1.

49. The method of any of claims 26-43, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs.

50. The method of any of claims 27-31, 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.

51. The method of any of claims 27-50, 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.

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52. The method of claim 51, wherein the plurality of separate containers comprises at least two separate containers.

53. The method of claim 51, wherein the plurality of separate containers comprises from two to twenty separate containers.

54. The method of claim 51, wherein the plurality of separate containers comprises from two to ten separate containers.

55. The method of claim 51, wherein the plurality of separate containers comprises from two to five separate containers.

56. The method of any of claims 51-55, wherein each of the separate containers comprises a first gas-permeable surface area.

57. The method of any of claims 27-50, wherein the multiple tumor fragments are distributed in a single container.

58. The method of claim 57, wherein the single container comprises a first gas-permeable surface area.

59. The method of claim 56 or 58, wherein in the step of the priming first expansion the cell culture medium comprises antigen-presenting cells (APCs) and the APCs are layered onto the first gas-permeable surface area at an average thickness of about one cell layer to about three cell layers.

60. The method of claim 58, 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.

61. The method of claim 58, wherein in the step of the priming first expansion the APCs are layered onto the first gas-permeable surface area at an average thickness of about 2 cell layers.

62. The method of any of claims 59-61, wherein in the step of the rapid second expansion the APCs are layered onto the first gas-permeable surface area at a thickness of about 3 cell layers to about 5 cell layers.

63. The method of claim 62, wherein in the step of the rapid second expansion the APCs are layered onto the first gas-permeable surface area at a thickness of about 3.5 cell layers to about 4.5 cell layers.

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64. The method of claim 63, wherein in the step of the rapid second expansion the APCs are layered onto the first gas-permeable surface area at a thickness of about 4 cell layers.

65. The method of any of claims 27-50, 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.

66. The method of claim 65, wherein the second container is larger than the first container.

67. The method of claim 65 or 66, wherein in the step of the priming first expansion the cell culture medium comprises antigen-presenting cells (APCs) and the APCs are layered onto the first gas-permeable surface area at an average thickness of about one cell layer to about three cell layers.

68. The method of claim 66, 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.

69. The method of claim 68, wherein in the step of the priming first expansion the APCs are layered onto the first gas-permeable surface area at an average thickness of about 2 cell layers.

70. The method of any of claims 65-69, 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.

71. The method of claim 70, 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 3.5 cell layers to about 4.5 cell layers.

72. The method of claim 70, 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 4 cell layers.

73. The method of any of claim 27-64, 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.

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74. The method of claim 73, wherein each container comprises a first gas-permeable surface area.

75. The method of claim 74, wherein in the step of the priming first expansion the cell culture medium comprises antigen-presenting cells (APCs) and the APCs are layered onto the first gas-permeable surface area at an average thickness of from about one cell layer to about three cell layers.

76. The method of claim 75, 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.

77. The method of claim 76, wherein in the step of the priming first expansion the APCs are layered onto the first gas-permeable surface area at an average thickness of about 2 cell layers.

78. The method of any of claims 74-77, 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.

79. The method of claim 78, 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 3.5 cell layers to about 4.5 cell layers.

80. The method of claim 79, 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 4 cell layers.

81. The method of any of claims 27-57, 65, 66 and 73, 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 in a range of about 1:1.1 to about 1:10.

82. The method of claim 81, 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 1:1.2 to about 1:8.

83. The method of claim 81, 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 raid second expansion is in a range of about 1:1.3 to about 1:7.

84. The method of claim 81, 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 1:1.4 to about 1:6.

85. The method of claim 81, 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 1:1.5 to about 1:5.

86. The method of claim 81, 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 1:1.6 to about 1:4.

87. The method of claim 81, 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 1:1.7 to about 1:3.5.

88. The method of claim 81, 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 1:1.8 to about 1:3.

89. The method of claim 81, 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 1:1.9 to about 1:2.5.

90. The method of claim 81, 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.

91. 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.

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

93. The method according to claim 26 or 50, further comprising the step of cryopreserving the infusion bag.

94. The method according to claim 92 or 93, wherein the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.

95. The method according to any of the preceding claims, wherein the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).

96. The method according to claim 95, wherein the PBMCs are irradiated and allogeneic.

97. 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 2.5×10^8.

98. The method according to any of preceding claims, 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 5×10^8.

99. The method according to any of claims 26-91, wherein the antigen-presenting cells are artificial antigen-presenting cells.

100. 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.

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

102. 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.

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103. The method according to any of the preceding claims, wherein the multiple fragments comprise about 30 to about 60 fragments with a total volume of about 1300 mm^3 to about 1500 mm^3.

104. The method according to claim 103, wherein the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3.

105. The method according to any of the preceding claims, wherein the multiple fragments comprise about 50 fragments with a total mass of about 1 gram to about 1.5 grams.

106. 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.

107. 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.

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

109. The method according to claim 26 or 50, 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.

110. The method according to any of claims 92-94, wherein the cryopreservation media comprises dimethlysulfoxide (DMSO).

111. The method according to claim 110, wherein the cryopreservation media comprises 7% to 10% DMSO.

112. The method according to any of the preceding claims, wherein 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.

113. The method according to any of claims 26-111, wherein the first period in the step of the priming first expansion is performed within a period of 5 days, 6 days, or 7 days.

114. The method according to any of claims 26-111, wherein the second period in the step of the rapid second expansion is performed within a period of 7 days, 8 days, or 9 days.

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115. The method according to any of claims 26-111, wherein 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.

116. The method according to any of claims 26-111, wherein steps of the priming first expansion through the harvesting of the therapeutic population of TILs are performed within a period of about 14 days to about 16 days.

117. The method according to any of claims 26-111, wherein 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.

118. The method according to any of claims 26-111, wherein steps of the priming first expansion through the harvesting of the therapeutic population of TILs are performed within a period of about 14 days.

119. The method according to any of claims 26-111, wherein steps of the priming first expansion through the harvesting of the therapeutic population of TILs are performed within a period of about 15 days.

120. The method according to any of claims 26-111, wherein steps the priming first expansion through the harvesting of the therapeutic population of TILs are performed within a period of about 16 days.

121. The method according to any of claims 26-111, further comprising the step of cryopreserving the harvested therapeutic population of TILs using a cryopreservation process, wherein steps of the priming first expansion through the harvesting of the therapeutic population of TILs and cryopreservation are performed in 16 days or less.

122. The method according to any one of claims 26 to 118, 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.

123. The method according to claim 122, wherein the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10}.

124. The method according to any one of claims 26 to 123, wherein 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.

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125. The method according to any one of claims 26 to 123, wherein 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.

126. The method according to any one of claims 26 to 123, wherein 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.

127. The method according to any one of claims 26 to 126, wherein the therapeutic population of TILs from the step of the harvesting of the therapeutic population of TILs are infused into a patient.

128. 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 either antigen presenting cells (APCs) and/or culture supernatant from a first culture of APCs comprising OKT-3 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 or 1 to 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 either antigen presenting cells (APCs) and/or culture supernatant from a second culture of APCs comprising OKT-3, 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;

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(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.

129. The method according to claim 128, wherein the number of TILs sufficient for administering a therapeutically effective dosage in step (f) is from about 2.3×10^{10} to about 13.7×10^{10}.

130. The method according to claim 128, wherein the antigen presenting cells (APCs) are PBMCs.

131. The method according to any of claims 128 to 130, wherein prior to administering a therapeutically effective dosage of TIL cells in step (f), a non-myeloablative lymphodepletion regimen has been administered to the patient.

132. The method according to claim 131, where 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.

133. The method according to any of claims 128 to 132, further comprising the step of treating the patient with a high-dose IL-2 regimen starting on the day after administration of the TIL cells to the patient in step (f).

134. The method according to claim 133, wherein 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.

135. The method according to any one of claims 128 to 134, wherein the third population of TILs in step (b) provides for increased efficacy, increased interferon-gamma production, and/or increased polyclonality.

136. The method according to any one of claims 128 to 134, wherein 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.

137. The method according to any one of claims 128 to 134, wherein the effector T cells and/or central memory T cells obtained from the third population of TILs in step (c)

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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).

138. The method according to any one of claims 128-137, wherein the cancer is a solid tumor.

139. The method according to any one of claims 128-137, wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, triple negative 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.

140. The method according to claim 139, wherein the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

141. The method according to claim 139, wherein the cancer is melanoma.

142. The method according to claim 139, wherein the cancer is HNSCC.

143. The method according to claim 139, wherein the cancer is a cervical cancer.

144. The method according to claim 139, wherein the cancer is NSCLC.

145. The method according to claim 139, wherein the cancer is glioblastoma (including GBM).

146. The method according to claim 139, wherein the cancer is gastrointestinal cancer.

147. The method according to any one of claims 128-146, wherein the cancer is a hypermutated cancer.

148. The method according to any one of claims 128-146, wherein the cancer is a pediatric hypermutated cancer.

149. The method according to any one of claims 128-148, wherein the container is a closed container.

150. The method according to any one of claims 128-149, wherein the container is a G-container.

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151. The method according to any one of claims 128-150, wherein the container is a GREX-10.

152. The method according to any one of claims 128-150, wherein the closed container comprises a GREX-100.

153. The method according to any one of claims 128-150, wherein the closed container comprises a GREX-500.

154. The therapeutic population of tumor infiltrating lymphocytes (TILs) made by the method of any of the preceding claims.

155. A 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.

156. The therapeutic population of TILs of claim 154 or claim 155 that provides for increased interferon-gamma production.

157. The therapeutic population of TILs of claim 154 or claim 155 that provides for increased polyclonality.

158. The therapeutic population of TILs of claim 154 or claim 155 that provides for increased efficacy.

159. The therapeutic population of TILs of any of claims 154-158, wherein the therapeutic population of TILs is capable of at least one-fold more interferon-gamma production as compared to TILs prepared by a process longer than 16 days.

160. The therapeutic population of TILs of any of claims 154-158, wherein the therapeutic population of TILs is capable of at least two-fold more interferon-gamma production as compared to TILs prepared by a process longer than 16 days.

161. The therapeutic population of TILs of any of claims 154-158, wherein the therapeutic population of TILs is capable of at least three-fold more interferon-gamma production as compared to TILs prepared by a process longer than 16 days.

162. 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).

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163. The therapeutic population of TILs of claim 162, wherein the therapeutic population of TILs 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.

164. The therapeutic population of TILs of claim 163, wherein the therapeutic population of TILs 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.

165. 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.

166. The therapeutic population of TILs of claim 165, wherein the therapeutic population of TILs 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.

167. The therapeutic population of TILs of claim 165, wherein the therapeutic population of TILs 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.

168. 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.

169. The therapeutic population of TILs of claim 168, wherein the therapeutic population of TILs 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.

170. The therapeutic population of TILs of claim 168, wherein the therapeutic population of TILs 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.

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171. A tumor infiltrating lymphocyte (TIL) composition comprising the therapeutic population of TILs of any of claims 154-170 and a pharmaceutically acceptable carrier.

172. A sterile infusion bag comprising the TIL composition of claim 168.

173. A cryopreserved preparation of the therapeutic population of TILs of any of clams 154-167.

174. A tumor infiltrating lymphocyte (TIL) composition comprising the therapeutic population of TILs of any of claims 154-170 and a cryopreservation media.

175. The TIL composition of claim 174, wherein the cryopreservation media contains DMSO.

176. The TIL composition of claim 175, wherein the cryopreservation media contains 7-10% DMSO.

177. A cryopreserved preparation of the TIL composition of any of claims 171 or 174-176.

178. The tumor infiltrating lymphocyte (TIL) composition of any of claims 171 or 174-177 for use as a medicament.

179. The tumor infiltrating lymphocyte (TIL) composition of any of claims 171 or 174-177 for use in the treatment of a cancer.

180. The tumor infiltrating lymphocyte (TIL) composition of any of claims 171 or 174-177 for use in the treatment of a solid tumor cancer.

181. The tumor infiltrating lymphocyte (TIL) composition of any of claims 171 or 174-177 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, triple negative 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.

182. The tumor infiltrating lymphocyte (TIL) composition of any of claims 171 or 174-177 for use in treatment of a cancer selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

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183. The TIL composition of any of claims 171 or 174-177 for use in treatment of a cancer wherein cancer is melanoma.

184. The TIL composition of any of claims 171 or 174-177 for use in treatment of a cancer wherein cancer is HNSCC.

185. The TIL composition of any of claims 171 or 174-177 for use in treatment of a cancer wherein a cervical cancer.

186. The TIL composition of any of claims 171 or 174-177 for use in treatment of a cancer wherein the cancer is NSCLC.

187. The TIL composition of any of claims 171 or 174-177 for use in treatment of a cancer wherein the cancer is glioblastoma (including GBM).

188. The TIL composition of any of claims 171 or 174-177 for use in treatment of a cancer wherein the cancer is gastrointestinal cancer.

189. The TIL composition of any of claims 171 or 174-177 for use in treatment of a cancer wherein the cancer is a hypermutated cancer.

190. The TIL composition of any of claims 171 or 174-177 for use in treatment of a cancer wherein the cancer is a pediatric hypermutated cancer.

191. The use of the tumor infiltrating lymphocyte (TIL) composition of any of claims 171 or 174-177 in a method of treating cancer in a subject comprising administering a therapeutically effective dosage of the TIL composition to the subject.

192. The use of the TIL composition of claim 191, wherein the cancer is a solid tumor.

193. The use of the TIL composition of claim 191, wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, triple negative 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.

194. The use of the TIL composition of claim 191, wherein the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

195. The use of the TIL composition of claim 191, wherein the cancer is melanoma.

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196. The use of the TIL composition of claim 191, wherein the cancer is HNSCC.

197. The use of the TIL composition of claim 191, wherein the cancer is a cervical cancer.

198. The use of the TIL composition of claim 191, wherein the cancer is NSCLC.

199. The use of the TIL composition of claim 191, wherein the cancer is glioblastoma (including GBM).

200. The use of the TIL composition of claim 191, wherein the cancer is gastrointestinal cancer.

201. The use of the TIL composition of claim 191, wherein the cancer is a hypermutated cancer.

202. The use of the TIL composition of claim 191, wherein the cancer is a pediatric hypermutated cancer.

203. The tumor infiltrating lymphocyte (TIL) composition of any of claims 171 or 174-177 for use in a method of treating cancer in a subject comprising administering a therapeutically effective dosage of the TIL composition to the subject.

204. The TIL composition of claim 203, wherein the cancer is a solid tumor.

205. The TIL composition of claim 203, wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, triple negative 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.

206. The TIL composition of claim 203 wherein the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

207. A method of treating cancer in a subject comprising administering to the subject a therapeutically effective dosage of the tumor infiltrating lymphocyte (TIL) composition of any of claims 171 or 174-177.

208. The method of claim 207, wherein the cancer is a solid tumor.

209. The method of claim 207, wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung

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cancer, bladder cancer, breast cancer, triple negative 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.

210. The method of claim 207, wherein the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

211. The method of claim 207, wherein the cancer is melanoma.

212. The method of claim 207, wherein the cancer is HNSCC.

213. The method of claim 207, wherein the cancer is a cervical cancer.

214. The method of claim 207, wherein the cancer is NSCLC.

215. The method of claim 207, wherein the cancer is glioblastoma (including GBM).

216. The method of claim 207, wherein the cancer is gastrointestinal cancer.

217. The method of claim 207, wherein the cancer is a hypermutated cancer.

218. The method of claim 207, wherein the cancer is a pediatric hypermutated cancer.

219. 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.

220. The method of claim 219, wherein the priming first expansion of step (a) is performed during a period of up to 7 days.

221. The method of claim 219 or 220, wherein the rapid second expansion of step (b) is performed during a period of up to 11 days.

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222. The method of claim 221, wherein the rapid second expansion of step (b) is performed during a period of up to 9 days.

223. The method of any of claims 219-222, 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.

224. The method of claim 219, wherein the priming first expansion of step (a) is performed during a period of up to 8 days.

225. The method of claim 219 or 220, wherein the rapid second expansion of step (b) is performed during a period of up to 8 days.

226. The method of any of claims 219-222, 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.

227. The method of any of claims 219-226, wherein in step (a) the first population of T cells is cultured in a first culture medium comprising OKT-3 and IL-2.

228. The method of claim 227, wherein the first culture medium comprises OKT-3, IL-2 and antigen-presenting cells (APCs).

229. The method of any of claims 219-226, wherein in step (b) the first population of T cells is cultured in a second culture medium comprising OKT-3, IL-2 and either antigen presenting cells (APCs) and/or culture supernatant from a culture of APCs comprising OKT-3.

230. The method of any of claims 219-226, wherein 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) or culture supernatant from a first culture of APCs comprising OKT-3, 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 or culture supernatant from a second culture of APCs comprising OKT-3, wherein the second population of APCs is exogenous to the donor of the first population of T cells

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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.

231. The method of claim 230, 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.

232. The method of claim 230 or 231, wherein the number of APCs in the first population of APCs is about 2.5×10^8 and the number of APCs in the second population of APCs is about 5×10^8.

233. The method of any of claims 230-232, wherein 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.

234. The method of any of claims 230-233, wherein 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.

235. The method of any of claims 230-234, 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.

236. The method of any of claims 230-235, wherein the APCs are peripheral blood mononuclear cells (PBMCs).

237. The method of any of claims 230-236, wherein APCs comprise PBMCs that are irradiated and exogenous to the donor of the first population of T cells.

238. The method of any of claims 227-234, wherein the T cells are tumor infiltrating lymphocytes (TILs).

239. The method of any of claims 227-234, wherein the T cells are marrow infiltrating lymphocytes (MILs).

240. The method of any of claims 227-234, wherein the T cells are peripheral blood lymphocytes (PBLs).

241. The method of any of the preceding claims wherein the cell culture medium is a defined medium and/or a serum free medium.

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242. The method of claim 241, wherein the defined medium comprises (optionally recombinant) transferrin, (optionally recombinant) insulin, and (optionally recombinant) albumin.

243. The method of any of claims 241-242, wherein the serum-free or defined medium comprises a basal cell culture medium and a serum supplement and/or a serum replacement.

244. The method of claim 243, wherein 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.

245. The method of any of claims 243-244, 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.

246. The method of any of claims 241-245, wherein the cell culture medium comprises one or more albumins or albumin substitutes.

247. The method of any of claims 241-246, wherein the cell culture medium comprises one or more amino acids.

248. The method of any of claims 241-247, wherein the cell culture medium comprises one or more vitamins, one or more transferrins or transferrin substitutes.

249. The method of any of claims 241-248, wherein the cell culture medium comprises one or more antioxidants, one or more insulins or insulin substitutes.

250. The method of any of claims 241-249, wherein the cell culture medium comprises one or more collagen precursors, one or more antibiotics, and one or more trace elements.

251. The method of any of claims 241-250, wherein the cell culture medium comprises albumin.

252. The method of any of claims 241-251, wherein the cell culture medium comprises albumin and one or more ingredients selected from the group consisting of glycine, L-

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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+}.

253. The method of any of claims 241-252, wherein the cell culture medium further comprises L-glutamine, sodium bicarbonate and/or 2-mercaptoethanol.

254. The method of any of claims 241-253, 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.

255. The method of any of claims 241-254, wherein 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.

256. The method of any of claims 241-255, 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.

257. The method of any of claims 241-256, wherein the cell culture medium further comprises glutamine (*i.e.*, GlutaMAX®) at a concentration of about 2mM.

258. The method of any of claims 241-257, wherein 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.

259. The method of any of claims 241-258, wherein the cell culture medium further comprises 2-mercaptoethanol at a concentration of about 55mM.

260. The method of any of claims 241-259, wherein the cell culture medium comprises the defined media described in International PCT Publication No. WO/1998/030679.

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261. The method of any of claims 241-260, 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.

262. The method of any of claims 241-261, wherein 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 4 provided herein.

263. The method of any of claims 241-262, wherein the osmolarity of the cell culture medium is between about 260 and 350 mOsmol.

264. The method of any of claims 241-263, wherein the cell culture medium further comprises about 3.7 g/L, or about 2.2 g/L sodium bicarbonate.

265. The method of any of claims 241-264, 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).

266. The method of any of claims 241-265, 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).

267. The method of any of claims 241-265, wherein the cell culture medium comprises CTS OpTmizer T-Cell Expansion SFM, 3% CTS Immune Cell Serum Replacement, 55mM BME, and optionally glutamine.

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268. The method of any of claims 241-265, 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, and 2 mM Glutamax, optionally further comprising 6,000 IU/mL of IL-2.

269. The method of any of claims 241-265, 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 optionaly further comprising 3,000 IU/mL of IL-2.

270. The method of any of the preceding claims, wherein the tumor sample is one or more small biopsies, core biopsies, or needle biopsies of the tumor in the subject.

271. 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 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 either antigen presenting cells (APCs) and/or culture supernatant from a first culture of APCs comprising OKT-3 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 either antigen presenting cells (APCs) and/or culture supernatant from a second culture of APCs comprising OKT-3, 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

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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.

272. 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 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 either antigen presenting cells (APCs) and/or culture supernatant from a first culture of APCs comprising OKT-3 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 either antigen presenting cells (APCs) and/or culture supernatant from a second culture of APCs comprising OKT-3, 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).

273. The method of 271 or 272 wherein after day 5 of the second period the culture is split into 2 or more subcultures, and each subculture is supplemented with an additional quantity of the third culture medium and cultured for about 6 days.

274. The method of claim 273 wherein after day 5 of the second period the culture is split into up to 5 subcultures.

275. The method of any of claims 271-274 wherein all steps in the method are completed in about 22 days.

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276. A method of expanding T cells comprising:

(i) performing a priming first expansion of a first population of T cells from a tumor sample 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.

277. The method of any of claims 270-276 wherein the tumor sample is obtained from a plurality of core biopsies.

278. The method of claim 277wherein the plurality of core biopsies is selected from the group consisting of 2, 3, 4, 5, 6, 7, 8, 9 and 10 core biopsies.

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

i) a population of tumor infiltrating lymphocytes (TILs) optionaly derived according to any of claims 1 to 278, and

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

280. The TIL or expanded TIL composition of claim 270, wherein the defined medium or serum free medium comprises (optionally recombinant) transferrin, (optionally recombinant) insulin, and (optionally recombinant) albumin.

281. The TIL or expanded TIL composition of any of claims 270-271, wherein the defined medium or serum free medium comprises a basal cell medium and a serum supplement and/or a serum replacement.

282. The TIL or expanded TIL composition of claim 272, 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

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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.

283. The TIL or expanded TIL composition of any of claims 281-282, 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.

284. The TIL or expanded TIL composition of any of claims 279-283, wherein the defined medium or serum free medium comprises one or more albumins or albumin substitutes.

285. The TIL or expanded TIL composition of any of claims 279-284, wherein the defined medium or serum free medium comprises one or more amino acids.

286. The TIL or expanded TIL composition of any of claims 289-285, wherein the defined medium or serum free medium comprises one or more vitamins, one or more transferrins or transferrin substitutes.

287. The TIL or expanded TIL composition of any of claims 279-286, wherein the defined medium or serum free medium comprises one or more antioxidants, one or more insulins or insulin substitutes.

288. The TIL or expanded TIL composition of any of claims 279-287, wherein the defined medium or serum free medium comprises one or more collagen precursors, one or more antibiotics, and one or more trace elements.

289. The TIL or expanded TIL composition of any of claims 279-288, wherein the defined medium or serum free medium comprises albumin.

290. The TIL or expanded TIL composition of any of claims 279-289, 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^{+},

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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+}.

291. The TIL or expanded TIL composition of any of claims 279-290, wherein the defined medium or serum free medium further comprises L-glutamine, sodium bicarbonate and/or 2-mercaptoethanol.

292. The TIL or expanded TIL composition of any of claims 279-291, 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.

293. The TIL or expanded TIL composition of any of claims 279-292, wherein 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.

294. The TIL or expanded TIL composition of any of claims 279-293, 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.

295. The TIL or expanded TIL composition of any of claims 279-294, wherein the defined medium or serum free medium further comprises glutamine (*i.e.*, GlutaMAX®) at a concentration of about 2mM.

296. The TIL or expanded TIL composition of any of claims 279-295, 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.

297. The TIL or expanded TIL composition of any of claims 279-296, wherein the defined medium or serum free medium further comprises 2-mercaptoethanol at a concentration of about 55mM.

298. The TIL or expanded TIL composition of any of claims 279-297, wherein the defined medium or serum free medium comprises the defined media described in International PCT Publication No. WO/1998/030679.

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299. The TIL or expanded TIL composition of any of claims 279-298, 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.

300. The TIL or xpanded TIL composition of any of claims 279-299, wherein 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 4 provided herein.

301. The TIL or expanded TIL composition of any of claims 279-300, wherein the osmolarity of the defined medium or serum free medium is between about 260 and 350 mOsmol.

302. The TIL or expanded TIL composition of any of claims 279-301, wherein the defined medium or serum free medium further comprises about 3.7 g/L, or about 2.2 g/L sodium bicarbonate.

303. The TIL or expanded TIL composition of any of claims 279-302, wherein 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).

304. The TIL or expanded TIL composition of any of claims 279-303, wherein 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).

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305. The TIL or expanded TIL composition of any of claims 279-304, wherein the cell culture medium comprises CTS OpTmizer T-Cell Expansion SFM, 3% CTS Immune Cell Serum Replacement, 55mM BME, and optionally glutamine.

306. The TIL or expanded TIL composition of any of claims 279-305, 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, and 2 mM Glutamax, optionally further comprising 6,000 IU/mL of IL-2.

307. The TIL or expanded TIL composition of any of claims 279-305, 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 optionaly further comprising 3,000 IU/mL of IL-2.

308. The TIL or expanded TIL composition of any of claims 279-207, wherein the population of TILs is a therapeutic population of TILs.

309. The TIL or expanded TIL composition of any of claims 279-308, wherein 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.

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

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

obtaining a first population of TILs from a tumor resected from a patient by processing a tumor sample obtained from the patient into a tumor digest;

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

(c) performing a first expansion or priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3,

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and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about to about 1 to 5 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 4 to 8 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

(f) harvesting the second plurality of subpopulations of TILs obtained from step (e), wherein the transition from step (e) to step (f) occurs without opening the system when optionally performed in a closed system; and

(g) transferring the harvested subpopulations of TILs from step (g) to one or more infusion bags, wherein the transition from step (f) to (g) occurs without opening the system when optionally performed in a closed system.

311. A tumor infiltrating lymphocyte (TIL) composition comprising a therapeutic population of infiltrating lymphocytes (TILs), wherein the TIL composition is produced by a method comprising:

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

obtaining a first population of TILs from a tumor resected from a patient by processing a tumor sample obtained from the patient into a tumor digest;

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

(c) performing a first expansion or a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2and antigen presenting cells (APCs), and optionally OKT-3, to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about to about 1 to 5 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 4 to 8 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

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(f) harvesting the second plurality of subpopulations of TILs obtained from step (e), wherein the transition from step (e) to step (f) occurs without opening the system when optionally performed in a closed system; and

(g) transferring the harvested subpopulations of TILs from step (g) to one or more infusion bags, wherein the transition from step (f) to (g) occurs without opening the system when optionally performed in a closed system.

312. The TIL composition of claim 311 wherein the TIL composition is a cryopreserved composition and wherein the method further comprises (h) cryopreserving the infusion bag comprising the harvested TIL population from step (g) using a cryopreservation process.

313. A method for treating a subject with cancer, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

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

obtaining a first population of TILs from a tumor resected from a patient by processing a tumor sample obtained from the patient into a tumor digest;

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

(c) performing a first expansion or a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 1 to 5 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second

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gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 4 to 8 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

(f) harvesting the second plurality of subpopulations of TILs obtained from step (e), wherein the transition from step (e) to step (f) occurs without opening the system when optionally performed in a closed system;

(g) transferring the harvested subpopulations of TILs from step (g) to one or more infusion bags, wherein the transition from step (f) to (g) occurs without opening the system when optionally performed in a closed system; and

(h) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (g) to the subject.

314. The method of claim 310-313, wherein the first expansion or the priming first expansion is performed for about 6-8 days.

315. The method of claim 310-313, wherein the rapid second expansion is performed for about 2-4 days.

316. The method of claim 310-313, wherein the third expansion is each performed for about 5-7 days.

317. The method of claim 310-313, wherein the first expansion or priming first expansion is performed for about 7 days, the rapid second expansion is performed for about 3 days, and the third expansion is performed for about 6 days.

318. The method of claim 310-313, wherein steps (c)-(e) are performed in about 14-18 days.

319. The method of claim 310-313, wherein steps (c)-(e) are performed in about 16 days.

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320. The method of claim 310-313, wherein steps (c)-(e) are performed in about 18 days or less.

321. The method of claim 310-313, wherein steps (c)-(e) are performed in about 16 days or less.

322. The method of any of claims 310-321, wherein step (e) comprises seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container providing a third gas-permeable surface area at a seeding density of about $2x10^6$ cells/cm^2.

323. The method of claim 313-322, wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, triple negative 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.

324. The method of claim 323, wherein the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

325. The method of claim 323, wherein the cancer is melanoma.

326. The method of claim 323, wherein the cancer is HNSCC.

327. The method of claim 323, wherein the cancer is a cervical cancer.

328. The method of claim 323, wherein the cancer is NSCLC.

329. The method of claim 323, wherein the cancer is glioblastoma (including GBM).

330. The method of claim 323, wherein the cancer is gastrointestinal cancer.

331. The method of claim 323, wherein the cancer is a hypermutated cancer.

332. The method of claim 323, wherein the cancer is a pediatric hypermutated cancer.

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

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

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obtaining a first population of TILs from a tumor resected from a patient by processing a tumor sample obtained from the patient into a tumor digest;

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

(c) performing a first expansion or a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the first expansion or the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

(f) harvesting the second plurality of subpopulations of TILs obtained from step (f), wherein the transition from step (e) to step (f) occurs without opening the system when optionally performed in a closed system; and

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(g) transferring the harvested subpopulations of TILs from step (f) to one or more infusion bags, wherein the transition from step (f) to (g) occurs without opening the system when optionally performed in a closed system.

334. A tumor infiltrating lymphocyte (TIL) composition comprising a therapeutic population of infiltrating lymphocytes (TILs), wherein the TIL composition is produced by a method comprising:

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

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

(c) performing a first expansion or a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the first expansion or the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for

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about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

(f) harvesting the second plurality of subpopulations of TILs obtained from step (f), wherein the transition from step (e) to step (f) occurs without opening the system when optionally performed in a closed system; and

(g) transferring the harvested subpopulations of TILs from step (f) to one or more infusion bags, wherein the transition from step (f) to (g) occurs without opening the system when optionally performed in a closed system.

335. The TIL composition of claim 334 wherein the TIL composition is a cryopreserved composition and wherein the method further comprises (h) cryopreserving the infusion bag comprising the harvested TIL population from step (g) using a cryopreservation process.

336. A method for treating a subject with cancer, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

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

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

(c) performing a first expansion or a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the first expansion or the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

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(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

(f) harvesting the second plurality of subpopulations of TILs obtained from step (f), wherein the transition from step (e) to step (f) occurs without opening the system when optionally performed in a closed system;

(g) transferring the harvested subpopulations of TILs from step (f) to one or more infusion bags, wherein the transition from step (f) to (g) occurs without opening the system when optionally performed in a closed system; and

(h) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (g) to the subject.

337. The method of claim 333-336, wherein in step (a) the tumor sample obtained from the patient is processed into multiple tumor fragments by (i) cryopreserving the tumor sample to produce a cryopreserved tumor sample; (ii) thawing the cryopreserved tumor sample to produce a thawed tumor sample; and (iii) fragmenting the thawed tumor sample into multiple tumor fragments.

338. The method of claim 333-336, wherein in step (a) the tumor sample obtained from the patient is processed into a tumor digest by (i) cryopreserving the tumor sample to produce a cryopreserved tumor sample; (ii) thawing the cryopreserved tumor sample to

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produce a thawed tumor sample; and (iii) digesting the thawed tumor sample to produce a tumor digest.

339. The method of claim 333-336, wherein in step (a) the tumor sample obtained from the patient is processed into a tumor digest by (i) cryopreserving the tumor sample to produce a cryopreserved tumor sample; (ii) thawing the cryopreserved tumor sample to produce a thawed tumor sample; (iii) fragmenting the thawed tumor sample into multiple tumor fragments; and (iv) digesting the multiple tumor fragments to produce a tumor digest.

340. The method of any of claims 333-336, wherein step (e) comprises seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container providing a third gas-permeable surface area at a seeding density of about $2x10^6$ cells/cm^2.

341. The method of claim 336-340, wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, triple negative 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.

342. The method of claim 341, wherein the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

343. The method of claim 341, wherein the cancer is melanoma.

344. The method of claim 341, wherein the cancer is HNSCC.

345. The method of claim 341, wherein the cancer is a cervical cancer.

346. The method of claim 341, wherein the cancer is NSCLC.

347. The method of claim 341, wherein the cancer is glioblastoma (including GBM).

348. The method of claim 341, wherein the cancer is gastrointestinal cancer.

349. The method of claim 341, wherein the cancer is a hypermutated cancer.

350. The method of claim 341, wherein the cancer is a pediatric hypermutated cancer.

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

(a) performing a first expansion or a priming first expansion by (i) thawing a cryopreserved tumor digest comprising a first population of TILs from a tumor that was resected from a subject, digested after the resection, and cryopreserved after the digestion, and (ii) culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the first expansion or the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) harvesting the second plurality of subpopulations of TILs obtained from step (c), wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system; and

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system.

352. A tumor infiltrating lymphocyte (TIL) composition comprising a therapeutic population of infiltrating lymphocytes (TILs), wherein the TIL composition is produced by a method comprising:

(a) performing a first expansion or a priming first expansion by (i) thawing a cryopreserved tumor comprising a first population of TILs from a tumor that was resected from a subject and cryopreserved after the resection, and (ii) culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the first expansion or the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

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(d) harvesting the second plurality of subpopulations of TILs obtained from step (c), wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system; and

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system.

353. The TIL composition of claim 352, wherein the TIL composition is a cryopreserved composition and wherein the method further comprises (f) cryopreserving the infusion bag comprising the harvested TIL population from step (e) using a cryopreservation process.

354. A method for treating a subject with cancer, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) performing a first expansion or a priming first expansion by (i) thawing a cryopreserved tumor digest comprising a first population of TILs from a tumor that was resected from a subject, digested after the resection, and cryopreserved after the digestion, and (ii) culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the first expansion or the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second

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plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) harvesting the second plurality of subpopulations of TILs obtained from step (c), wherein the transition from step (d) to step (e) occurs without opening the system when optionally performed in a closed system;

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system;(f) administering a therapeutically effective dosage of the harvested population of TILs from the infusion bag in step (e) to the subject.

355. The method of claim 351-354, wherein step (a)(i) comprises thawing a cryopreserved tumor comprising a first population of TILs from a tumor that was resected from a subject and cryopreserved after the resection to produce a thawed tumor, and fragmenting the thawed tumor into multiple tumor fragments, and wherein step (a)(ii) comprises culturing the multiple tumor fragments comprising the first population of TILs.

356. The method of any of claims 351-355, wherein step (c) comprises seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container providing a third gas-permeable surface area at a seeding density of about $2x10^6$ cells/cm^2.

357. The method of any of claims 351-356, wherein the first expansion or priming first expansion is performed for about 6 to 8 days.

358. The method of any of claims 351-356, wherein the rapid second expansion is performed for about 6 to 8 days.

359. The method of any of claims 351-356, wherein the third expansion is performed for about 6 to 8 days.

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360. The method of any of claims 351-356, wherein the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 18 to 24 days.

361. The method of any of claims 351-356, wherein the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 20 to 22 days.

362. The method of any of claims 351-356, wherein the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 21 days.

363. The method of any of claims 351-356, wherein the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 24 days or less.

364. The method of any of claims 351-356, wherein the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 22 days or less.

365. The method of any of claims 351-356, wherein the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 21 days or less.

366. The method of claim 354-365, wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, triple negative 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.

367. The method of claim 366, wherein the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

368. The method of claim 366, wherein the cancer is melanoma.

369. The method of claim 366, wherein the cancer is HNSCC.

370. The method of claim 366, wherein the cancer is a cervical cancer.

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371. The method of claim 366, wherein the cancer is NSCLC.

372. The method of claim 366, wherein the cancer is glioblastoma (including GBM).

373. The method of claim 366, wherein the cancer is gastrointestinal cancer.

374. The method of claim 366, wherein the cancer is a hypermutated cancer.

375. The method of claim 366, wherein the cancer is a pediatric hypermutated cancer.

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

(a) performing a first expansion or a priming first expansion by culturing a tumor sample comprising a first population of TILs from a tumor resected from a patient in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 1 to 5 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 4 to 8 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

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(d) harvesting the second plurality of subpopulations of TILs obtained from step (c), wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system; and

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system.

377. A tumor infiltrating lymphocyte (TIL) composition comprising a therapeutic population of infiltrating lymphocytes (TILs), wherein the TIL composition is produced by a method comprising:

(a) performing a first expansion or a priming first expansion by culturing a tumor sample comprising a first population of TILs from a tumor resected from a patient in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 1 to 5 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 4 to 8 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

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(d) harvesting the second plurality of subpopulations of TILs obtained from step (c), wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system; and

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system.

378. The TIL composition of claim 377, wherein the TIL composition is a cryopreserved composition and wherein the method further comprises (f) cryopreserving the infusion bag comprising the harvested TIL population from step (e) using a cryopreservation process.

379. A method for treating a subject with cancer, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) performing a first expansion or a priming first expansion by culturing a tumor sample comprising a first population of TILs from a tumor resected from a patient in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 1 to 5 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 4 to 8 days, wherein optionally each separate container is a closed container

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providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) harvesting the second plurality of subpopulations of TILs obtained from step (c), wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system; and

(f) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (e) to the subject.

380. The method of claim 376-379, wherein before culturing in step (a) the tumor sample is fragmenting into multiple tumor fragments comprising the first population of TILs.

381. The method of claim 376-379, wherein before culturing in step (a) the tumor sample is digested to produce a tumor digest comprising the first population of TILs.

382. The method of any of claims 376-381, wherein the first expansion or priming first expansion is performed for about 6-8 days.

383. The method of any of claims 376-381, wherein the rapid second expansionis performed for about 2-4 days.

384. The method of any of claims 376-381, wherein the third expansion is each performed for about 5-7 days.

385. The method of any of claims 376-381, wherein the first expansion or priming first expansion is performed for about 7 days, the rapid second expansion is performed for about 3 days, and the third expansion is performed for about 6 days.

386. The method of any of claims 376-381, wherein steps (a)-(c) are performed in about 14-18 days.

387. The method of any of claims 376-381, wherein steps (a)-(c) are performed in about 16 days.

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388. The method of any of claims 376-381, wherein steps (a)-(c) are performed in about 18 days or less.

389. The method of any of claims 376-381, wherein steps (a)-(c) are performed in about 16 days or less.

390. The method of any of claims 376-381, wherein step (c) comprises seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container providing a third gas-permeable surface area at a seeding density of about $2x10^6$ cells/cm^2.

391. The method of claim 379-390, wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, triple negative 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.

392. The method of claim 391, wherein the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

393. The method of claim 391, wherein the cancer is melanoma.

394. The method of claim 391, wherein the cancer is HNSCC.

395. The method of claim 391, wherein the cancer is a cervical cancer.

396. The method of claim 391, wherein the cancer is NSCLC.

397. The method of claim 391, wherein the cancer is glioblastoma (including GBM).

398. The method of claim 391, wherein the cancer is gastrointestinal cancer.

399. The method of claim 391, wherein the cancer is a hypermutated cancer.

400. The method of claim 391, wherein the cancer is a pediatric hypermutated cancer.

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

(a) performing a first expansion or a priming first expansion by culturing a tumor sample comprising a first population of TILs from a tumor resected from a patient in a

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cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) harvesting the second plurality of subpopulations of TILs obtained from step (f), wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system; and

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system.

402. A tumor infiltrating lymphocyte (TIL) composition comprising a therapeutic population of infiltrating lymphocytes (TILs), wherein the TIL composition is produced by a method comprising:

(a) performing a first expansion or a priming first expansion by culturing a tumor sample comprising a first population of TILs from a tumor resected from a patient in a

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cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) harvesting the second plurality of subpopulations of TILs obtained from step (f), wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system; and

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system.

403. The method of claim 402, wherein the TIL composition is a cryopreserved composition and wherein the method further comprises (f) cryopreserving the infusion bag comprising the harvested TIL population from step (e) using a cryopreservation process.

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404. A method for treating a subject with cancer, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) performing a first expansion or a priming first expansion by culturing a tumor sample comprising a first population of TILs from a tumor resected from a patient in a cell culture medium comprising IL-2, and optionally OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed for about 5 to 9 days to obtain the second population of TILs, wherein the first expansion or the priming first expansion is optionally performed in a closed container providing a first gas-permeable surface area;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and APCs, to produce a third population of TILs, wherein the second expansion is performed for about 5 to 9 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (a) to step (b) occurs without opening the system when optionally performed in a closed system;

(c) performing a third expansion by dividing the third population of TILs into a first plurality of subpopulations of TILs, seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container, adding a cell culture medium supplemented with IL-2, and optionally OKT-3, and culturing to produce a second plurality of subpopulations of TILs, wherein the third expansion is performed for about 5 to 9 days, wherein optionally each separate container is a closed container providing a third gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system when optionally performed in a closed system;

(d) harvesting the second plurality of subpopulations of TILs obtained from step (f), wherein the transition from step (c) to step (d) occurs without opening the system when optionally performed in a closed system;

(e) transferring the harvested subpopulations of TILs from step (d) to one or more infusion bags, wherein the transition from step (d) to (e) occurs without opening the system when optionally performed in a closed system; and

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(f) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (e) to the subject.

405. The method of claim 401-404, wherein before culturing in step (a) the tumor sample is fragmenting into multiple tumor fragments comprising the first population of TILs.

406. The method of claim 401-404, wherein before culturing in step (a) the tumor sample is digested to produce a tumor digest comprising the first population of TILs.

407. The method of any of claims 401-406, wherein the first expansion or priming first expansion is performed for about 6 to 8 days.

408. The method of any of claims 401-406, wherein the rapid second expansion is performed for about 6 to 8 days.

409. The method of any of claims 401-406, wherein the third expansion is performed for about 6 to 8 days.

410. The method of any of claims 401-406, wherein the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 18 to 24 days.

411. The method of any of claims 401-406, wherein the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 20 to 22 days.

412. The method of any of claims 401-406, wherein the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 21 days.

413. The method of any of claims 401-406, wherein the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 24 days or less.

414. The method of any of claims 401-406, wherein the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 22 days or less.

415. The method of any of claims 401-406, wherein the first expansion or priming first expansion, the rapid second expansion, and the third expansion are performed in about 21 days or less.

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416. The method of any of claims 401-406, wherein step (c) comprises seeding each subpopulation of the first plurality of subpopulations of TILs into a separate container providing a third gas-permeable surface area at a seeding density of about $2x10^6$ cells/cm^2.

417. The method of claim 404-416, wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, triple negative 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.

418. The method of claim 417, wherein the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

419. The method of claim 417, wherein the cancer is melanoma.

420. The method of claim 417, wherein the cancer is HNSCC.

421. The method of claim 417, wherein the cancer is a cervical cancer.

422. The method of claim 417, wherein the cancer is NSCLC.

423. The method of claim 417, wherein the cancer is glioblastoma (including GBM).

424. The method of claim 417, wherein the cancer is gastrointestinal cancer.

425. The method of claim 417, wherein the cancer is a hypermutated cancer.

426. The method of claim 417, wherein the cancer is a pediatric hypermutated cancer.

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Figure 1A

Process 2A: about 22 days from Steps A - E	Process GEN 3: about 14-18 days from Steps A - E
STEP A Obtain Patient Tumor Sample (optionally can be frozen before Step B; optionally tumor sample can be a core/small biopsy)	**STEP A** Obtain Patient Tumor Sample (optionally can be frozen before Step B)
STEP B First Expansion (physical fragmentation to at least 40 fragments per container grown for about 3 days to 14 days with media comprising IL-2)	**STEP B** Priming First Expansion (physical fragmentation of up to 60 fragments per container grown for about 1 days to 7/8 days with media comprising IL-2, OKT-3, and optionally antigen-presenting feeder cells)
STEP C First Expansion to Second Expansion Transition (Step B TILs directly move to Step D, optionally on Step B day 11)	**STEP C** Priming First Expansion to Rapid Second Expansion Transition (Step B TILs directly move to Step D on day 7/8)
STEP D Second Expansion (TILs grown in growth media medium comprising IL-2, OKT-3, and antigen-presenting feeder cells in a closed container)	**STEP D** Rapid Second Expansion (TILs grown in growth media medium comprising IL-2, OKT-3, and 2X antigen-presenting feeder cells; Days 10-11 scale up and add additional IL-2)
STEP E Harvest TILS from Step D (TILs harvested via closed system)	**STEP E** Harvest TILS from Step D
STEP F Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)	**STEP F** Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)

Figure 1B

Process GEN 3: about 14-18 days from Steps A - E

STEP A
Obtain Patient Tumor Sample
(optionally can be frozen before Step B)

STEP B
Priming First Expansion
(physical fragmentation of up to 60 fragments per container grown for about 1 days to 7/8 days with media comprising IL-2, OKT-3, and optionally antigen-presenting feeder cells)

STEP C
Priming First Expansion to Rapid Second Expansion Transition
(Step B TILs directly move to Step D on day 7/8)

STEP D
Rapid Second Expansion
(TILs grown in growth media medium comprising IL-2, OKT-3, and 2X antigen-presenting feeder cells; Days 10-11 scale up and add additional IL-2)

STEP E
Harvest TILS from Step D

STEP F
Final Formulation and/or Transfer to Infusion Bag
(optionally cryopreserve)

Figure 1C

Embodiment GEN 3.0: about 14-18 days from Steps A - E	**Embodiment GEN 3.1 control: about 14-18 days from Steps A - E**	**Embodiment GEN 3.1 Test/F: about 14-18 days from Steps A - E**
STEP A Obtain Patient Tumor Sample (optionally can be frozen before Step B)	**STEP A** Obtain Patient Tumor Sample (optionally can be frozen before Step B)	**STEP A** Obtain Patient Tumor Sample (optionally can be frozen before Step B)
STEP B Priming First Expansion (physical fragmentation of up to 60 fragments per container grown for about 1 days to 7/8 days with media comprising IL-2)	**STEP B** Priming First Expansion (physical fragmentation of up to 60 fragments per container grown for about 1 days to 7/8 days with media comprising IL-2, and OKT-3)	**STEP B** Priming First Expansion (physical fragmentation of up to 60 fragments per container grown for about 1 days to 7/8 days with media comprising IL-2, OKT-3, and antigen-presenting feeder cells)
STEP C Priming First Expansion to Rapid Second Expansion Transition (Step B TILs directly move to Step D on day 7/8)	**STEP C** Priming First Expansion to Rapid Second Expansion Transition (Step B TILs directly move to Step D on day 7/8)	**STEP C** Priming First Expansion to Rapid Second Expansion Transition (Step B TILs directly move to Step D on day 7/8)
STEP D Rapid Second Expansion (TILs grown in growth media medium comprising IL-2, OKT-3, and antigen-presenting feeder cells; Days 10-11 scale up and add additional IL-2)	**STEP D** Rapid Second Expansion (TILs grown in growth media medium comprising IL-2, OKT-3, and 2X antigen-presenting feeder cells; Days 10-11 scale up and add additional IL-2)	**STEP D** Rapid Second Expansion (TILs grown in growth media medium comprising IL-2, OKT-3, and 2X antigen-presenting feeder cells; Days 10-11 scale up and add additional IL-2)
STEP E Harvest TILS from Step D	**STEP E** Harvest TILS from Step D	**STEP E** Harvest TILS from Step D
STEP F Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)	**STEP F** Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)	**STEP F** Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)

Figure 1D

Modified Gen 2-like Process: about 22 days from Steps A - E

STEP A
Obtain Patient Tumor Sample
(optionally can be frozen before Step B;
optionally tumor sample can be a core/small biopsy)

STEP B1
Initial Culture
physical fragmentation of up to 60 tumor fragments or up to 10 cores/small biopsies per container, TILs grown for 3 days in growth medium comprising IL-2

STEP B2
Priming First Expansion
TILs grown for 8 days in growth medium comprising IL-2, OKT-3, and antigen-presenting feeder cells)

STEP C
Priming First Expansion to Rapid Second Expansion Transition
(Step B TILs directly move to Step D on day 11)

STEP D
Rapid Second Expansion
(volume reduced; TILs grown in growth media medium comprising IL-2, OKT-3, and 50X antigen-presenting feeder cells; Day 16 scale up and add additional IL-2)

STEP E
Harvest TILS from Step D

STEP F
Final Formulation and/or Transfer to Infusion Bag
(optionally cryopreserve)

Figure 1E

Second Generation Process GEN 3 (A): about 14-18 days from Steps A – E

STEP A
Obtain Patient Tumor Sample
(optionally can be frozen before Step B)

STEP B
Priming First Expansion
(physical fragmentation of up to 60 fragments per container grown for about 1 day to 7/8 days with media comprising IL-2 and culture supernatant obtained from a culture of antigen-presenting feeder cells (APCs))

STEP C
Priming First Expansion to Rapid Second Expansion
Transition
(Step B TILs directly move to Step D on day 7/8)

STEP D
Rapid Second Expansion
(TILs grown in growth media medium comprising IL-2, OKT-3 and APCs
Days 10-11 scale up and add additional IL-2)

STEP E
Harvest TILS from Step D

STEP F
Final Formulation and/or Transfer to Infusion Bag
(optionally cryopreserve)

Figure 1F

Second Generation Process GEN 3 (B): about 14-18 days from Steps A – E

STEP A
Obtain Patient Tumor Sample
(optionally can be frozen before Step B)

STEP B
Priming First Expansion
(physical fragmentation of up to 60 fragments per container grown for about 1 day to 7/8 days with media comprising IL-2, OKT-3 and APCs)

STEP C
Priming First Expansion to Rapid Second Expansion Transition
(Step B TILs directly move to Step D on day 7/8)

STEP D
Rapid Second Expansion
(TILs grown in growth media medium comprising IL-2 and culture supernatant obtained from a culture of APCs; Days 10-11 scale up and add additional IL-2)

STEP E
Harvest TILS from Step D

STEP F
Final Formulation and/or Transfer to Infusion Bag
(optionally cryopreserve)

Figure 1G

Second Generation Process GEN 3 (C): about 14-18 days from Steps A – E

STEP A
Obtain Patient Tumor Sample
(optionally can be frozen before Step B)

STEP B
Priming First Expansion
(physical fragmentation of up to 60 fragments per container grown for about 1 day to 7/8 days with media comprising IL-2 and culture supernatant obtained from a culture of APCs)

STEP C
Priming First Expansion to Rapid Second Expansion Transition
(Step B TILs directly move to Step D on day 7/8)

STEP D
Rapid Second Expansion
(TILs grown in growth media medium comprising IL-2 and culture supernatant obtained from a culture of APCs; Days 10-11 scale up and add additional IL-2)

STEP E
Harvest TILS from Step D

STEP F
Final Formulation and/or Transfer to Infusion Bag
(optionally cryopreserve)

Figure 2

EXEMPLARY GEN 3

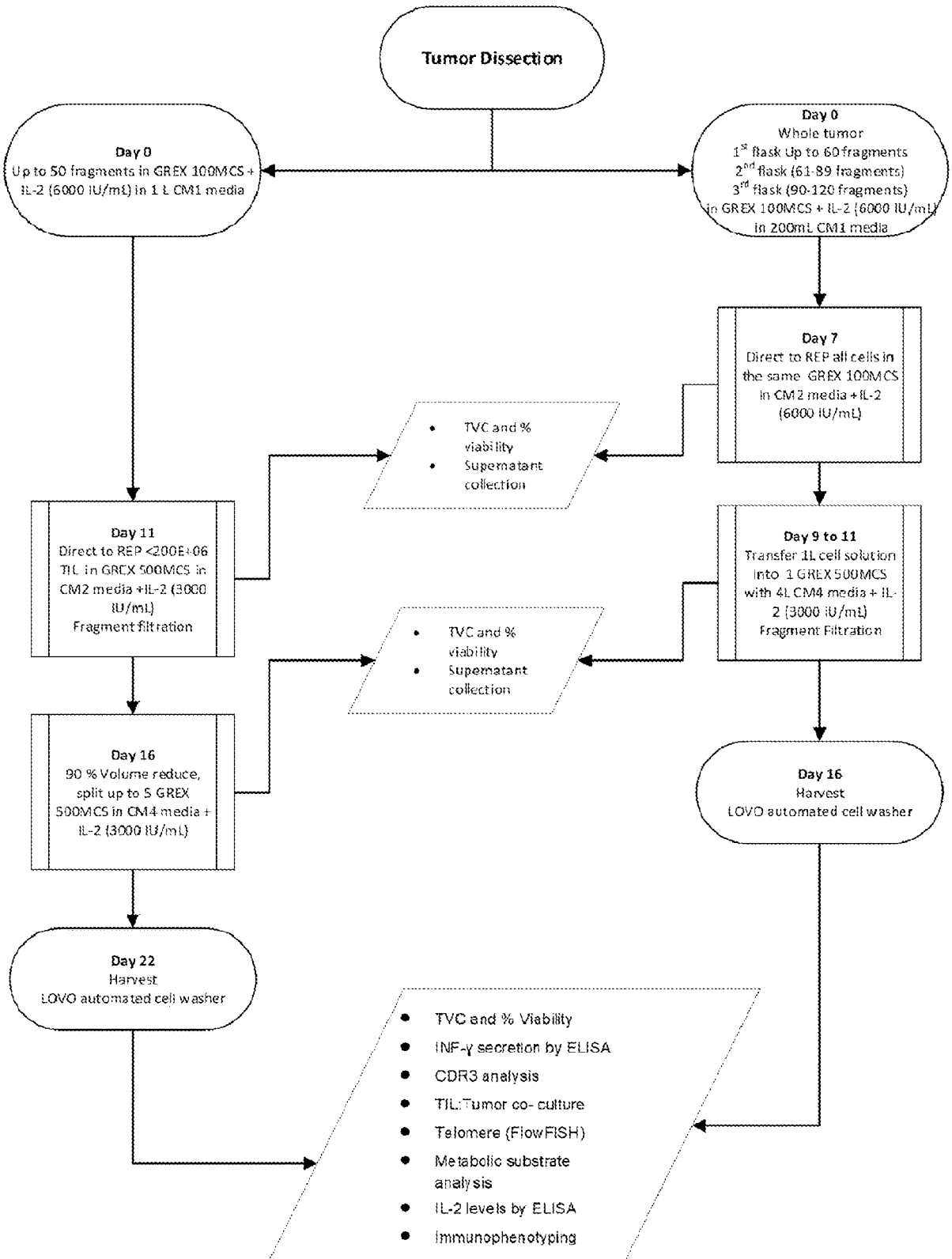


Figure 3

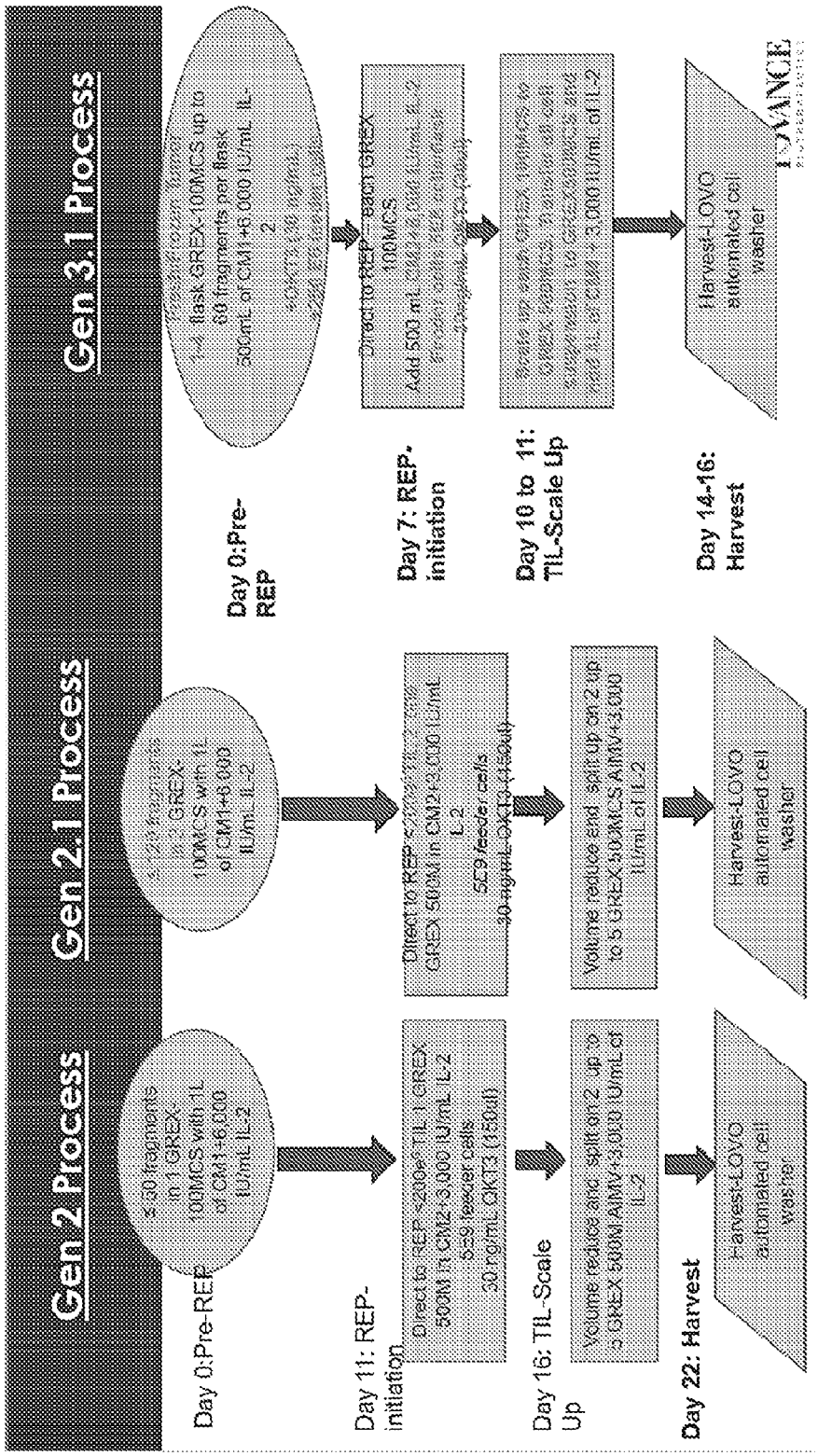

Gen 2 Process
Gen 2.1 Process
Gen 3.1 Process
Day 0:Pre-REP
Day 11: REP-initiation
Day 16: TIL-Scale Up
Day 22: Harvest
Harvest-LOVO automated cell washer
Day 0:Pre-REP
Day 7: REP-initiation
Day 10 to 11: TIL-Scale Up
Day 14-16: Harvest
Harvest-LOVO automated cell washer
LOVANCE

Figure 4

STEP	Gen 2	Gen 2A	Gen 3.0 Optimized
Pre REP- day 0	≤ 50 fragments/ 1 G-Rex 100MCS - **11 days**	≤ 180 fragments/ 3 G-Rex, Pre-formulated CM1 warmed media 100MCS - **11 days**	***Fresh or Frozen Tumor*** Whole tumor with ≤ 30 fragments up to 60 fragments per 1 G-Rex 100MCS (up to 4 G-Rex), preformulated warmed media - **7 days. Pre REP. Feeders 2.5 E8 cells + OKT-3 (30ng/mL)**
REP Initiation	Direct to REP- Day 11- **<200 E6 TIL** 1 G-Rex 500MCS	Direct to REP- Day 11- **<200 E6 TIL** Pre-formulated CM2 warmed media in one G-Rex 500MCS	Direct to REP- Day **7-all cells TIL- same G-Rex 100MCS (100MCS up to 4 GREX), Standard media or Defined Media (Serum free). Addition Feeders 5 E8 cells +OKT-3 (30ng/mL)**
TIL propagation or Scale up	1 to 5 G-REX 500MCS **Split day 16**	2 to 5 G-REX 500MCS Pre-formulated CM4 warmed media **Split day 16**	From G-REX 100MCS transfer TIL suspension to G-REX 500MCS , up to 4 GREX 500 MCS- Standard media or Defined Media (Serum Free) **Scale up on day 10 or 11**
Harvest	**Harvest day 22,** LOVO-automated cell washer	**Harvest day 22,** LOVO-automated cell washer (5 wash cycle)	**Harvest day 14 or 16** LOVO- automated cell washer (5 wash cycle)
Final formulation	Cryopreserved Product 300IU/ml IL2- CS10 in LN_2, multiple aliquots	Cryopreserved Product 300IU/ml IL2- CS10 in LN_2, multiple aliquots	Cryopreserved product 300IU/ml IL-2-CS10 in LN_2, multiple aliquots
Process time	**22 days**	**22 days**	**16 days**

Figure 5

Process Day	Conditions	Gen 3.1
Day 0- pre REP initiation	Media CM1	500 mL
	IL-2 (6000 IU/mL)	+
	OKT-3 (30ng/mL)	+
	Feeders (250 E+06)	+

Process Day	Conditions	Gen 3.1
Day 7- REP initiation	Media CM2	500 mL
	IL-2 (6000 IU/mL)	+
	OKT-3 (30ng/mL) added on Day 7	+
	Feeders Added on Day 7	500 E06
	Total Feeders at Day	750 E+06

Process Day	Conditions	Gen 3.1
Day 9-11 –Scale Up	From G-REX 100MCS transfer TIL suspension to 1 G-REX 500MCS (up to 3 GREX 500MCS)	Yes
Day 16- Harvest	LOVO- automated cell washer	Yes

Figure 6

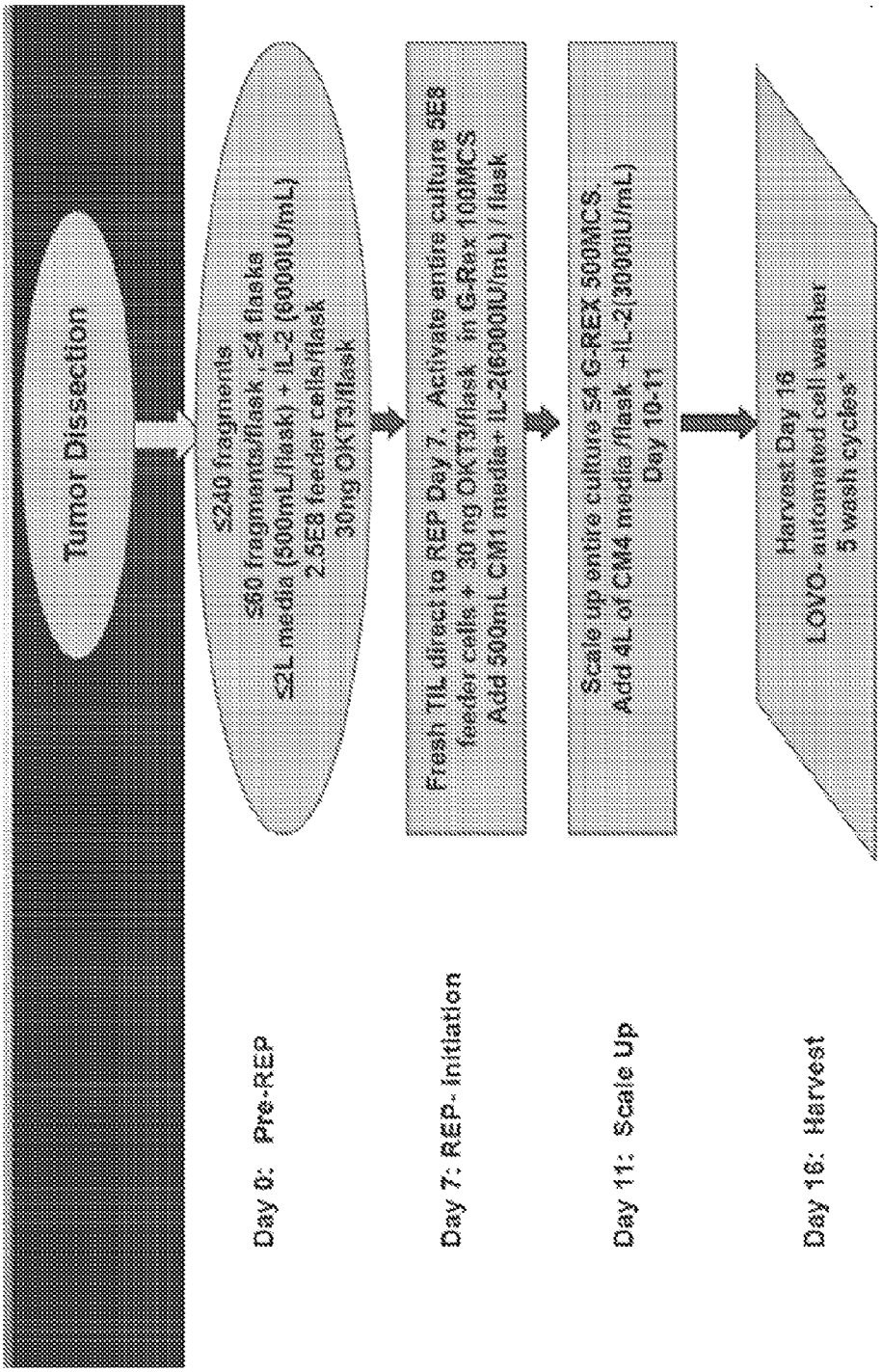

Tumor Dissection
Day 0: Pre-REP
≤240 fragments
≤30 fragments/flask ,≤4 flasks
≤2L media (500mL/flask) + IL-2 (6000IU/mL)
2.5E8 feeder cells/flask
30ng OKT3/flask
Day 7: REP- initiation
Fresh TIL direct to REP Day 7. Activate entire culture 5E8
feeder cells + 30 ng OKT3/flask in G-Rex 100MCS
Add 500mL CM1 media+ IL-2(6000IU/mL) / flask
Day 11: Scale Up
Scale up entire culture 24 G-REX 500MCS
Add 4L of CM4 media /flask +IL-2(3000IU/mL)
Day 10-11
Day 16: Harvest
Harvest Day 16
LOVO- automated cell washer
5 wash cycles*

Figure 7

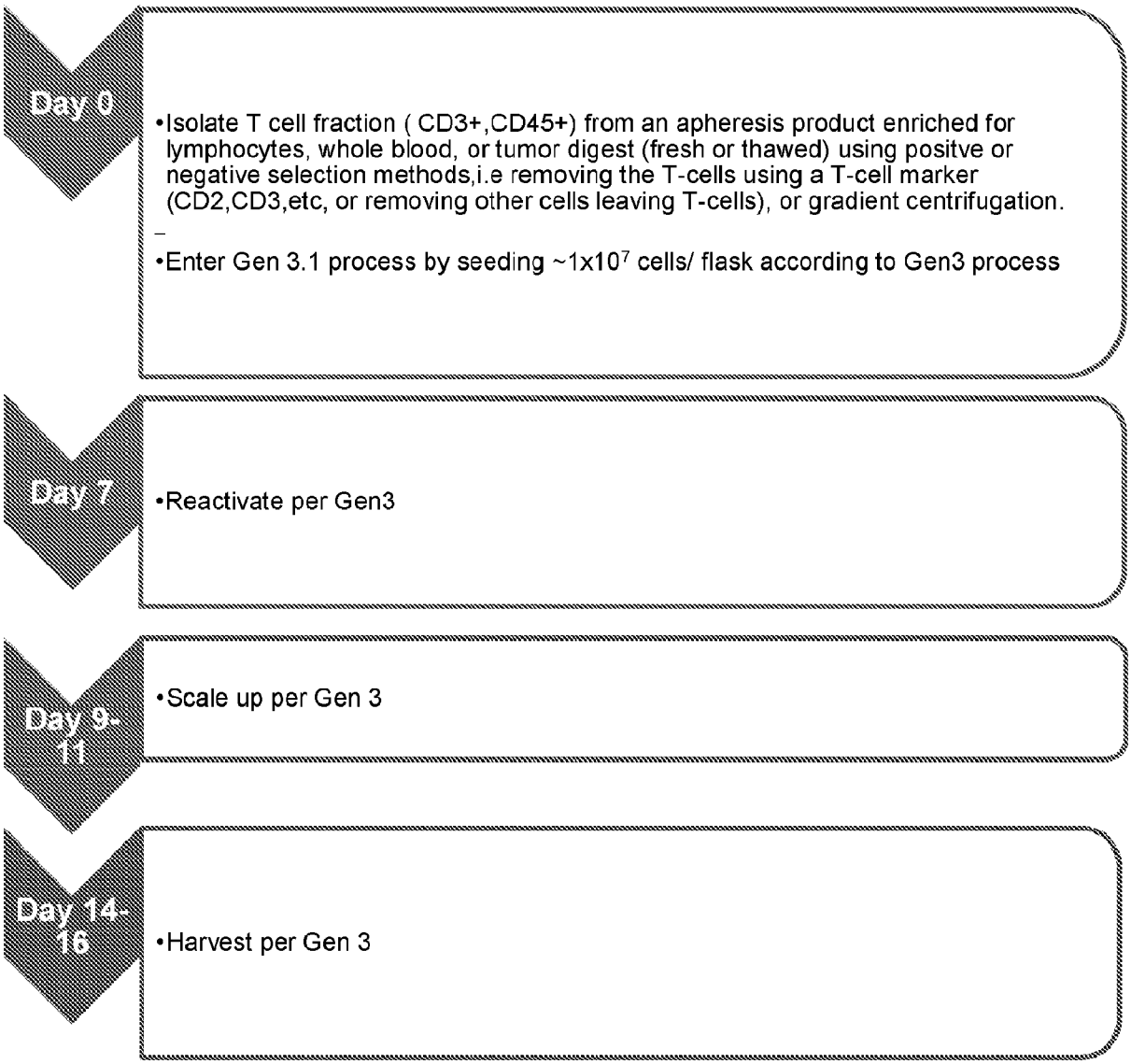

Day 0
•Isolate T cell fraction (CD3+,CD45+) from an apheresis product enriched for lymphocytes, whole blood, or tumor digest (fresh or thawed) using positve or negative selection methods,i.e removing the T-cells using a T-cell marker (CD2,CD3,etc, or removing other cells leaving T-cells), or gradient centrifugation.
•Enter Gen 3.1 process by seeding ~1x10^7 cells/ flask according to Gen3 process
Day 7
•Reactivate per Gen3
Day 9-11
•Scale up per Gen 3
Day 14-16
•Harvest per Gen 3

Figure 8

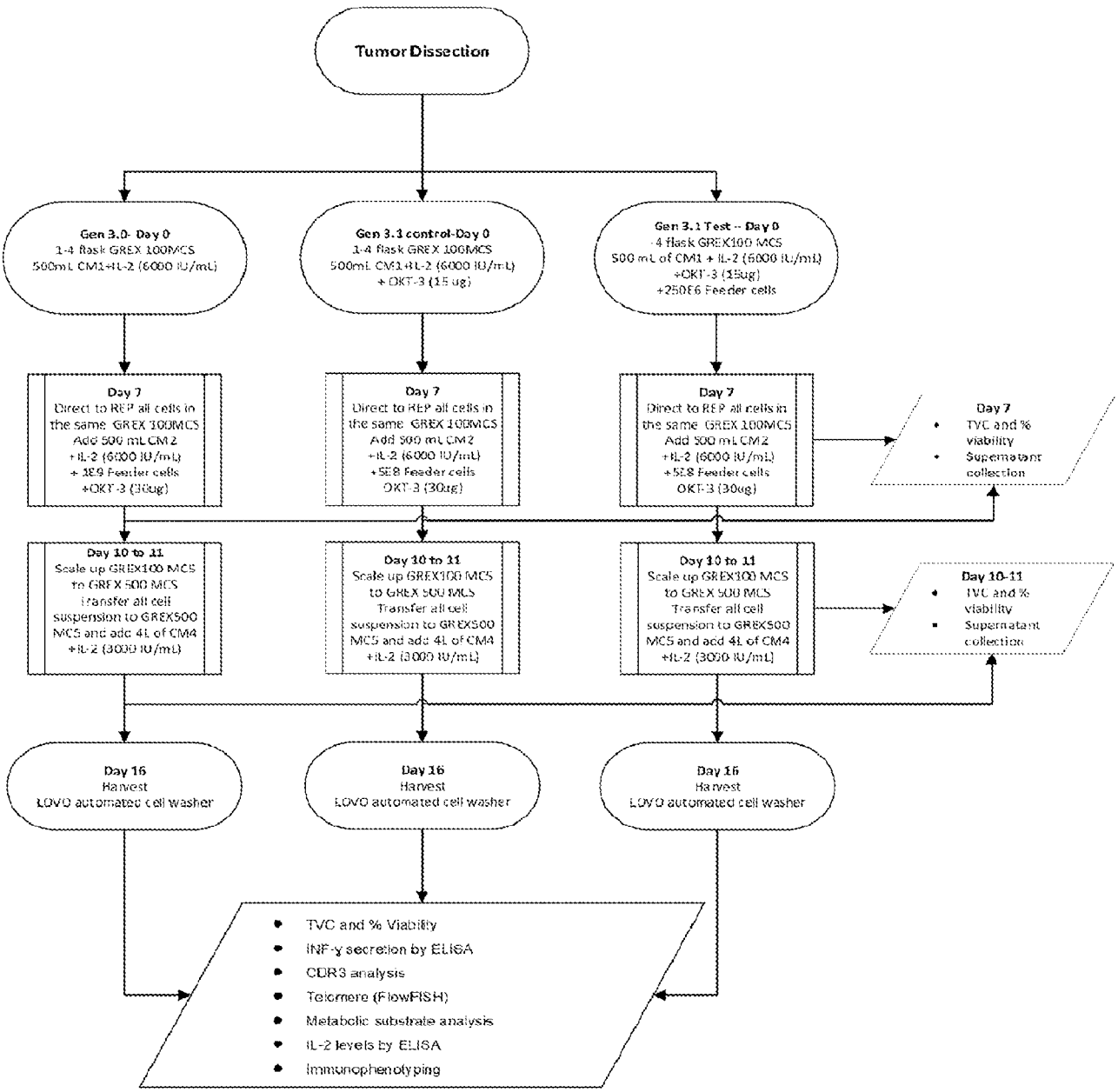

Tumor Dissection
Gen 3.0- Day 0
1-4 flask GREX 100MCS
500mL CM1+IL-2 (6000 IU/mL)
Gen 3.1 control-Day 0
1-4 flask GREX 100MCS
500mL CM1+IL-2 (6000 IU/mL)
+ OKT-3 (15 ug)
Gen 3.1 Test -- Day 0
-4 flask GREX100 MCS
500 mL of CM1 + IL-2 (6000 IU/mL)
+OKT-3 (15ug)
+250E6 Feeder cells
Day 7
Direct to REP all cells in the same GREX 100MCS
Add 500 mL CM2
+IL-2 (6000 IU/mL)
+ 1E9 Feeder cells
+OKT-3 (30ug)
Day 7
Direct to REP all cells in the same GREX 100MCS
Add 500 mL CM2
+IL-2 (6000 IU/mL)
+5E8 Feeder cells
OKT-3 (30ug)
Day 7
Direct to REP all cells in the same GREX 100MCS
Add 500 mL CM2
+IL-2 (6000 IU/mL)
+5E8 Feeder cells
OKT-3 (30ug)
Day 7
TVC and % viability
Supernatant collection
Day 10 to 11
Scale up GREX100 MCS to GREX 500 MCS
Transfer all cell suspension to GREX500 MCS and add 4L of CM4
+IL-2 (3000 IU/mL)
Day 10 to 11
Scale up GREX100 MCS to GREX 500 MCS
Transfer all cell suspension to GREX500 MCS and add 4L of CM4
+IL-2 (3000 IU/mL)
Day 10 to 11
Scale up GREX100 MCS to GREX 500 MCS
Transfer all cell suspension to GREX500 MCS and add 4L of CM4
+IL-2 (3000 IU/mL)
Day 10-11
TVC and % viability
Supernatant collection
Day 16
Harvest
LOVO automated cell washer
Day 16
Harvest
LOVO automated cell washer
Day 16
Harvest
LOVO automated cell washer
TVC and % Viability
INF-γ secretion by ELISA
CDR3 analysis
Telomere (FlowFISH)
Metabolic substrate analysis
IL-2 levels by ELISA
Immunophenotyping

Figure 9

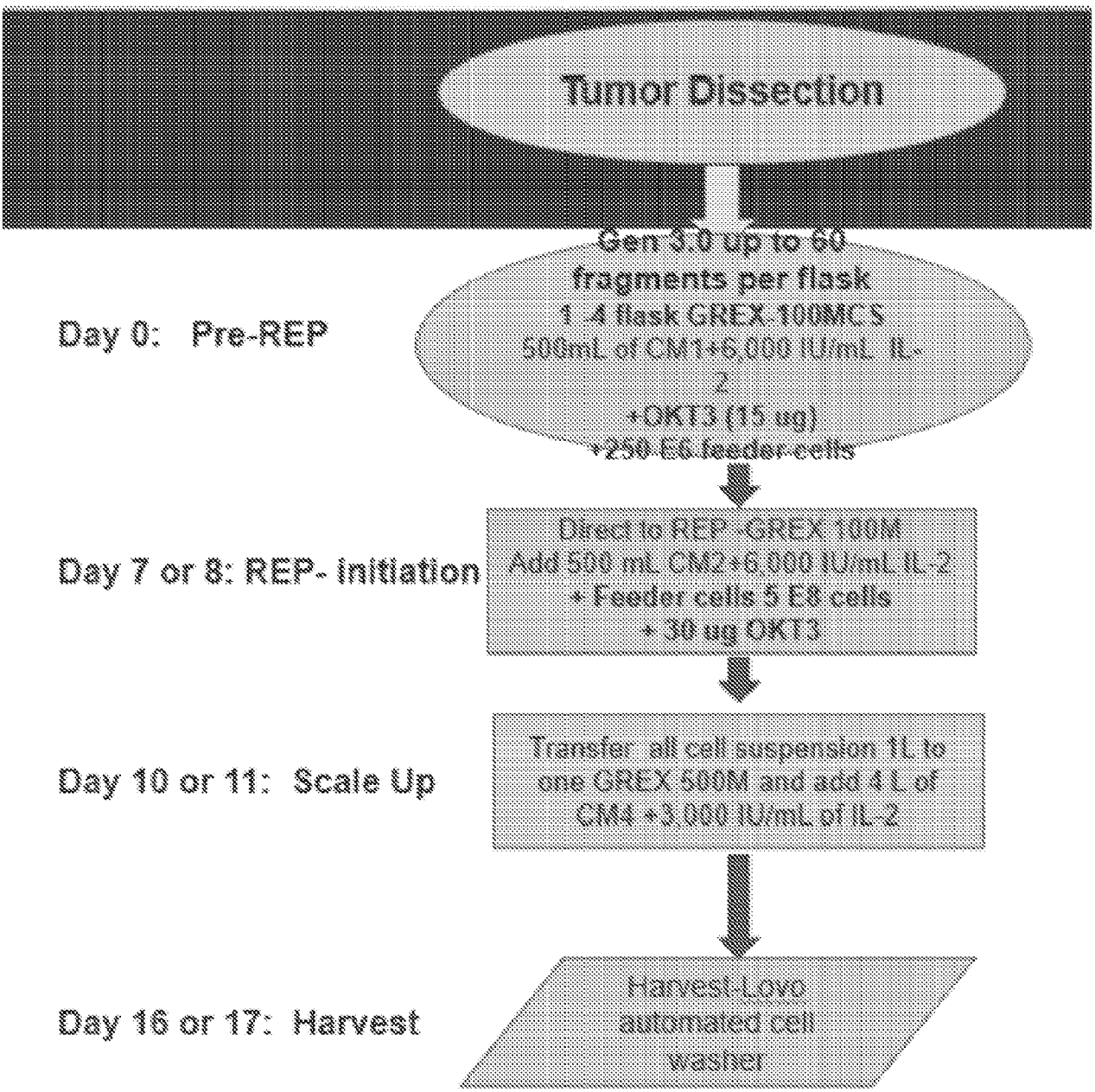

Tumor Dissection
Day 0: Pre-REP
Gen 3.0 up to 60 fragments per flask
1 -4 flask GREX-100MCS
500mL of CM1+6,000 IU/mL IL-2
+OKT3 (15 ug)
+250 E6 feeder cells
Day 7 or 8: REP- initiation
Direct to REP -GREX 100M
Add 500 mL CM2 +6,000 IU/mL IL-2
+ Feeder cells 5 E8 cells
+ 30 ug OKT3
Day 10 or 11: Scale Up
Transfer all cell suspension 1L to one GREX 500M and add 4 L of CM4 +3,000 IU/mL of IL-2
Day 16 or 17: Harvest
Harvest-Lovo automated cell washer

Figure 10A

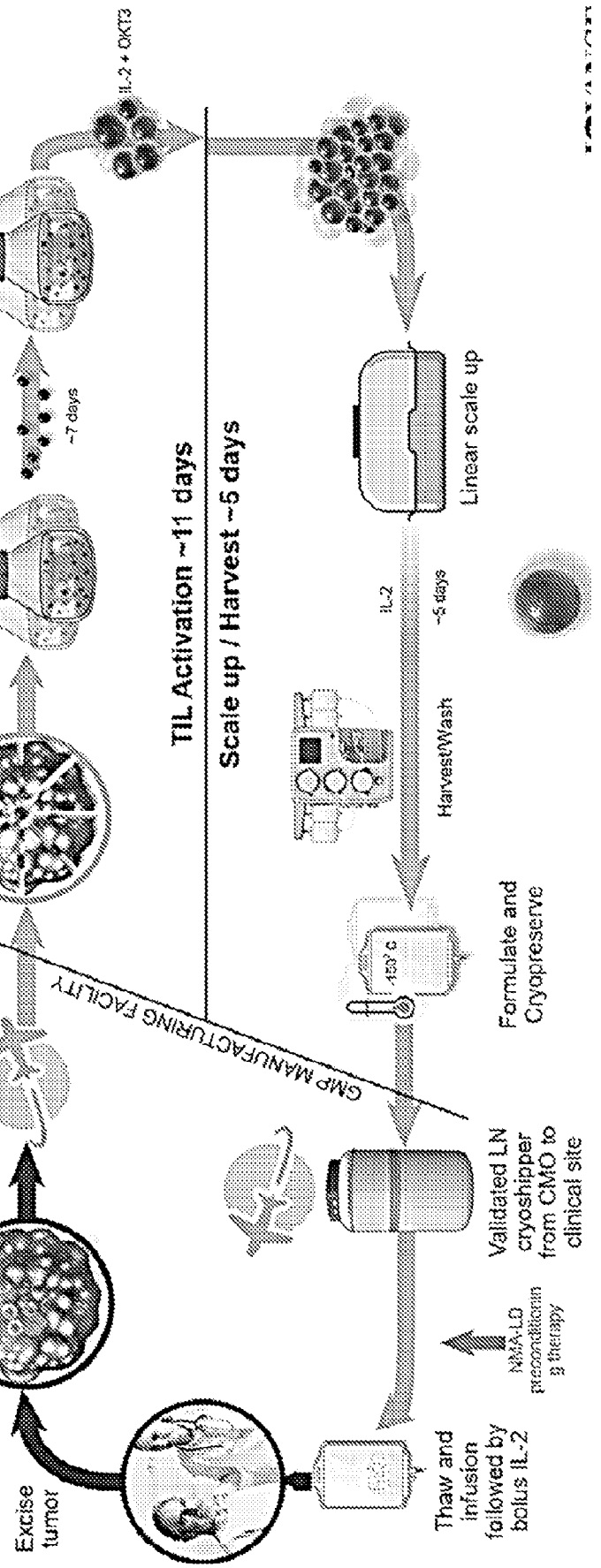

Excise tumor
Express courier from clinical site to CMO
GMP MANUFACTURING FACILITY
Cut into fragments
Primary culture and activation
IL-2 + OKT3
~7 days
Secondary culture and activation
IL-2 + OKT3
TIL Activation ~11 days
Scale up / Harvest ~5 days
Linear scale up
IL-2
~5 days
Harvest/Wash
Formulate and Cryopreserve
Validated LN cryoshipper from CMO to clinical site
Thaw and infusion followed by bolus IL-2

Figure 10B

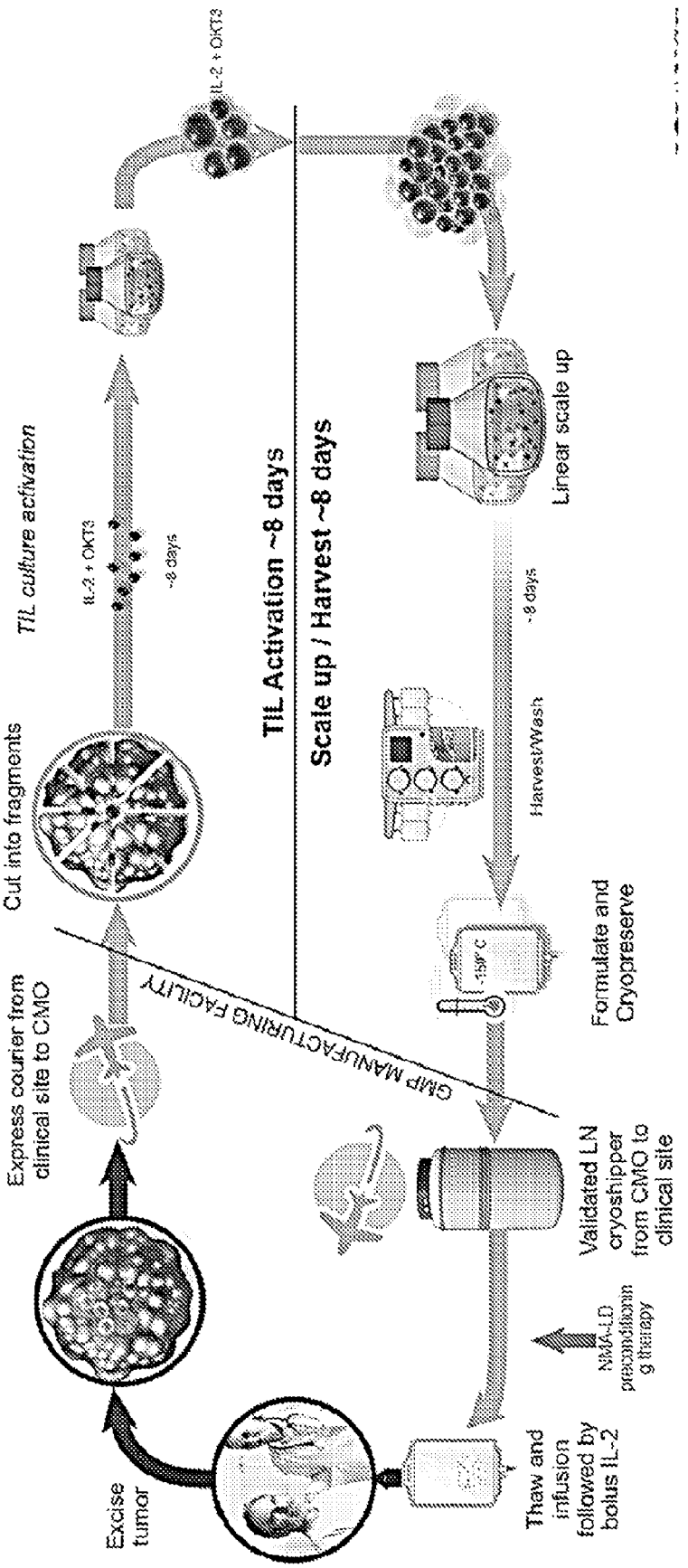
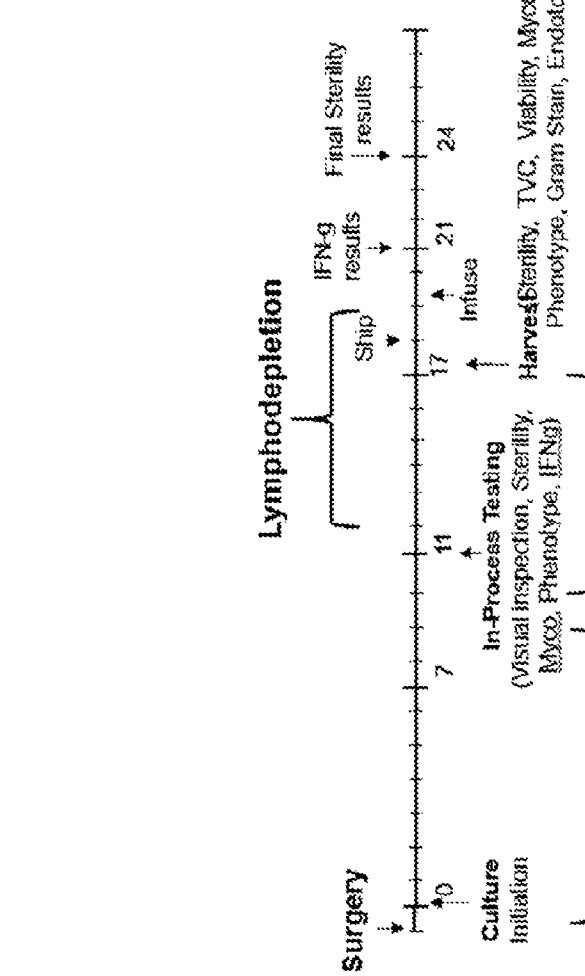

Excise tumor
Thaw and infusion followed by bolus IL-2
NMA-LD preconditioning therapy
Validated LN cryoshipper from CMO to clinical site
Express courier from clinical site to CMO
GMP MANUFACTURING FACILITY
Cut into fragments
TIL culture activation
IL-2 + OKT3
~8 days
TIL Activation ~8 days
Scale up / Harvest ~8 days
Linear scale up
~8 days
Harvest/Wash
Formulate and Cryopreserve
IL-2 + OKT3

Figure 11



LD on:
Day 10-11 Viability
Day 10-11 Phenotype
Day 10-11 myco
Day 10-11 IFNg*

Release for infusion on:
Gram Stain
Day 10-11 Sterility
Day 16-17 Mycoplasma
Day 16-17 Endotoxin
Day 16-17 Phenotyping
Day 16-17 TVC count
Day 16-17 Viability

Final Release (CoA)
Day 16-17 Frozen Thawed IFNg (ELISA)
Day 16-17 Endotoxin
Day 16-17 Phenotyping
Day 16-17 TVC count
Day 16-17 Viability
Day 16-17 Sterility

*** Restimulation for ELISA on Day 10-11 will be from a fresh / frozen in-process sample reportable On Day 14**

Figure 12

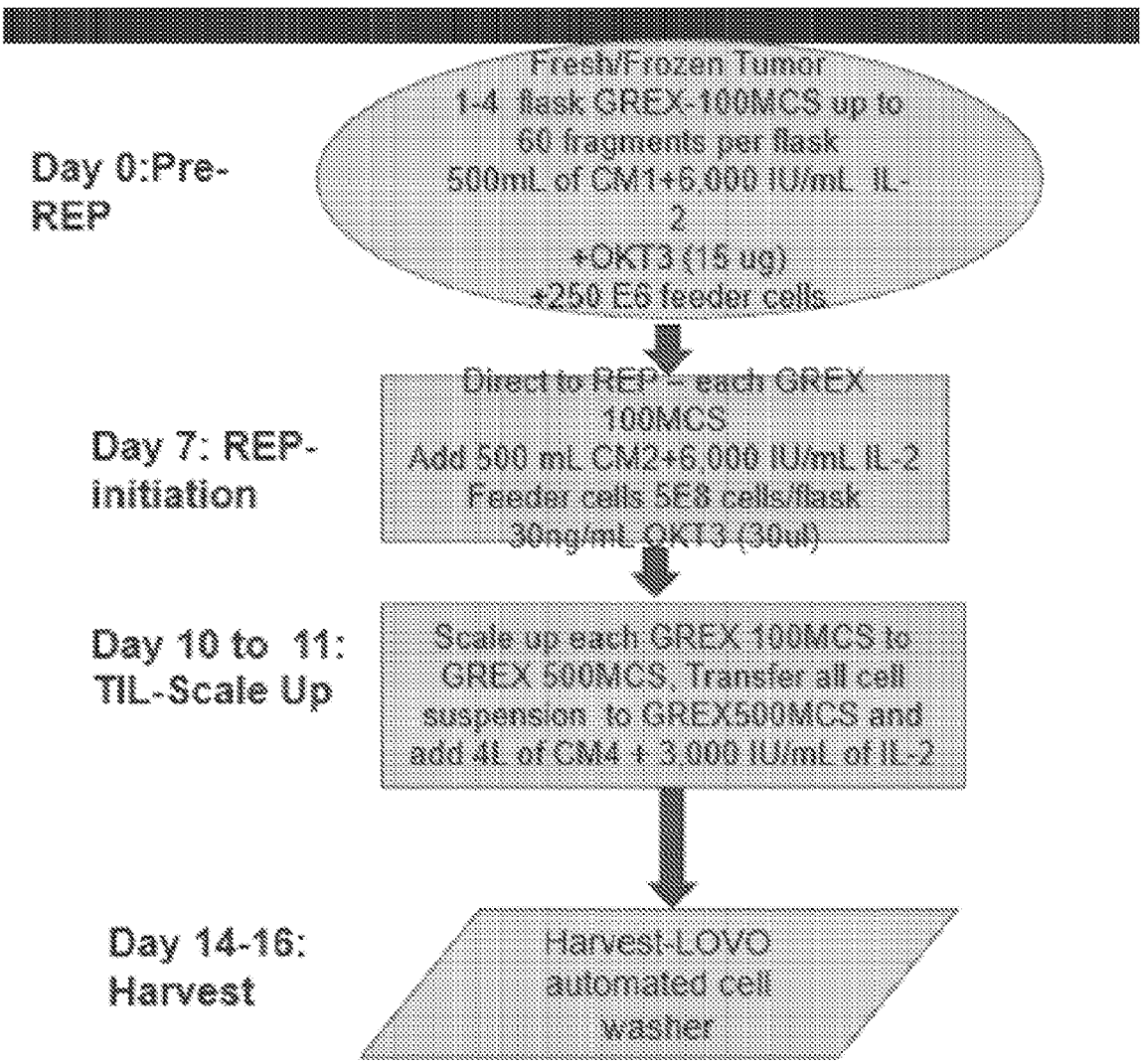

Day 0:Pre-REP
Fresh/Frozen Tumor
1-4 flask GREX-100MCS up to 60 fragments per flask
500mL of CM1+6,000 IU/mL IL-2
+OKT3 (15 ug)
+250 E6 feeder cells
Day 7: REP-initiation
Direct to REP – each GREX 100MCS
Add 500 mL CM2+6,000 IU/mL IL-2
Feeder cells 5E8 cells/flask
30ng/mL OKT3 (30ul)
Day 10 to 11: TIL-Scale Up
Scale up each GREX 100MCS to GREX 500MCS. Transfer all cell suspension to GREX500MCS and add 4L of CM4 + 3,000 IU/mL of IL-2
Day 14-16: Harvest
Harvest-LOVO automated cell washer

Figure 13A

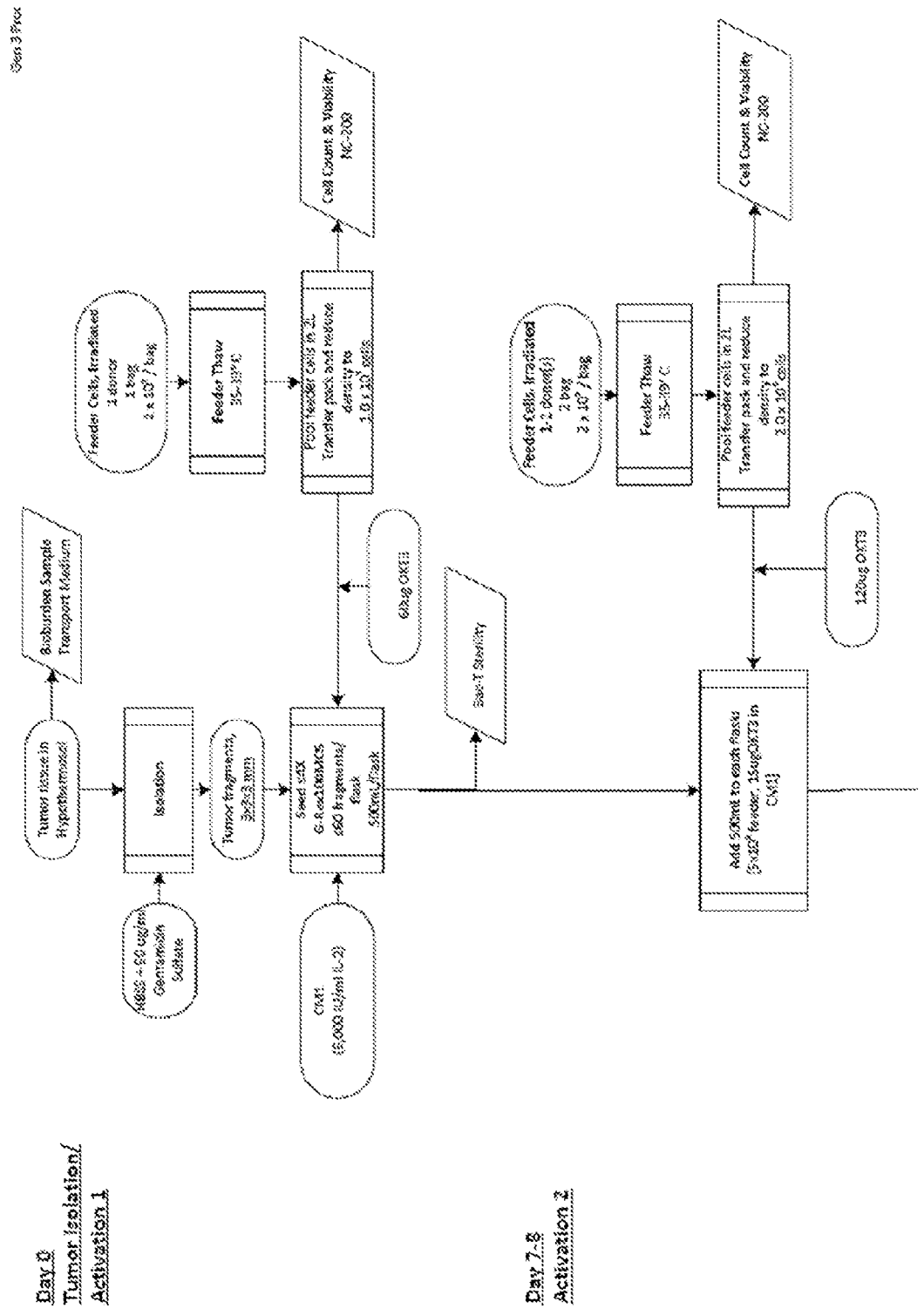

Day 0
Tumor Isolation/
Activation 1
Day 7-8
Activation 2
Tumor tissue in Hypothermosol
Biobanked Sample Transport Medium
Isolation
Tumor fragments, 3x3x3 mm
Seed 10X G-Rex100MCS 400 fragments/ flask 500mL/flask
CM1
Gibco CM11
Bio-T Stability
Add 500ml to each flask (5x10^8 feeder, 1ug/OKT3 in CM1)
120ug OKT3
Feeder Cells, Irradiated
Feeder Thaw
Post feeder cells in 2L
Cell Count & Viability NC-200

Figure 13B

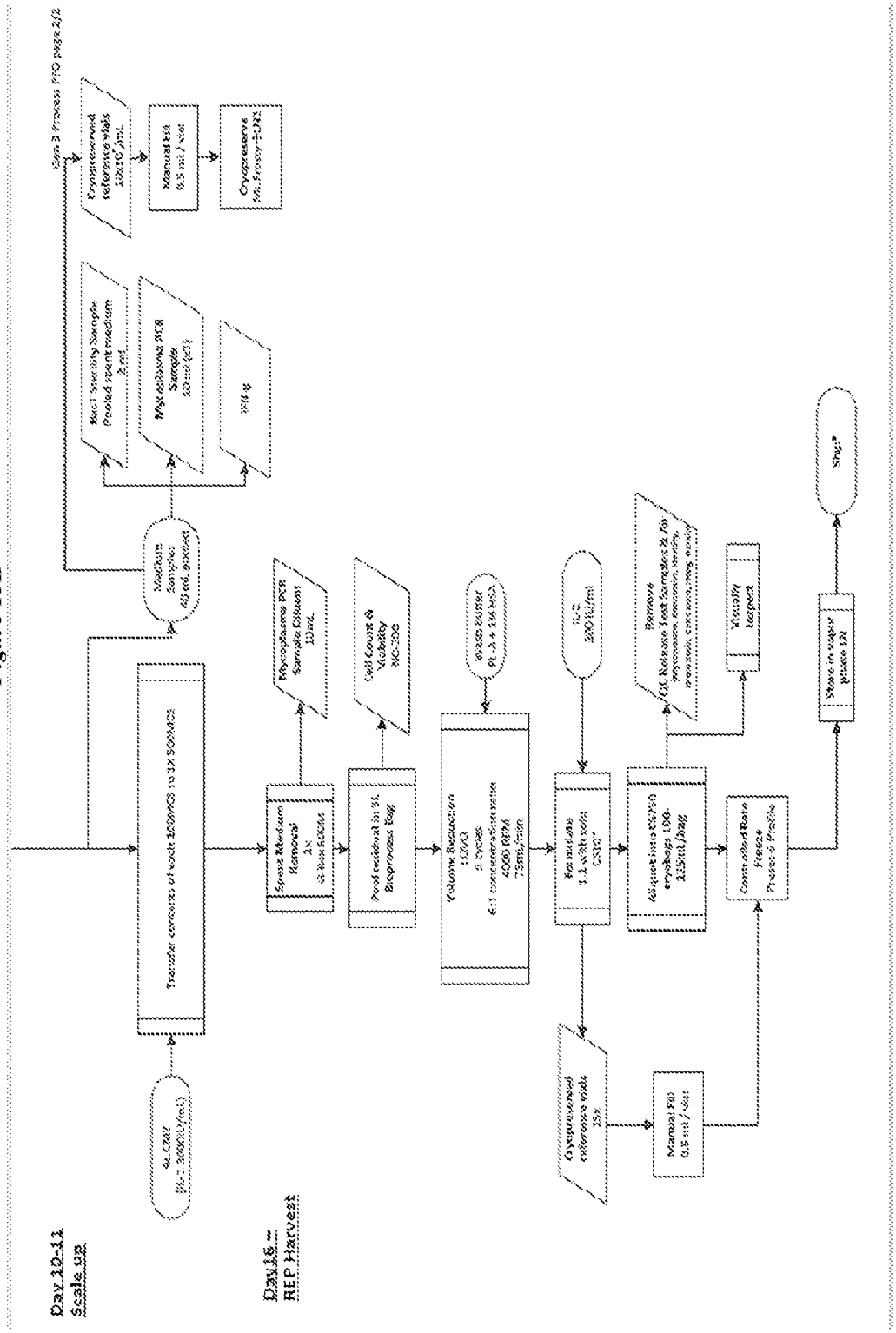

Day 10-11
Scale up
Day 16 –
REP Harvest
Transfer contents of vials 3000MCB to 3X 500MCB
Serum Medium Removal 1X Culture 5000L
Post-culture in 3L Bioprocess Bag
Volume Reduction 1.0X/3 5 cycles 4:1 concentration ratio 4000 RPM 75mL/min
Resuspend 1.1 X WB with CS07
Aliquot into 5X250 cryobags 100-125mL/bag
Controlled Rate Freeze Process by Profile
Store in vapor phase LN
Ship*
Cryopreserved reference vials 25x
Manual Fill 0.5 ml / Vial
Cryopreserved reference vials 100x10^6/mL
Manual Fill 0.5 ml / Vial
Cryopreserve 10% DMSO
Bact Sterility Sample Pooled spent medium 2 mL
Myc-plasma PCR Sample 40 ml (pool)
WB 4g
Medium Sample 40 ml (pool)
Mycoplasma PCR Sample Effluent 10mL
Cell Count & Viability NC-200
Wash Buffer 9L x 3 135mL/min
IL-2 200 IU/ml
Remove QC Release Test Samples & Jig (mycoplasma, endotoxin, sterility, viability, cell count, identity)
Viability Report

Figure 14

STEP	Gen 2	Gen 2.1	Gen 3.0
Pre REP- day 0	≤ 50 fragments/ 1 G-Rex 100MCS - **11 days**	≤180 fragments/ 3 G-Rex, Pre-formulated CM1 warmed media 100MCS - **11 days**	***Fresh or Frozen Tumor*** Whole tumor with ≤ 30 fragments up to 60 fragments per 1 G-Rex 100MCS (up to 4 G-Rex), preformulated warmed media - **7 days. Pre REP. Feeders 250e6 cells + OKT-3 (15ug)**
REP Initiation	Direct to REP- Day 11- **<200e^6 TIL** 1 G-Rex 500MCS	Direct to REP- Day 11- **<200 e^6 TIL** Pre-formulated CM2 warmed media in one G-Rex 500MCS	Direct to REP- Day 7-all cells TIL- same G-Rex **100MCS (100MCS up to 4 GREX), Standard media or Defined Media (Serum free). Addition Feeders 500e6 cells +OKT-3 (30ug)**
TIL propagation or Scale up	1 to 5 G-REX 500MCS **Split day 16**	2 to 5 G-REX 500MCS Pre-formulated CM4 warmed media **Split day 16**	From G-REX 100MCS transfer TIL suspension to G-REX 500MCS up to 4 GREX 500 MCS- Standard media or Defined Media (Serum Free) **Scale up on day 10 or 11**
Harvest	**Harvest day 22.** LOVO-automated cell washer	**Harvest day 22,** LOVO-automated cell washer (5 wash cycle)	**Harvest day 14 or 16** LOVO- automated cell washer (5 wash cycle)
Final formulation	Cryopreserved Product 300IU/ml IL2- CS10 in LN_2 multiple aliquots	Cryopreserved Product 300IU/ml IL2- CS10 in LN_2 multiple aliquots	Cryopreserved product 300IU/ml IL-2-CS10 in LN_2 multiple aliquots
Process time	**22 days**	**22 days**	**16 days**

Figure 15

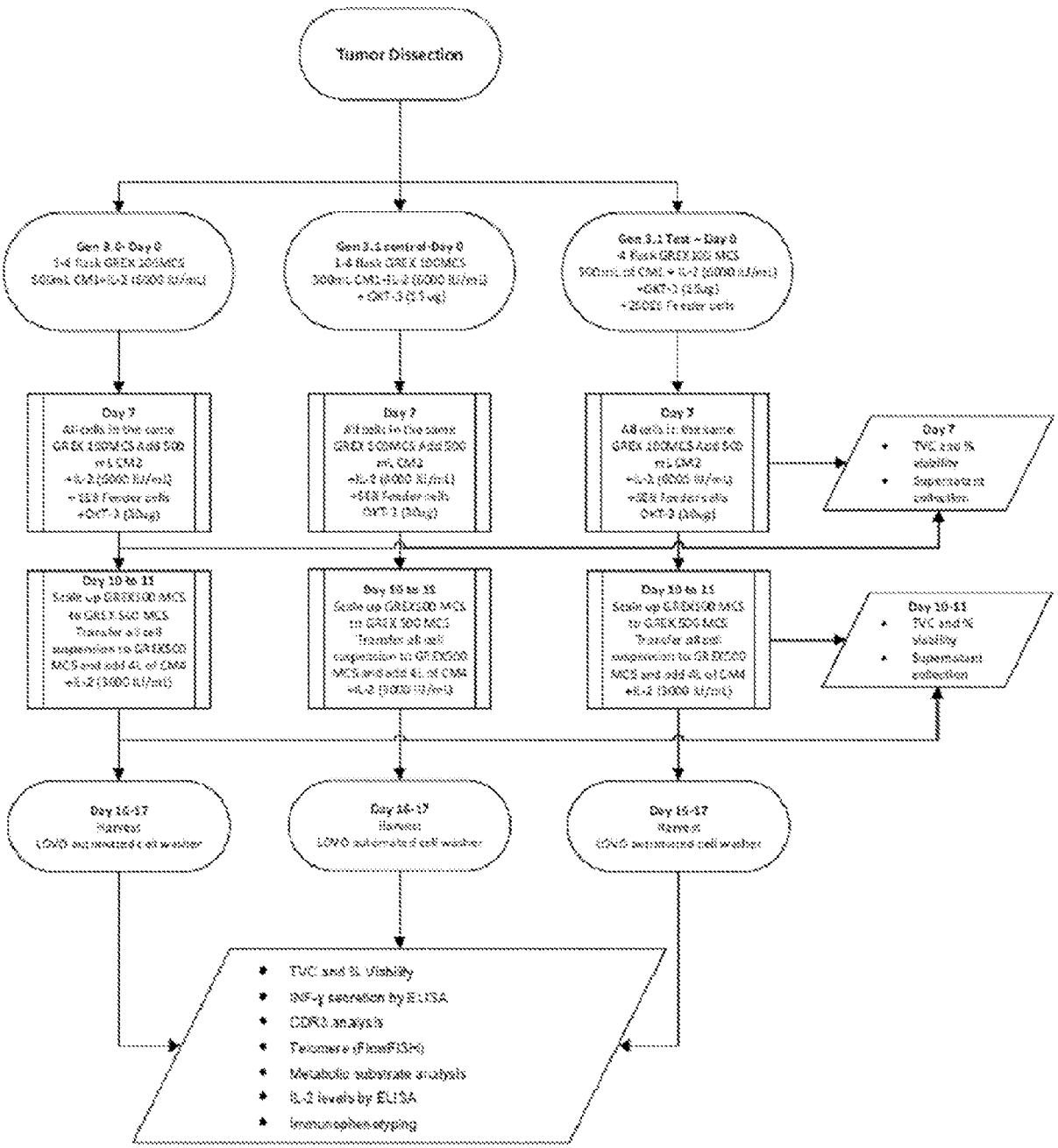
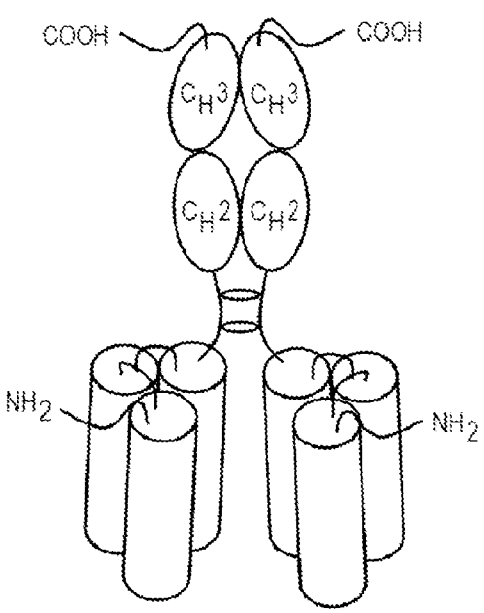

Tumor Dissection
Day 7
Day 7
Day 7
Day 10 to 15
Day 10 to 15
Day 10 to 15
Day 16-17
Day 16-17
Day 16-17
TVC and % Viability
INF-γ secretion by ELISA
CDR3 analysis
Telomere (FlowFISH)
Metabolic substrate analysis
IL-2 levels by ELISA
Immunophenotyping

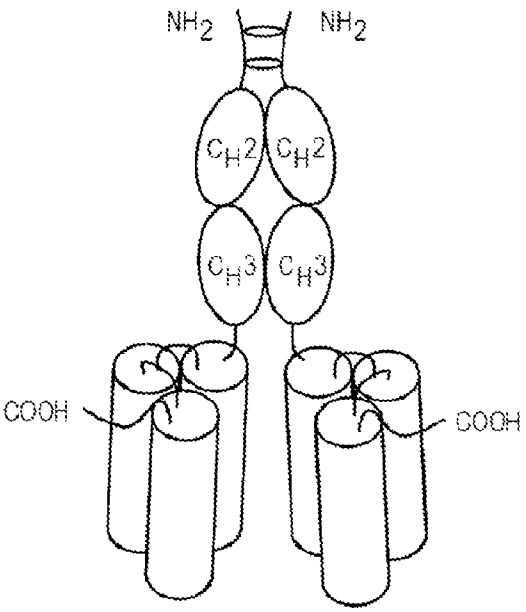
Figure 16

Step	Process Gen 3-Optimized
Day 0 Tumor isolation and Activation	≤240 fragments ≤60 fragments/flask ≤4 flasks ≤2L media (500mL/flask) IL-2 (6000IU/mL) $2.5x10^8$ feeder cells/flask 15ug OKT3/flask
Day 7 - 8 Reactivation	Fresh TIL direct to REP Activate entire culture $5x10^8$ feeder cells 30 ug OKT3/flask G-Rex 100MCS Add 500mL media+ IL-2(6000IU/mL)
Day 10 – 11 Scale up or TIL Sub-culture	≤4 G-REX 500MCS Scale up entire culture transferring 1L from GREX 100MCS into GREX 500MCS and add 4L of media +IL-2 (3000 IU/mL) /flask
Day 16 -17 Harvest	Harvest LOVO- automated cell washer Cryopreservation on Plasmalyte 1% HSA: CS10

Figure 17



Structure I-A



Structure I-B

Figure 18

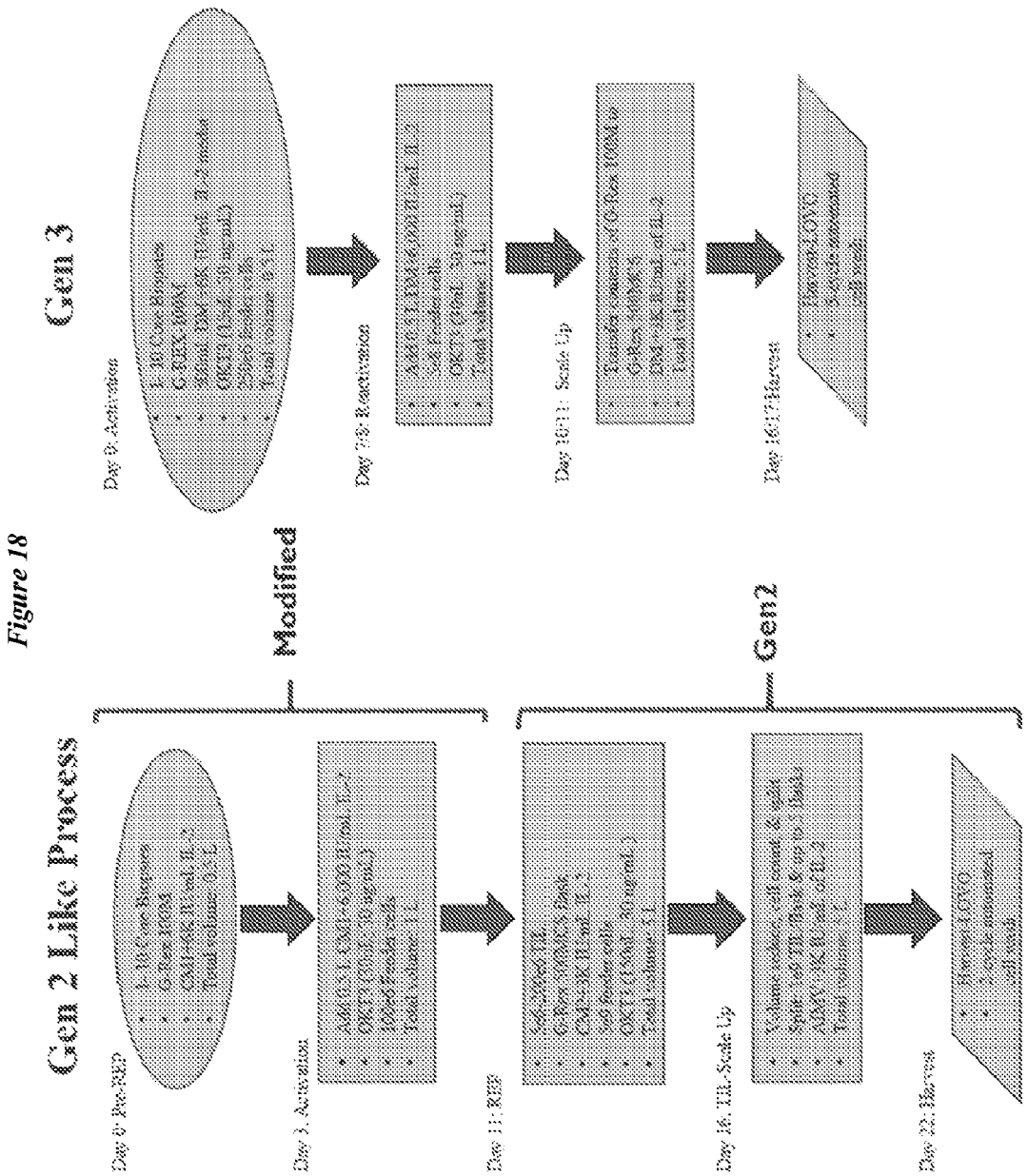

Gen 2 Like Process
Modified
Gen 3
Gen2
Day 0: Pre-REP
Day 3: Activation
Day 11: REP
Day 16: TIL Scale Up
Day 22: Harvest
Day 0: Activation
Day 7/8: Reactivation
Day 10/11: Scale Up
Day 16/17: Harvest

Figure 19

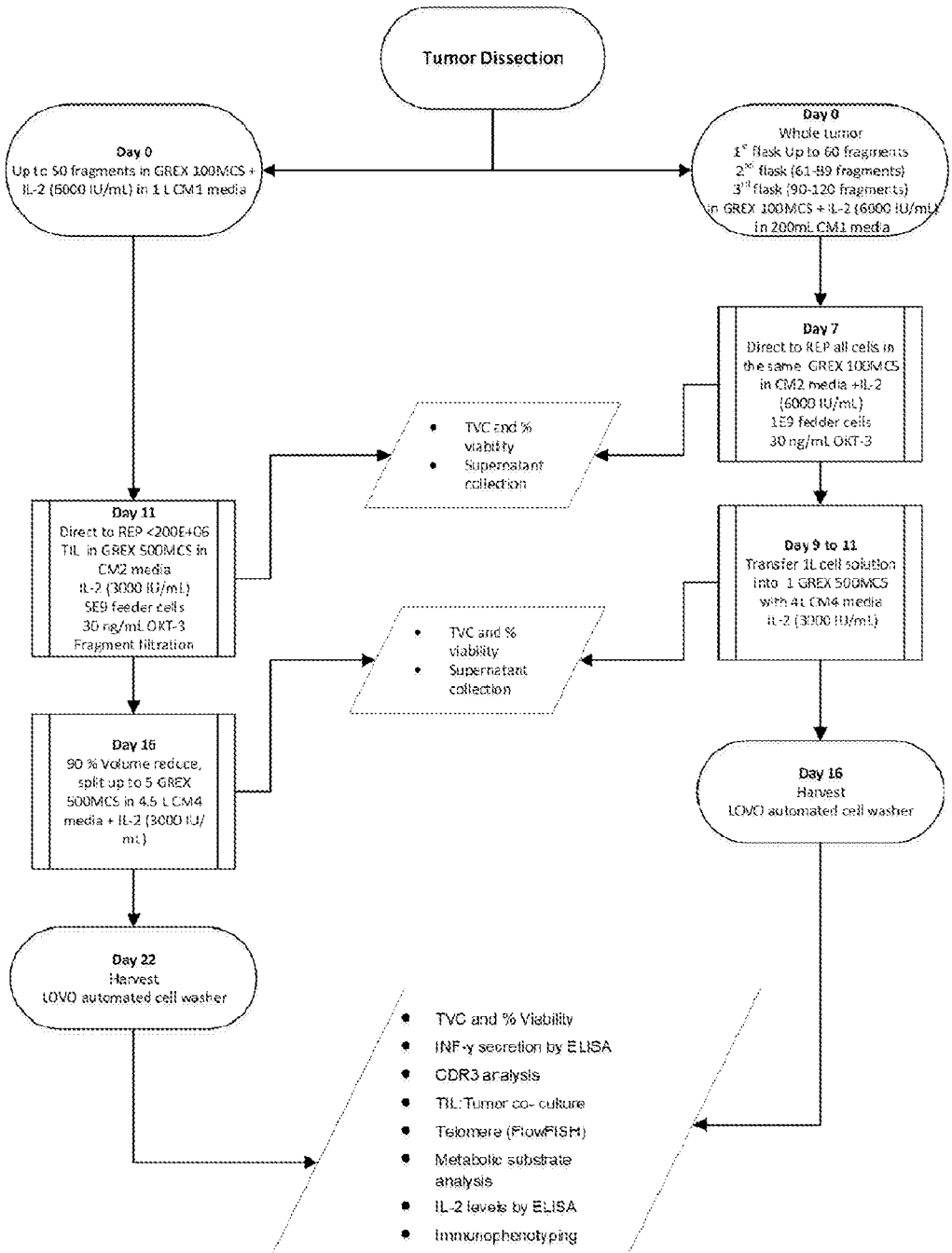

Tumor Dissection
Day 0
Up to 50 fragments in GREX 100MCS + IL-2 (6000 IU/mL) in 1 L CM1 media
Day 0
Whole tumor
1st flask Up to 60 fragments
2nd flask (61-89 fragments)
3rd flask (90-120 fragments)
in GREX 100MCS + IL-2 (6000 IU/mL) in 200mL CM1 media
Day 7
Direct to REP all cells in the same GREX 100MCS in CM2 media +IL-2 (6000 IU/mL)
1E9 feeder cells
30 ng/mL OKT-3
TVC and % viability
Supernatant collection
Day 11
Direct to REP <200E+06 TIL in GREX 500MCS in CM2 media
IL-2 (3000 IU/mL)
5E9 feeder cells
30 ng/mL OKT-3
Fragment filtration
Day 9 to 11
Transfer 1L cell solution into 1 GREX 500MCS with 4L CM4 media
IL-2 (3000 IU/mL)
TVC and % viability
Supernatant collection
Day 16
90 % Volume reduce, split up to 5 GREX 500MCS in 4.5 L CM4 media + IL-2 (3000 IU/mL)
Day 16
Harvest
LOVO automated cell washer
Day 22
Harvest
LOVO automated cell washer
TVC and % Viability
INF-γ secretion by ELISA
CDR3 analysis
TIL:Tumor co- culture
Telomere (FlowFISH)
Metabolic substrate analysis
IL-2 levels by ELISA
Immunophenotyping

Figure 20

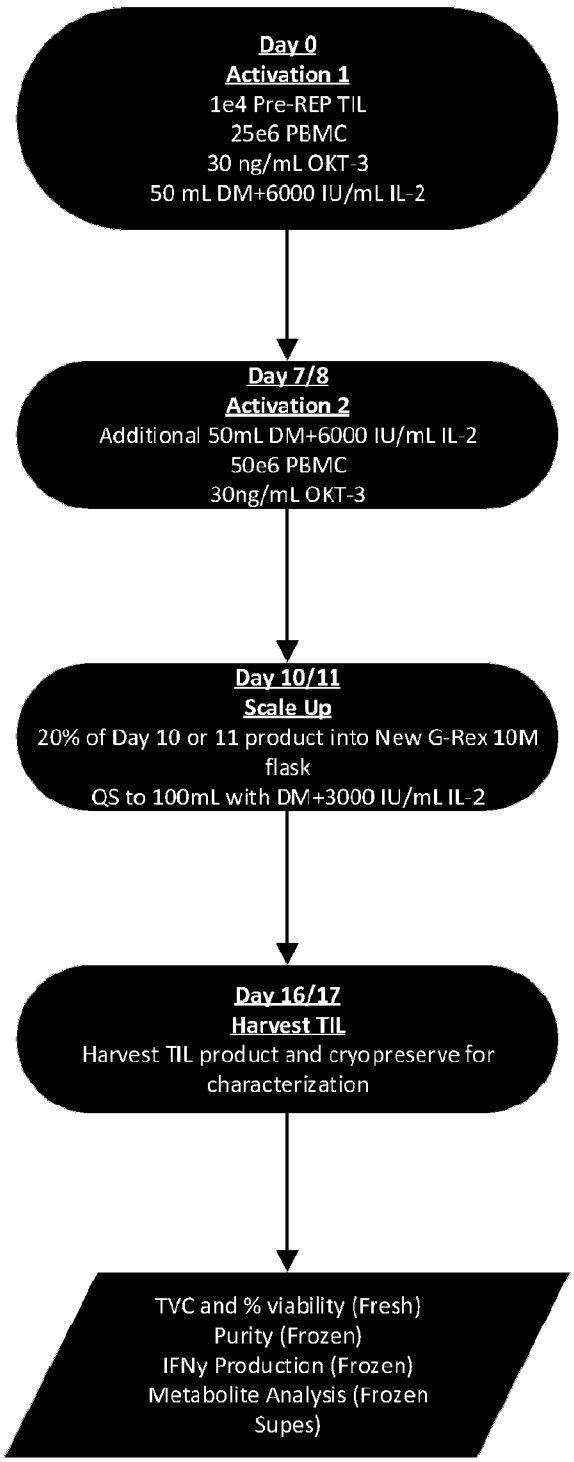

Day 0
Activation 1
1e4 Pre-REP TIL
25e6 PBMC
30 ng/mL OKT-3
50 mL DM+6000 IU/mL IL-2
Day 7/8
Activation 2
Additional 50mL DM+6000 IU/mL IL-2
50e6 PBMC
30ng/mL OKT-3
Day 10/11
Scale Up
20% of Day 10 or 11 product into New G-Rex 10M flask
QS to 100mL with DM+3000 IU/mL IL-2
Day 16/17
Harvest TIL
Harvest TIL product and cryopreserve for characterization
TVC and % viability (Fresh)
Purity (Frozen)
IFNγ Production (Frozen)
Metabolite Analysis (Frozen Supes)

Figure 21

Process day	Gen 3.0 Current process	Gen 3.0 + Feeder soup on Day 0	Gen 3.0 + Feeder soup on Day 7/8	Gen 3.0 + Feeder soup on Day 0 and Day 7/8	Feeders flask #1	Feeders flask #2
Day -3	NA	NA	NA	NA	Culture **500e6** feeders DM1 media +IL-2 (6000IU/mL)+OKT3 **30ng/mL in** 1L total volume of media in GREX 100 flask.	NA
Day 0	4 fragments IL-2 (6e3IU/mL) 50 mL DM1 media Grex10M 25e6 feeders OKT-3 (30ng/mL)	4 fragments IL-2 (6e3IU/mL) 25 mL DM1 media Grex10M +25 mL of feeder soup from only feeders flask #1 (Day -3)	4 fragments IL-2 (6e3IU/mL) 50 mL DM1 media Grex10M 25e6 feeders OKT-3 (30ng/mL)	4 fragments IL-2 (6e3IU/mL) 25 mL DM1 media Grex10M +25 mL of feeder soup from only feeders flask #1 (Day -3)	Collect Soup and add the specific volume to A and C conditions. Collect soup for additional testing (next section)	NA
Day 4	NA	NA	NA	NA	Collect soup for additional testing (next section)	Culture **1e7** feeders DM1 media +IL-2 (6000IU/mL)+OKT3 **30ng/mL in** 1L total volume of media in GREX 100 flask.
Day 7/8	Add 50 mL DM1 media + IL-2 (6e3 IU/mL) +50e6 feeders OKT-3 (30ng/mL)	Add 50 mL DM1 media + IL-2 (6e3 IU/mL) +50e6 feeders OKT-3 (30ng/mL)	Add 25 mL DM1 media + IL-2 (6e3 IU/mL) +25 mL of feeder soup from only feeders flask #2 (Day 4)	Add 25 mL DM1 media + IL-2 (6e3 IU/mL) +25 mL of feeder soup from only feeders flask #2 (Day 4)	Collect soup for additional testing (next section)	Collect Soup and add the specific volume to B and C conditions. Collect soup for additional testing)
Day 10/11	Transfer to a new Grex10M 20% of the cell culture and QS to 100mL with DM2 +IL-2 (3e3IU/mL)	Transfer to a new Grex10M 20% of the cell culture and QS to 100mL with DM2 +IL-2 (3e3IU/mL)	Transfer to a new Grex10M 20% of the cell culture and QS to 100mL with DM2 +IL-2 (3e3IU/mL)	Transfer to a new Grex10M 20% of the cell culture and QS to 100mL with DM2 +IL-2 (3e3IU/mL)	Collect soup for additional testing (next section)	Collect soup for additional testing
Day 16/17	Harvest (TVC and %viability)	Harvest (TVC and %viability)	Harvest (TVC and %viability)	Harvest (TVC and %viability)	NA	Collect soup for additional testing

Figure 22

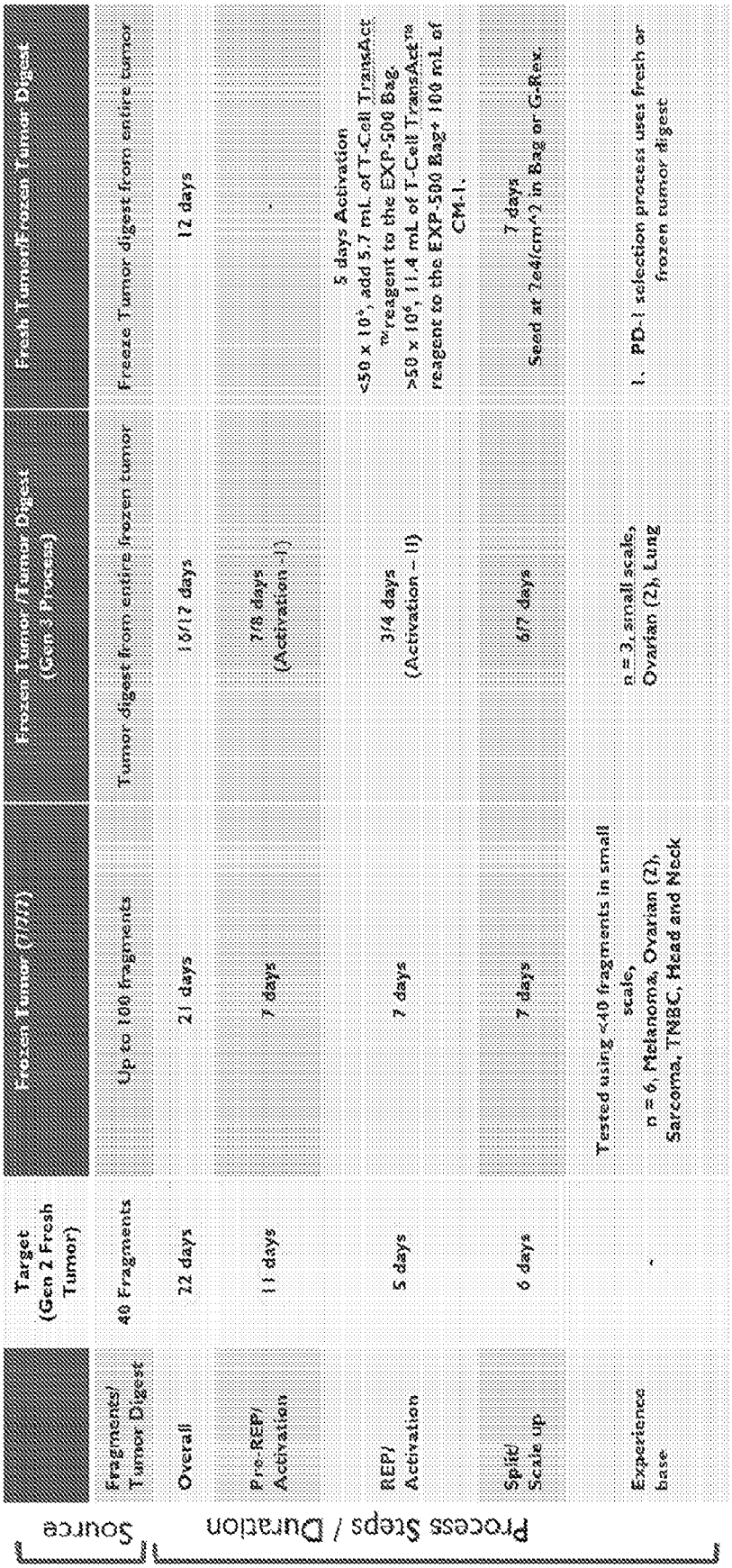

Source
Process Steps / Duration
Target (Gen 2 Fresh Tumor)
Frozen Tumor (TIL1)
Frozen Tumor/Frozen Digest (Gen 1 Process)
Fresh Tumor/Fresh Tumor Digest
Fragments/ Tumor Digest
40 Fragments
Up to 100 fragments
Tumor digest from entire frozen tumor
Freeze Tumor digest from entire tumor
Overall
22 days
21 days
10/12 days
12 days
Pre-REP/ Activation
11 days
7 days
7/8 days (Activation -1)
REP/ Activation
5 days
7 days
3/4 days (Activation – 1))
5 days Activation
<50 x 10^9, add 5.7 mL of T-Cell TransAct™ reagent to the EXP-500 Bag.
>50 x 10^9, 11.4 mL of T-Cell TransAct™ reagent to the EXP-500 Bag+ 100 mL of CM-1.
Split/ Scale up
6 days
7 days
6/7 days
7 days
Seed at 7e4/cm^2 in Bag or G-Rex
Experience base
Tested using <40 fragments in small scale,
n = 6, Melanoma, Ovarian (2), Sarcoma, TNBC, Head and Neck
n = 3, small scale, Ovarian (2), Lung
1. PD-1 selection process uses fresh or frozen tumor digest

Figure 23

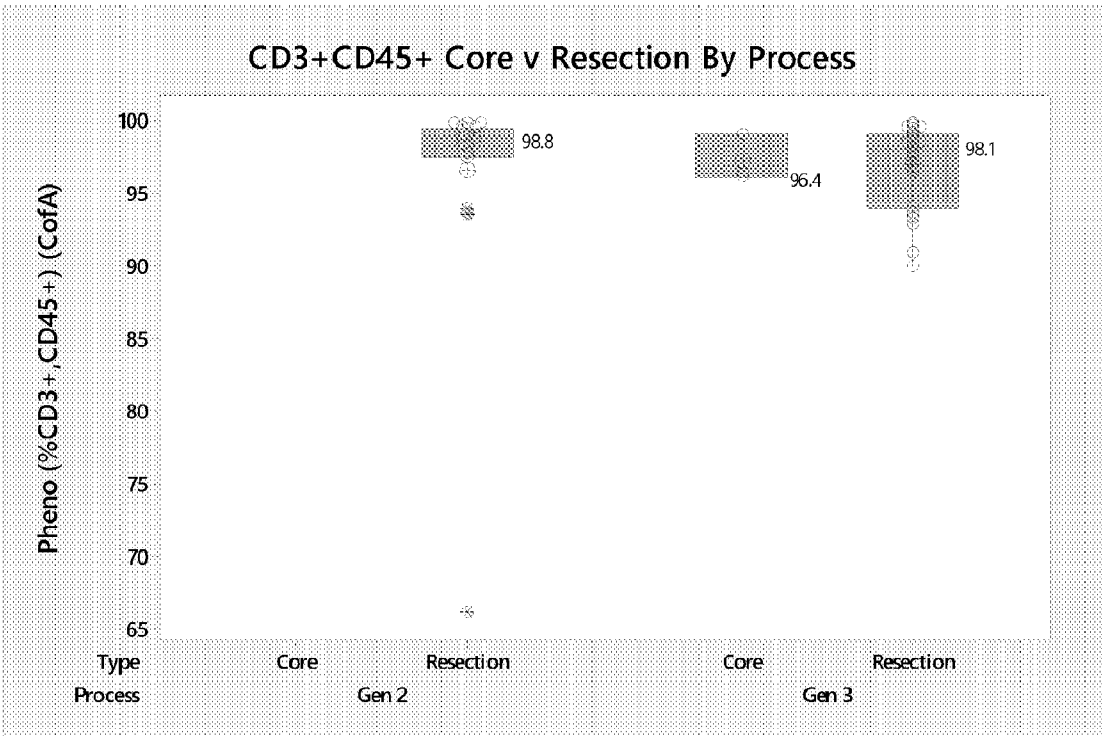

CD3+CD45+ Core v Resection By Process
Pheno (%CD3+,CD45+) (CofA)
100
95
90
85
80
75
70
65
98.8
96.4
98.1
Type
Core
Resection
Core
Resection
Process
Gen 2
Gen 3

Figure 24

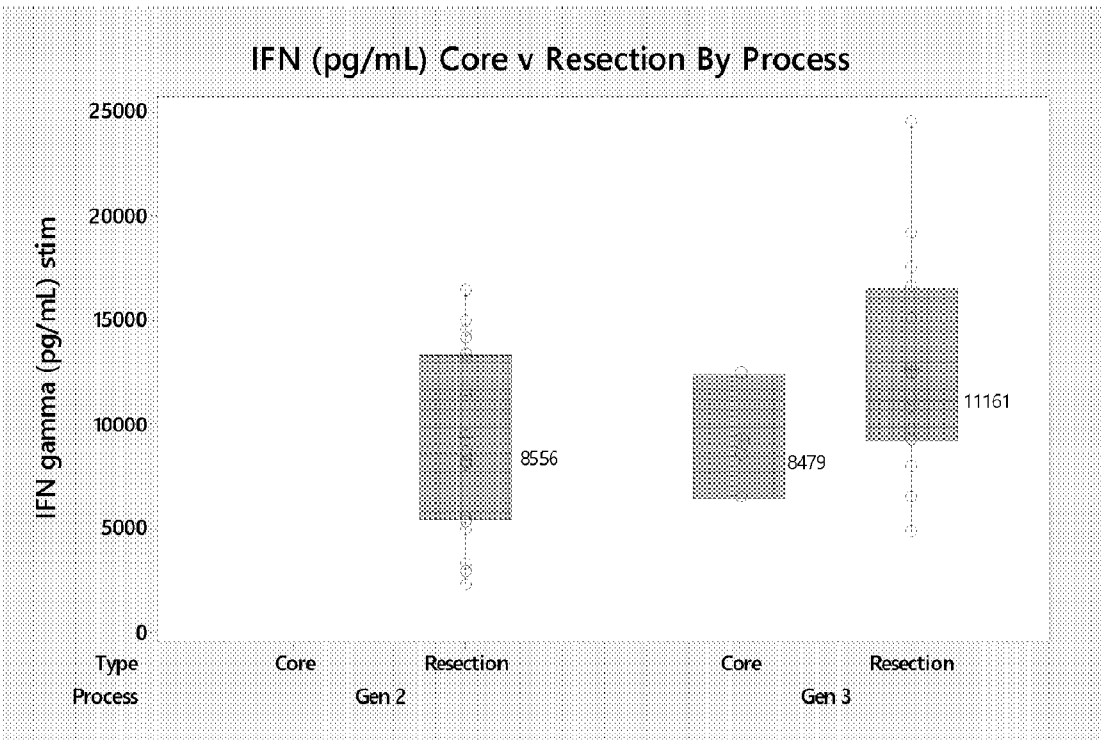

IFN (pg/mL) Core v Resection By Process
IFN gamma (pg/mL) stim
25000
20000
15000
10000
5000
0
8556
8479
11161
Type
Core
Resection
Core
Resection
Process
Gen 2
Gen 3

Figure 25

		Gen2 Target	Gen2 like Process				Gen 3 Process		
			PT21010	PT21011	P7055	P7058	P7058	END22000	PT21009
Pre-REP /Activation	Number of Cores	N/A	8	2	3	5	5	2	3
	TVC harvested (Day 11)	N/A	31.4e6	510e6	47.3e6	52.4e6	NA[1]	NA[1]	NA[1]
	TVC seeded	5 - 200e6	31.4e6	200e6[2]	47.3e6	52.4e6	NA[1]	NA[1]	NA[1]
REP /Reactivation	TVC harvested (Pre-LOVO)	N/A	27.1e9	N/A	18.4e9	30.5e9	12.8e9	11.8e9	21.2e9
	TVC harvested (Post-LOVO)	1-150e9	Day 22 25.5e9	Day 22 50.2e9[2]	Day 22 16.8e9	Day 22 31.1e9	Day 16 10.9e9	Day 16 9.7e9	Day 17 20.5e9
	% LOVO Recovery	N/A	94.1	N/A	91.3	102	85.2	82.2	96.7
	# Cell Doublings	N/A	9.3	6.6	7.8	9.2	-	-	-
	REP Fold expansion	N/A	649	98	219	593	-	-	-
	% Viability	> 70%	94.0%	96.9%	93.3%	95.4%	73.3%	72.0%	88.0%
	%CD45+/CD3+	> 90%	99.9%	99.7%	99.4%	99.8%	98.3%	97.4%	99.8%
	IFNg (pg/mL)	> 500	11290	4938	6340	5977	9703	3891	12332
	Granzyme B (pg/mL)	N/A	Ongoing	21688	10559	30958	41967	10005	14125
	% CD4+CD107A (Stimulated)	N/A	6.8%	69.9%	39.9%	34.2%	60.2%	32.1%	42.0%
	% CD8+CD107A (Stimulated)	N/A	42.4%	83.9%	42.0%	75.9%	39.3%	31.8%	60.9%

Figure 26

Characteristic		Cell 24 Life Process				Cell Therapeutics		
		PT21010	PT21011	P7055	P7058	P7058	END2200	PT21009
Purity	NK cells (CD3-CD56+CD16+) (%)	0.2	1.0	0.1	0.2	2.8	0.3	0.1
	B cells (CD3-CD19+) (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Monocytes (CD14+) (%)	1.3	0.8	0.9	1.2	0.4	0.2	0.4
Identity T cells	TCRα/β (%)	91.9	92.7	90.9	95.1	90	95.4	97.3
	TCRγ/δ (%)	1.8	1.3	2.2	1.5	0.8	0.3	1.0
	TCRα/β+ CD4+ (%)	0.9	79	92.2	89.2	79.6	81.4	93.0
	TCRα/β+ CD8+ (%)	77.5	20.1	6.3	10.3	19.7	16.5	6.2
Memory Phenotype-TCRαβ+	Naïve: CCR7+CD45RA+ (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	T-EM: CCR7-CD45RA-(%)	99.8	99.8	98.4	99.8	96.6	97.3	99.7
	T-CM: CCR7+CD45RA-(%)	0.2	0.1	1.5	0.1	1.2	2.1	0.2
	T-EFF/TEMRA: CCR7-CD45RA+(%)	0.0	0.2	0.0	0.0	2.0	0.6	0.2

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Figure 27

Characteristic (Gated on Live, CD3+)	Historical values from Melanoma (Range from n = 64)	Gen 2-Like Process				Gen 3 Process		
		PT21010	PT21011	P7055	P7058	P7058	END2200	PT21009
CD27+ (%)	0.7-59.0	1.4	1.1	0.5	0.8	5.2	2.3	0.5
CD28+ (%)	12.7-92.0	90.9	91.4	96.0	93.9	82.7	84.6	93.7
CD56+ (%)	0.7-35.4	3.2	2.9	6.1	5.1	12.6	10.2	1.9
CD57+ (%)	1.1-35.5	17.7	65.1	31.2	20.7	2.9	3.9	14.8
BTLA+ (%)	33.3-99.2	99.4	99.5	99.2	99.6	92.1	97.1	99.2
CD25+ (%)	1.0-96.7	4.0	6.0	19.1	2.9	71.4	46.7	11.6
CD69+ (%)	13.2-82.9	29.7	36.0	33.7	40.7	40.4	34.3	22.3

SUBSTITUTE SHEET (RULE 26)

Figure 28A

CD4+		Gen2 Like Process			Gen3 Process			
		PT21010	PT21011	P7055	P7058	P7058	END2200	PT21009
Differentiation	CD27+ (%)	25.2	1.9	2.2	2.0	33.7	14.6	2.6
	CD28+ (%)	97.3	95.4	96.7	95.0	88.0	88.7	95.4
	CD57+ (%)	4.7	64.0	30.1	21.9	2.1	3.6	15.1
	KLRG1+ (%)	3.4	31.0	5.6	7.0	4.4	3.6	3.2
Activation	284+ (%)	5.1	1.5	10.5	1.3	2.3	1.4	1.3
	BTLA4+ (%)	98.3	99.3	99.1	99.4	89.6	96.2	99.1
	CD25+ (%)	7.8	5.2	17.7	2.3	73.6	43.6	9.7
	CD69+ (%)	27.9	37.8	35.5	42.7	40.1	34.9	24.5
	CD95+ (%)	99.6	99.4	98.7	99.1	99.5	98.8	99.7
Exhaustion	LAG3+ (%)	0.3	0.1	1.2	0.1	3.1	1.0	0.8
	PD1+ (%)	1.2	10.6	2.2	4.1	7.9	5.3	6.1
	TIGIT+ (%)	3.1	8.2	22.4	6.1	23.6	11.3	8.1
	TIM3+ (%)	76.8	68.5	73.5	81.8	93.2	92.7	88.8

Figure 28B

	CD8+	CD8 Like Process			CD4 Like Process		
		PT21010	PT21011	P7055	P7058	P7058	END2200	PT21009
Differentiation	CD27+ (%)	4.9	10.1	3.5	6.4	3.6	7.4	6.2
	CD28+ (%)	96.6	94.4	98.0	97.1	86.5	90.6	92.0
	CD57+ (%)	14.1	61.2	14.1	6.0	5.0	3.2	3.5
	KLRG1+ (%)	10.0	48.4	13.2	10.4	8.7	5.4	8.0
	2B4+ (%)	3.0	1.8	12.5	2.1	2.9	0.5	0.2
	BTLA4+ (%)	99.6	99.7	99.8	99.4	97.8	98.4	99.0
Activation	CD25+ (%)	2.0	3.0	13.0	2.4	48.4	39.8	15.8
	CD69+ (%)	41.2	45.5	53.1	65.1	63.7	57.0	38.3
	CD95+ (%)	92.9	94.3	91.0	93.0	93.4	89.1	91.3
	LAG3+ (%)	3.1	0.4	2.6	1.8	5.2	4.2	8.4
Exhaustion	PD1+ (%)	0.1	2.0	0.3	7.5	9.1	1.6	2.1
	TIGIT+ (%)	51.7	85.8	96.8	91.5	94.3	94.0	87.7
	TIM3+ (%)	98.2	95.4	97.2	96.9	99.1	99.2	98.2