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CA 3232700 A1 2023/03/30

(21) 3 232 700

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2022/09/23
(87) Date publication PCT/PCT Publication Date: 2023/03/30
(85) Entrée phase nationale/National Entry: 2024/03/21
(86) N° demande PCT/PCT Application No.: US 2022/076966
(87) N° publication PCT/PCT Publication No.: 2023/049862
(30) Priorités/Priorities: 2021/09/24 (US63/248,350); 2021/09/28 (US63/249,459); 2022/01/28 (US63/304,361)

(51) Cl.Int./Int.Cl. *A61K 45/00* (2006.01), *A61P 35/00* (2006.01), *C12N 5/0783* (2010.01)

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(54) Titre : PROCESSUS D'EXPANSION ET AGENTS POUR LYMPHOCYTES INFILTRANT LA TUMEUR
(54) Title: EXPANSION PROCESSES AND AGENTS FOR TUMOR INFILTRATING LYMPHOCYTES

(57) Abrégé/Abstract:
The present invention provides a method of treating a cancer in a patient or subject in need thereof comprising administering a population of modified tumor infiltrating lymphocytes (TILs), wherein the population of TILs has been modified by adding an epigenetic reprogramming agent to the cell culture medium used for expanding the TILs.

Date Submitted: 2024/03/21

CA App. No.: 3232700

Abstract:

The present invention provides a method of treating a cancer in a patient or subject in need thereof comprising administering a population of modified tumor infiltrating lymphocytes (TILs), wherein the population of TILs has been modified by adding an epigenetic reprogramming agent to the cell culture medium used for expanding the TILs.

DEMANDE OU BREVET VOLUMINEUX

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NOTE POUR LE TOME / VOLUME NOTE:

EXPANSION PROCESSES AND AGENTS FOR TUMOR INFILTRATING LYMPHOCYTES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/248,350, filed on September 24, 2021, U.S. Provisional Patent Application No. 63/249,459, filed on September 28, 2021, and U.S. Provisional Patent Application No. 63/304,361, filed on January 28, 2022, each of which are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in XML file format and is hereby incorporated by reference in its entirety. Said XML file, created on September 23, 2022, is named 116983-5095-WO_Sequence_Listing.XML and is 312 kilobytes in size.

BACKGROUND OF THE INVENTION

[0003] Treatment of melanoma remains challenging, particularly for patients that do not respond to commonly-used initial lines of therapy, including nivolumab monotherapy, pembrolizumab monotherapy, therapy using a combination of nivolumab and ipilimumab, ipilimumab monotherapy, therapy using a combination of dabrafenib and trametinib, vemurafenib monotherapy, or pegylated interferon (preinterferon) alfa-2b. Adoptive cell therapy using autologous tumor-infiltrating lymphocytes (TIL) has shown durable responses in a subset of patients with metastatic melanoma (Sarnaik AA et al, *J Clin Oncol* 2021), cervical carcinoma (Jazaeri AA et al, ASCO 2019 #182), and other epithelial malignancies.

[0004] While TIL can be reactivated and expanded ex vivo, their epigenetic programming could be keeping TIL in a more differentiated and less functional state. Strategies aimed at expanding TIL with less differentiated and more stem-like attributes are therefore needed to improve persistence, functionality, and more effective tumor responses.

BRIEF SUMMARY OF THE INVENTION

[0005] Provided herein are methods for generating modified TILs which can then be employed in the treatment of cancer patients or subjects by adding an epigenetic reprogramming agent to the cell culture medium used for expanding the TILs.

[0006] The present invention provides a method of treating a cancer in a patient or subject in need thereof comprising administering a population of modified tumor infiltrating lymphocytes (TILs), wherein the population of TILs has been modified by adding an epigenetic reprogramming agent to the cell culture medium used for expanding the TILs.

[0007] The present invention provides a method of treating a cancer in a subject in need thereof comprising administering a population of modified tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

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

(b) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-14 days to obtain the second population of TILs;

(c) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-14 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (b) to step (c) optionally occurs without opening the system;

(d) harvesting the third population of TILs obtained from step (c), wherein the transition from step (c) to step (d) optionally occurs without opening the system;

(e) administering a therapeutically effective dosage of the third population of TILs obtained in step (d) to the subject; and

(f) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

[0008] In aspect, the present invention provides a method of treating a cancer in a patient or subject in need thereof comprising administering a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(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 or processing a tumor sample obtained from the subject into a tumor digest;

(b) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-11 days to obtain the second population of TILs;

(c) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-11 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 (b) to step (c) optionally occurs without opening the system;

(d) harvesting the third population of TILs obtained from step (c), wherein the transition from step (c) to step (d) optionally occurs without opening the system;

(e) transferring the harvested third TIL population from step (d) to an infusion bag, wherein the transition from step (d) to (e) optionally occurs without opening the system;

(f) cryopreserving the infusion bag comprising the harvested TIL population from step (e) using a cryopreservation process;

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

(h) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

[0009] In another aspect, the present invention provides a method of treating a cancer in a patient or subject in need thereof comprising administering a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) obtaining and/or receiving a first population of TILs from surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from the cancer in the patient or subject,

(b) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-11 days to obtain the second population of TILs;

(c) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-11 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 (b) to step (c) optionally occurs without opening the system;

(d) harvesting the third population of TILs obtained from step (c), wherein the transition from step (c) to step (d) optionally occurs without opening the system;

(e) transferring the harvested third TIL population from step (d) to an infusion bag, wherein the transition from step (d) to (e) optionally occurs without opening the system;

(f) cryopreserving the infusion bag comprising the harvested TIL population from step (e) using a cryopreservation process;

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

(h) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

[0010] In another aspect, the present invention provides a method of treating a cancer in a patient or subject in need thereof comprising administering a population of modified tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) resecting a tumor from the subject or patient, the tumor comprising a first population of TILs, optionally from surgical resection, needle biopsy, core biopsy, small

biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from the cancer;

(b) processing the tumor into multiple tumor fragments;

(c) enzymatically digesting the multiple tumor fragments to obtain the first population of TILs;

(d) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-11 days to obtain the second population of TILs;

(e) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-11 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 (d) to step (e) optionally occurs without opening the system;

(f) harvesting the third population of TILs obtained from step (e), wherein the transition from step (e) to step (f) optionally occurs without opening the system;

(g) transferring the harvested third TIL population from step (f) to an infusion bag, wherein the transition from step (f) to (g) optionally occurs without opening the system;

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

(i) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (h) to the subject or patient with the cancer; and

(j) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (d) and/or step (e).

[0011] In another aspect, the present invention provides a method of treating a cancer in a patient or subject in need thereof comprising administering a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) obtaining and/or receiving a first population of TILs from surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from the subject or patient;

(b) performing an initial expansion (or priming first expansion) of the first population of TILs in the first cell culture medium to obtain a second population of TILs, optionally OKT-3 (anti-CD3 antibody), and optionally antigen presenting cells (APCs), where the priming first expansion occurs for a period of 1 to 8 days;

(c) performing a rapid second expansion of the second population of TILs in a second cell culture medium to obtain a third population of TILs, wherein the second cell culture medium comprises IL-2, OKT-3 (anti-CD3 antibody), and APCs; and wherein the rapid expansion is performed over a period of 14 days or less, optionally the rapid second 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;

(d) harvesting the third population of TILs;

(e) administering a therapeutically effective portion of the third population of TILs to the subject or patient with the cancer; and

(h) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

[0012] In another aspect, the present invention provides a method of treating a cancer in a patient or subject in need thereof comprising administering a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) resecting a tumor from the cancer in the subject or patient, the tumor comprising a first population of TILs, optionally from surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from the cancer;

(b) fragmenting the tumor into tumor fragments or processing the tumor into a tumor digest;

(c) contacting the tumor fragments or tumor digest with a first cell culture medium;

(d) performing an initial expansion (or priming first expansion) of the first population of TILs in the first cell culture medium to obtain a second population of TILs,

optionally OKT-3 (anti-CD3 antibody), and optionally antigen presenting cells (APCs), where the priming first expansion occurs for a period of 1 to 8 days;

(e) performing a rapid second expansion of the second population of TILs in a second cell culture medium to obtain a third population of TILs wherein the second cell culture medium comprises IL-2, OKT-3 (anti-CD3 antibody), and APCs; and wherein the rapid expansion is performed over a period of 14 days or less, optionally the rapid second 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;

(f) harvesting the third population of TILs;

(g) administering a therapeutically effective portion of the third population of TILs to the subject or patient with the cancer; and

(h) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (d) and/or step (e).

[0013] In another aspect, the present invention provides a method of treating a cancer in a patient or subject in need thereof comprising administering a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) obtaining and/or receiving a first population of TILs from surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from the cancer in the patient or subject,

(b) culturing the first population of TILs in a culture medium comprising IL-2 for 1 to 3 days;

(c) performing a priming first expansion by culturing the first population of TILs in a first cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for first period of about 1 to 11 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

(d) performing a rapid second expansion by culturing the modified second population of TILs in a second 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 14 days or less to obtain the therapeutic population of TILs, wherein the third population of TILs is a therapeutic population of TILs;

(e) harvesting the third population of TILs;

(f) administering a therapeutically effective portion of the third population of TILs to the subject or patient with the cancer, and

(g) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

[0014] In another aspect, 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 cancer in a subject by processing a tumor sample obtained from the tumor into multiple tumor fragments or processing a tumor sample obtained from the subject into a tumor digest;

(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 antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for first period of about 1 to 7/8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

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

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

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

(f) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

[0015] In another aspect, the present invention provides a method of expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs, the method comprising the steps of:

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

(b) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-14 days to obtain the second population of TILs;

(c) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-14 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (b) to step (c) optionally occurs without opening the system;

(d) harvesting the third population of TILs obtained from step (c), wherein the transition from step (c) to step (d) optionally occurs without opening the system;

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

(h) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

[0016] In another aspect, the present invention provides a method of expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs, the method comprising the steps of:

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

(b) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-11 days to obtain the second population of TILs;

(c) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-11 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 (b) to step (c) optionally occurs without opening the system;

(d) harvesting the third population of TILs obtained from step (c), wherein the transition from step (c) to step (d) optionally occurs without opening the system;

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

(h) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

[0017] In another aspect, the present invention provides a method of expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs, the method comprising the steps of:

(a) obtaining and/or receiving a first population of TILs from surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from a cancer in a patient or subject,

(b) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is optionally performed in a closed container providing a first gas-permeable

surface area, wherein the first expansion is performed for about 3-11 days to obtain the second population of TILs;

(c) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-11 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 (b) to step (c) optionally occurs without opening the system;

(d) harvesting the third population of TILs obtained from step (c), wherein the transition from step (c) to step (d) optionally occurs without opening the system;

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

(f) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

[0018] In another aspect, the present invention provides a method of expanding tumor infiltrating lymphocytes (TILs) to a therapeutic population of TILs, the method comprising the steps of:

(a) resecting a tumor from a cancer in subject or patient, the tumor comprising a first population of TILs, optionally from surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from the cancer;

(b) processing the tumor into multiple tumor fragments;

(c) enzymatically digesting the multiple tumor fragments to obtain the first population of TILs;

(d) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-11 days to obtain the second population of TILs;

(e) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (d) to step (e) optionally occurs without opening the system;

(f) harvesting the third population of TILs obtained from step (e), wherein the transition from step (e) to step (f) optionally occurs without opening the system;

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

(j) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (d) and/or step (e).

[0019] In another aspect, the present invention provides a method of expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs, the method comprising the steps of:

(a) obtaining and/or receiving a first population of TILs from surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from a cancer in the subject or patient;

(b) performing an initial expansion (or priming first expansion) of the first population of TILs in the first cell culture medium to obtain a second population of TILs, optionally OKT-3 (anti-CD3 antibody), and optionally antigen presenting cells (APCs), where the priming first expansion occurs for a period of 1 to 8 days;

(c) performing a rapid second expansion of the second population of TILs in a second cell culture medium to obtain a third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second cell culture medium comprises IL-2, OKT-3 (anti-CD3 antibody), and APCs; and wherein the rapid expansion is performed over a period of 14 days or less, optionally the rapid second 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;

(d) harvesting the third population of TILs; and

(e) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

[0020] In another aspect, the present invention provides a method of expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs, the method comprising the steps of:

(a) resecting a tumor from the cancer in the subject or patient, the tumor comprising a first population of TILs, optionally from surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from the cancer;

(b) fragmenting the tumor into tumor fragments or into a tumor digest;

(c) contacting the tumor fragments or tumor digest with a first cell culture medium;

(d) performing an initial expansion (or priming first expansion) of the first population of TILs in the first cell culture medium to obtain a second population of TILs, wherein the first cell culture medium comprises IL-2, optionally OKT-3 (anti-CD3 antibody), and optionally antigen presenting cells (APCs), where the priming first expansion occurs for a period of 1 to 8 days;

(e) performing a rapid second expansion of the second population of TILs in a second cell culture medium to obtain a third population of TILs; wherein the third population of TILs is a therapeutic population of TILs, wherein the second cell culture medium comprises IL-2, OKT-3 (anti-CD3 antibody), and APCs; and wherein the rapid expansion is performed over a period of 14 days or less, optionally the rapid second 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;

(f) harvesting the third population of TILs; and

(g) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (d) and/or step (e).

[0021] In another aspect, 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 cancer in a subject by processing a tumor sample obtained from the tumor into multiple tumor fragments or processing a tumor sample obtained from the subject into a tumor digest;

(b) performing a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, optionally OKT-3, and optionally comprising antigen presenting cells (APCs), to produce a second population of TILs, wherein the priming first expansion is performed for a first period of about 1 to 11 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

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

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

(g) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c). In some embodiments, 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 (d).

[0022] In another aspect, 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 surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from a cancer in a patient or subject,

(b) culturing the first population of TILs in a culture medium comprising IL-2 for 1 to 3 days;

(c) performing a priming first expansion by culturing the first population of TIL in a first cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a

container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for first period of about 1 to 11 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

(d) performing a rapid second expansion by culturing the second population of TILs in a second culture medium comprising IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the rapid second expansion is performed for a second period of about 14 days or less to obtain the therapeutic population of TILs;

(e) harvesting the therapeutic population of TILs; and

(f) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b), step (c) and/or step (d).

[0023] In some embodiment, the subject being treated has a cancer 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)), renal cancer, and renal cell carcinoma.

[0024] In another aspect, 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 in a cell culture medium comprising IL-2, optionally OKT-3, and optionally comprising antigen presenting cells (APCs), to produce a second population of TILs, wherein the priming first expansion is performed for a first period of about 1 to 11 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

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

(c) harvesting the third population of TILs obtained from step (b); and

(d) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (a) and/or step (b). In some embodiments, 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).

[0025] In another aspect, the present 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 population of 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;

(c) harvesting the second population of T cells; and

(d) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (a) and/or step (b).

[0026] In another aspect, the present invention provides a method of expanding T cells comprising:

(a) 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;

(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; and

(d) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (a) and/or step (b).

[0027] In some embodiments, the second population of TILs and/or the third population of TILs has an increased frequency of CD8 TILs and/or an increased ratio of CD4 TILs to CD8 TILs when compared to a corresponding population of TILs expanded in a cell culture medium without the epigenetic reprogramming agent.

[0028] In some embodiments, the second population of TILs is at least 5-fold greater in number than the first population of TILs, wherein the first cell culture medium comprises IL-2.

[0029] In some embodiments, the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7-8 days from the start of the rapid expansion.

[0030] In some embodiments, the cell permeating agent comprises an octyl ester or a disodium salt. In some embodiments, the octyl ester is selected from (2S)-Octyl-a-hydroxyglutarate and (2R)-Octyl-a-hydroxyglutarate. In some embodiments, the disodium salt is selected from R-2-hydroxyglutaric acid disodium salt and S-2-hydroxyglutaric acid disodium salt.

[0031] In some embodiments, the epigenetic reprogramming agent includes one or more of a DNA hypomethylating agent, a MEK inhibitor, a HDAC inhibitor, an EZH2 inhibitor, a bromodomain inhibitor, an AKT inhibitor, and/or a TET inhibitor.

[0032] In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof.

[0033] In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof.

[0034] In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG).

[0035] In some embodiments, the TET inhibitor includes C35.

[0036] In some embodiments, the bromodomain inhibitor is one or more selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof.

[0037] In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

[0038] In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof.

[0039] In some embodiments, the MEK inhibitor inhibits MEK1 and/or MEK2. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof.

[0040] In some embodiments, the second population of TILs and/or the third population of TILs has an increased expression of IL-7 receptor when compared to a corresponding population of TILs expanded in a cell culture medium without the epigenetic reprogramming agent.

[0041] In some embodiments, the second population of TILs and/or the third population of TILs has an increased expression of at least one of CD25, CD28, ICOS, Ki-67 and GZMB, when compared to a corresponding population of TILs expanded in a cell culture medium without the epigenetic reprogramming agent.

[0042] In some embodiments, the second population of TILs and/or the third population of TILs has a reduced expression of at least one of PD1 and TIGIT, when compared to a corresponding population of TILs expanded in a cell culture medium without the epigenetic reprogramming agent.

[0043] In some embodiments, the second population of TILs and/or the third population of TILs has an increased expression of at least one of TCFI, EOMES and KLF2, when compared to a corresponding population of TILs expanded in a cell culture medium without the epigenetic reprogramming agent.

[0044] In some embodiments, the epigenetic reprogramming agent is a DNA hypomethylating agent. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is decitabine.

[0045] In some embodiments, the epigenetic reprogramming agent is a MEK inhibitor. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is trametinib.

[0046] In some embodiments, the epigenetic reprogramming agent is an HDAC inhibitor. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is ricolinistat.

[0047] In some embodiments, the epigenetic reprogramming agent is a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is JQ1.

[0048] In some embodiments, the epigenetic reprogramming agent is an EZH2 inhibitor. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

[0049] In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is ipatasertib.

[0050] In some embodiments, the epigenetic reprogramming agent is a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35.

[0051] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and a MEK inhibitor. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[0052] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and an HDAC inhibitor. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527,

apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[0053] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and an EZH2 inhibitor. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[0054] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[0055] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[0056] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[0057] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and an HDAC inhibitor. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib,

cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[0058] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and an EZH2 inhibitor. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[0059] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[0060] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[0061] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[0062] In some embodiments, the epigenetic reprogramming agent is a combination of an HDAC inhibitor and an EZH2 inhibitor. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat.

[0063] In some embodiments, the epigenetic reprogramming agent is a combination of an HDAC inhibitor and a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat.

[0064] In some embodiments, the epigenetic reprogramming agent is a combination of an HDAC inhibitor and an AKT inhibitor. In some embodiments, the AKT inhibitor is

selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat.

[0065] In some embodiments, the epigenetic reprogramming agent is a combination of an HDAC inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat.

[0066] In some embodiments, the epigenetic reprogramming agent is a combination of an EZH2 inhibitor and a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

[0067] In some embodiments, the epigenetic reprogramming agent is a combination of EZH2 inhibitor and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183,

AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

[0068] In some embodiments, the epigenetic reprogramming agent is a combination of EZH2 inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

[0069] In some embodiments, the epigenetic reprogramming agent is a combination of bromodomain inhibitor and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1.

[0070] In some embodiments, the epigenetic reprogramming agent is a combination of bromodomain inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1.

[0071] In some embodiments, the epigenetic reprogramming agent is a combination of an AKT inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib.

[0072] The present invention provide uses according to any of the methods described in any of the preceding claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0073] **Figure 1:** Exemplary Gen 2 (process 2A) chart providing an overview of Steps A through F.

[0074] **Figure 2A-2C:** Process Flow Chart of some embodiments of Gen 2 (process 2A) for TIL manufacturing.

[0075] **Figure 3:** Shows a diagram of some embodiments of a cryopreserved TIL exemplary manufacturing process (~22 days).

[0076] **Figure 4:** Shows a diagram of some embodiments of Gen 2 (process 2A), a 22-day process for TIL manufacturing.

[0077] **Figure 5:** Comparison table of Steps A through F from exemplary embodiments of process 1C and Gen 2 (process 2A) for TIL manufacturing.

[0078] **Figure 6:** Detailed comparison of some embodiments of process 1C and some embodiments of Gen 2 (process 2A) for TIL manufacturing.

[0079] **Figure 7:** Exemplary GEN 3 type TIL manufacturing process.

[0080] **Figure 8A** Shows a comparison between the 2A process (approximately 22-day process) and some embodiments of the Gen 3 process for TIL manufacturing (approximately 14-days to 16-days process).

[0081] **Figure 8B:** Illustrates an exemplary Process Gen 3 chart providing an overview of Steps A through F (approximately 14-days to 16-days process).

[0082] **Figure 8C:** Shows a chart providing three exemplary Gen 3 processes with an overview of Steps A through F (approximately 14-days to 16-days process) for each of the three process variations.

[0083] **Figure 8D:** Illustrates an exemplary modified Gen 2-like process providing an overview of Steps A through F (approximately 22-days process).

[0084] **Figure 8E:** Illustrates an exemplary Gen 3 process providing an overview of Steps A through E (about 14-18 days process from Steps A-E).

[0085] **Figure 8F:** Illustrates 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.

[0086] **Figure 8G:** Illustrates an exemplary modified Gen 2-like process providing an overview of Steps A through F (approximately 22 days process).

[0087] **Figure 9:** Provides an experimental flow chart for comparability between Gen 2 (process 2A) versus Gen 3 processes.

[0088] **Figure 10:** Shows a comparison between various Gen 2 (process 2A) and the Gen 3.1 process embodiment.

[0089] **Figure 11:** Table describing various features of embodiments of the Gen 2, Gen 2.1 and Gen 3.0 process.

[0090] **Figure 12:** Overview of the media conditions for some embodiments of the Gen 3 process, referred to as Gen 3.1.

[0091] **Figure 13:** Table describing various features of embodiments of the Gen 2, Gen 2.1 and Gen 3.1 process.

[0092] **Figure 14:** Table comparing various features of embodiments of the Gen 2 and Gen 3.0 processes.

[0093] **Figure 15:** Table providing media uses in the various embodiments of the described expansion processes.

[0094] **Figure 16:** Schematic of an exemplary embodiment of the Gen 3 process (a 16-day process).

[0095] **Figure 17:** Schematic of an exemplary embodiment of a method for expanding T cells from hematopoietic malignancies using Gen 3 expansion platform.

[0096] **Figure 18:** 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 CH_3 and CH_2 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.

[0097] **Figure 19:** Schematic of an exemplary embodiment of the Gen 3 process (a 16-day process).

[0098] **Figure 20:** Provides a processs overview for an exemplary embodiment of the Gen 3.1 process (a 16 day process).

[0099] **Figure 21:** Schematic of an exemplary embodiment of the Gen 3.1 Test (Gen 3.1 optimized) process (a 16-17 day process).

[00100] **Figure 22:** Schematic of an exemplary embodiment of the Gen 3 process (a 16-day process).

[00101] **Figure 23A:** Comparison table for exemplary Gen 2 and exemplary Gen 3 processes with exemplary differences highlighted.

[00102] **Figure 24:** Schematic of an exemplary embodiment of the Gen 3 process (a 16-17 day process) preparation timeline.

[00103] **Figure 25:** Schematic of an exemplary embodiment of the Gen 3 process (a 14-16 day process).

[00104] **Figure 26A-26B:** Schematic of an exemplary embodiment of the Gen 3 process (a 16 day process).

[00105] **Figure 27:** Schematic of an exemplary embodiment of the Gen 3 process (a 16 day process).

[00106] **Figure 28:** Comparison of Gen 2, Gen 2.1 and some embodiments of the Gen 3 process (a 16 day process).

[00107] **Figure 29:** Comparison of Gen 2, Gen 2.1 and some embodiments of the Gen 3 process (a 16 day process).

[00108] **Figure 30:** Gen 3 embodiment components.

[00109] **Figure 31:** Gen 3 embodiment flow chart comparison (Gen 3.0, Gen 3.1 control, Gen 3.1 test).

[00110] **Figure 32:** Shown are the components of an exemplary embodiment of the Gen 3 process (Gen 3-Optimized, a 16-17 day process).

[00111] **Figure 33:** Acceptance criteria table.

[00112] **Figure 34:** Schematic showing the different time points at which epigenetic reprogramming agents can be added to the cell culture medium during an expansion step described herein. Non-solid lines indicate optional process.

[00113] **Figure 35:** Schematic illustration of decitabine (DAC) added to the culture during *ex vivo* expansion either during the pre-REP and REP stages or during the REP stage only.

[00114] **Figure 36A:** Shown are the fold-expansion and viability when DAC was added to during the pre-REP and REP stages or during the REP stage only at the end of the 22-day expansion process.

[00115] **Figure 36B:** Shown is frequency of CD8+, CD4+, and CD4+ (Foxp3+) cells after the expansion process on cryopreserved cells. *$P < 0.05$, **$P < 0.01$. TIL were left untreated or treated with increasing concentrations of DAC. Treatment was added either during the pre-REP and REP or during the REP stage only.

[00116] **Figure 37A:** Shown are the subsets of $CD8^+$ TIL after expansion in control- and DAC-treated TIL. Frequency of Tcm (CD45RA-CCR7+), Tem (CD45RA-CCR7-), and Temra (CD45+CCR7-) cells. *$P < 0.05$, **$P < 0.01$.

[00117] **Figure 37B:** Shown are the subsets of $CD4^+$ TIL after expansion in control- and DAC-treated TIL. Frequency of Tcm (CD45RA-CCR7+), Tem (CD45RA-CCR7-), and Temra (CD45+CCR7-) cells. *$P < 0.05$, **$P < 0.01$.

[00118] **Figure 38A:** Shown are expression of CD25, ICOS, CD28, and IL-7R on CD8+ TIL. Control- or DAC-treated cryopreserved TIL were thawed and stained for flow cytometry analysis. Similar results were observed for CD4+ TIL. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

[00119] **Figure 38B:** Shown are expression of inhibitory receptors PD-1 and TIGIT on CD8+ TIL. Control- or DAC-treated cryopreserved TIL were thawed and stained for flow cytometry analysis. Similar results were observed for CD4+ TIL. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

[00120] **Figure 39:** Expression of transcription factors in DAC-treated TIL. Control- or DAC-treated cryopreserved TIL were thawed and stained for flow cytometry analysis. Expression of Eomes, KLF2, BATF, and T-bet on CD8+ TIL are shown. *P < 0.05, **P < 0.01.

[00121] **Figure 40:** Cytokine expression in control- or DAC-treated TIL following in vitro stimulation. Cryopreserved control- and DAC-treated TIL were stimulated overnight with anti-CD3/CD28 beads at a bead-to-cell ratio of 1:5. Expression of IFNγ, TNFα, and GZMB on CD8+ TIL are shown. *P < 0.05, **P < 0.01.

[00122] **Figure 41A:** Cryopreserved control and TIL treated at REP with 100 nM DAC were cocultured for 24 h with KILR® THP-1 cells (Eurofins DiscoverX, Fremont, CA, USA) at a 10:1 E:T cell ratio to measure cytotoxicity in an allogeneic setting.

[00123] **Figure 41B:** Control- and DAC-treated TIL were stimulated every 5 days with TransAct™ (Miltentyi Biotech, Germany). One day after the third stimulation, cells were washed and cocultured at a 10:1 effector-to-target cell ratio with KILR THP-1 cells for 24 h to measure cytotoxicity. *P < 0.05.

[00124] **Figure 42A:** Expression of IL-7R, PD-1, and TIM3 in TIL after repeated stimulation. Control- and DAC-treated TIL were stimulated every 5 days with TransAct™ (Miltentyi Biotech, Germany). One day after the third stimulation, cells were washed and stained for flow cytometry analysis. *P < 0.05, **P < 0.01.

[00125] **Figure 42B:** Expression levels of transcription factors in TIL after repeated stimulation. Control- and DAC-treated TIL were stimulated every 5 days with TransAct™ (Miltentyi Biotech, Germany). One day after the third stimulation, cells were washed and stained for flow cytometry analysis. *P < 0.05, **P < 0.01.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

[00126] SEQ ID NO:1 is the amino acid sequence of the heavy chain of muromonab.

[00127] SEQ ID NO:2 is the amino acid sequence of the light chain of muromonab.

[00128] SEQ ID NO:3 is the amino acid sequence of a recombinant human IL-2 protein.

[00129] SEQ ID NO:4 is the amino acid sequence of aldesleukin.

[00130] SEQ ID NO:5 is an IL-2 form.

[00131] SEQ ID NO:6 is the amino acid sequence of nemvaleukin alfa.

[00132] SEQ ID NO:7 is an IL-2 form.

[00133] SEQ ID NO:8 is a mucin domain polypeptide.

[00134] SEQ ID NO:9 is the amino acid sequence of a recombinant human IL-4 protein.

[00135] SEQ ID NO:10 is the amino acid sequence of a recombinant human IL-7 protein.

[00136] SEQ ID NO:11 is the amino acid sequence of a recombinant human IL-15 protein.

[00137] SEQ ID NO:12 is the amino acid sequence of a recombinant human IL-21 protein.

[00138] SEQ ID NO:13 is an IL-2 sequence.

[00139] SEQ ID NO:14 is an IL-2 mutein sequence.

[00140] SEQ ID NO:15 is an IL-2 mutein sequence.

[00141] SEQ ID NO:16 is the HCDR1_IL-2 for IgG.IL2R67A.H1.

[00142] SEQ ID NO:17 is the HCDR2 for IgG.IL2R67A.H1.

[00143] SEQ ID NO:18 is the HCDR3 for IgG.IL2R67A.H1.

[00144] SEQ ID NO:19 is the HCDR1_IL-2 kabat for IgG.IL2R67A.H1.

[00145] SEQ ID NO:20 is the HCDR2 kabat for IgG.IL2R67A.H1.

[00146] SEQ ID NO:21 is the HCDR3 kabat for IgG.IL2R67A.H1.

[00147] SEQ ID NO:22 is the HCDR1_IL-2 clothia for IgG.IL2R67A.H1.

[00148] SEQ ID NO:23 is the HCDR2 clothia for IgG.IL2R67A.H1.

[00149] SEQ ID NO:24 is the HCDR3 clothia for IgG.IL2R67A.H1.

[00150] SEQ ID NO:25 is the HCDR1_IL-2 IMGT for IgG.IL2R67A.H1.

[00151] SEQ ID NO:26 is the HCDR2 IMGT for IgG.IL2R67A.H1.

[00152] SEQ ID NO:27 is the HCDR3 IMGT for IgG.IL2R67A.H1.

[00153] SEQ ID NO:28 is the V_H chain for IgG.IL2R67A.H1.

[00154] SEQ ID NO:29 is the heavy chain for IgG.IL2R67A.H1.

[00155] SEQ ID NO:30 is the LCDR1 kabat for IgG.IL2R67A.H1.

[00156] SEQ ID NO:31 is the LCDR2 kabat for IgG.IL2R67A.H1.

[00157] SEQ ID NO:32 is the LCDR3 kabat for IgG.IL2R67A.H1.

[00158] SEQ ID NO:33 is the LCDR1 chothia for IgG.IL2R67A.H1.

[00159] SEQ ID NO:34 is the LCDR2 chothia for IgG.IL2R67A.H1.

[00160] SEQ ID NO:35 is the LCDR3 chothia for IgG.IL2R67A.H1.

[00161] SEQ ID NO:36 is a V_L chain.

[00162] SEQ ID NO:37 is a light chain.

[00163] SEQ ID NO:38 is a light chain.

[00164] SEQ ID NO:39 is a light chain.

[00165] SEQ ID NO:40 is the amino acid sequence of human 4-1BB.

[00166] SEQ ID NO:41 is the amino acid sequence of murine 4-1BB.

[00167] SEQ ID NO:42 is the heavy chain for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00168] SEQ ID NO:43 is the light chain for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00169] SEQ ID NO:44 is the heavy chain variable region (V_H) for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00170] SEQ ID NO:45 is the light chain variable region (V_L) for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00171] SEQ ID NO:46 is the heavy chain CDR1 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00172] SEQ ID NO:47 is the heavy chain CDR2 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00173] SEQ ID NO:48 is the heavy chain CDR3 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00174] SEQ ID NO:49 is the light chain CDR1 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00175] SEQ ID NO:50 is the light chain CDR2 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00176] SEQ ID NO:51 is the light chain CDR3 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00177] SEQ ID NO:52 is the heavy chain for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00178] SEQ ID NO:53 is the light chain for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00179] SEQ ID NO:54 is the heavy chain variable region (VH) for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00180] SEQ ID NO:55 is the light chain variable region (VL) for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00181] SEQ ID NO:56 is the heavy chain CDR1 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00182] SEQ ID NO:57 is the heavy chain CDR2 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00183] SEQ ID NO:58 is the heavy chain CDR3 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00184] SEQ ID NO:59 is the light chain CDR1 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00185] SEQ ID NO:60 is the light chain CDR2 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00186] SEQ ID NO:61 is the light chain CDR3 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00187] SEQ ID NO:62 is an Fc domain for a TNFRSF agonist fusion protein.

[00188] SEQ ID NO:63 is a linker for a TNFRSF agonist fusion protein.

[00189] SEQ ID NO:64 is a linker for a TNFRSF agonist fusion protein.

[00190] SEQ ID NO:65 is a linker for a TNFRSF agonist fusion protein.

[00191] SEQ ID NO:66 is a linker for a TNFRSF agonist fusion protein.

[00192] SEQ ID NO:67 is a linker for a TNFRSF agonist fusion protein.

[00193] SEQ ID NO:68 is a linker for a TNFRSF agonist fusion protein.

[00194] SEQ ID NO:69 is a linker for a TNFRSF agonist fusion protein.

[00195] SEQ ID NO:70 is a linker for a TNFRSF agonist fusion protein.

[00196] SEQ ID NO:71 is a linker for a TNFRSF agonist fusion protein.

[00197] SEQ ID NO:72 is a linker for a TNFRSF agonist fusion protein.

[00198] SEQ ID NO:73 is an Fc domain for a TNFRSF agonist fusion protein.

[00199] SEQ ID NO:74 is a linker for a TNFRSF agonist fusion protein.

[00200] SEQ ID NO:75 is a linker for a TNFRSF agonist fusion protein.

[00201] SEQ ID NO:76 is a linker for a TNFRSF agonist fusion protein.

[00202] SEQ ID NO:77 is a 4-1BB ligand (4-1BBL) amino acid sequence.

[00203] SEQ ID NO:78 is a soluble portion of 4-1BBL polypeptide.

[00204] SEQ ID NO:79 is a heavy chain variable region (V_H) for the 4-1BB agonist antibody 4B4-1-1 version 1.

[00205] SEQ ID NO:80 is a light chain variable region (V_L) for the 4-1BB agonist antibody 4B4-1-1 version 1.

[00206] SEQ ID NO:81 is a heavy chain variable region (V_H) for the 4-1BB agonist antibody 4B4-1-1 version 2.

[00207] SEQ ID NO:82 is a light chain variable region (V_L) for the 4-1BB agonist antibody 4B4-1-1 version 2.

[00208] SEQ ID NO:83 is a heavy chain variable region (V_H) for the 4-1BB agonist antibody H39E3-2.

[00209] SEQ ID NO:84 is a light chain variable region (V_L) for the 4-1BB agonist antibody H39E3-2.

[00210] SEQ ID NO:85 is the amino acid sequence of human OX40.

[00211] SEQ ID NO:86 is the amino acid sequence of murine OX40.

[00212] SEQ ID NO:87 is the heavy chain for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00213] SEQ ID NO:88 is the light chain for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00214] SEQ ID NO:89 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00215] SEQ ID NO:90 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00216] SEQ ID NO:91 is the heavy chain CDR1 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00217] SEQ ID NO:92 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00218] SEQ ID NO:93 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00219] SEQ ID NO:94 is the light chain CDR1 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00220] SEQ ID NO:95 is the light chain CDR2 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00221] SEQ ID NO:96 is the light chain CDR3 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00222] SEQ ID NO:97 is the heavy chain for the OX40 agonist monoclonal antibody 11D4.

[00223] SEQ ID NO:98 is the light chain for the OX40 agonist monoclonal antibody 11D4.

[00224] SEQ ID NO:99 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 11D4.

[00225] SEQ ID NO:100 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 11D4.

[00226] SEQ ID NO:101 is the heavy chain CDR1 for the OX40 agonist monoclonal antibody 11D4.

[00227] SEQ ID NO:102 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody 11D4.

[00228] SEQ ID NO:103 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody 11D4.

[00229] SEQ ID NO:104 is the light chain CDR1 for the OX40 agonist monoclonal antibody 11D4.

[00230] SEQ ID NO:105 is the light chain CDR2 for the OX40 agonist monoclonal antibody 11D4.

[00231] SEQ ID NO:106 is the light chain CDR3 for the OX40 agonist monoclonal antibody 11D4.

[00232] SEQ ID NO:107 is the heavy chain for the OX40 agonist monoclonal antibody 18D8.

[00233] SEQ ID NO:108 is the light chain for the OX40 agonist monoclonal antibody 18D8.

[00234] SEQ ID NO:109 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 18D8.

[00235] SEQ ID NO:110 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 18D8.

[00236] SEQ ID NO:111 is the heavy chain CDR1 for the OX40 agonist monoclonal antibody 18D8.

[00237] SEQ ID NO:112 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody 18D8.

[00238] SEQ ID NO:113 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody 18D8.

[00239] SEQ ID NO:114 is the light chain CDR1 for the OX40 agonist monoclonal antibody 18D8.

[00240] SEQ ID NO:115 is the light chain CDR2 for the OX40 agonist monoclonal antibody 18D8.

[00241] SEQ ID NO:116 is the light chain CDR3 for the OX40 agonist monoclonal antibody 18D8.

[00242] SEQ ID NO:117 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody Hu119-122.

[00243] SEQ ID NO:118 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody Hu119-122.

[00244] SEQ ID NO:119 is the heavy chain CDR1 for the OX40 agonist monoclonal antibody Hu119-122.

[00245] SEQ ID NO:120 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody Hu119-122.

[00246] SEQ ID NO:121 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody Hu119-122.

[00247] SEQ ID NO:122 is the light chain CDR1 for the OX40 agonist monoclonal antibody Hu119-122.

[00248] SEQ ID NO:123 is the light chain CDR2 for the OX40 agonist monoclonal antibody Hu119-122.

[00249] SEQ ID NO:124 is the light chain CDR3 for the OX40 agonist monoclonal antibody Hu119-122.

[00250] SEQ ID NO:125 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody Hu106-222.

[00251] SEQ ID NO:126 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody Hu106-222.

[00252] SEQ ID NO:127 is the heavy chain CDR1 for the OX40 agonist monoclonal antibody Hu106-222.

[00253] SEQ ID NO:128 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody Hu106-222.

[00254] SEQ ID NO:129 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody Hu106-222.

[00255] SEQ ID NO:130 is the light chain CDR1 for the OX40 agonist monoclonal antibody Hu106-222.

[00256] SEQ ID NO:131 is the light chain CDR2 for the OX40 agonist monoclonal antibody Hu106-222.

[00257] SEQ ID NO:132 is the light chain CDR3 for the OX40 agonist monoclonal antibody Hu106-222.

[00258] SEQ ID NO:133 is an OX40 ligand (OX40L) amino acid sequence.

[00259] SEQ ID NO:134 is a soluble portion of OX40L polypeptide.

[00260] SEQ ID NO:135 is an alternative soluble portion of OX40L polypeptide.

[00261] SEQ ID NO:136 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 008.

[00262] SEQ ID NO:137 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 008.

[00263] SEQ ID NO:138 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 011.

[00264] SEQ ID NO:139 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 011.

[00265] SEQ ID NO:140 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 021.

[00266] SEQ ID NO:141 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 021.

[00267] SEQ ID NO:142 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 023.

[00268] SEQ ID NO:143 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 023.

[00269] SEQ ID NO:144 is the heavy chain variable region (V_H) for an OX40 agonist monoclonal antibody.

[00270] SEQ ID NO:145 is the light chain variable region (V_L) for an OX40 agonist monoclonal antibody.

[00271] SEQ ID NO:146 is the heavy chain variable region (V_H) for an OX40 agonist monoclonal antibody.

[00272] SEQ ID NO:147 is the light chain variable region (V_L) for an OX40 agonist monoclonal antibody.

[00273] SEQ ID NO:148 is the heavy chain variable region (V_H) for a humanized OX40 agonist monoclonal antibody.

[00274] SEQ ID NO:149 is the heavy chain variable region (V_H) for a humanized OX40 agonist monoclonal antibody.

[00275] SEQ ID NO:150 is the light chain variable region (V_L) for a humanized OX40 agonist monoclonal antibody.

[00276] SEQ ID NO:151 is the light chain variable region (V_L) for a humanized OX40 agonist monoclonal antibody.

[00277] SEQ ID NO:152 is the heavy chain variable region (V_H) for a humanized OX40 agonist monoclonal antibody.

[00278] SEQ ID NO:153 is the heavy chain variable region (V_H) for a humanized OX40 agonist monoclonal antibody.

[00279] SEQ ID NO:154 is the light chain variable region (V_L) for a humanized OX40 agonist monoclonal antibody.

[00280] SEQ ID NO:155 is the light chain variable region (V_L) for a humanized OX40 agonist monoclonal antibody.

[00281] SEQ ID NO:156 is the heavy chain variable region (V_H) for an OX40 agonist monoclonal antibody.

[00282] SEQ ID NO:157 is the light chain variable region (V_L) for an OX40 agonist monoclonal antibody.

[00283] SEQ ID NO:158 is the heavy chain amino acid sequence of the PD-1 inhibitor nivolumab.

[00284] SEQ ID NO:159 is the light chain amino acid sequence of the PD-1 inhibitor nivolumab.

[00285] SEQ ID NO:160 is the heavy chain variable region (V_H) amino acid sequence of the PD-1 inhibitor nivolumab.

[00286] SEQ ID NO:161 is the light chain variable region (V_L) amino acid sequence of the PD-1 inhibitor nivolumab.

[00287] SEQ ID NO:162 is the heavy chain CDR1 amino acid sequence of the PD-1 inhibitor nivolumab.

[00288] SEQ ID NO:163 is the heavy chain CDR2 amino acid sequence of the PD-1 inhibitor nivolumab.

[00289] SEQ ID NO:164 is the heavy chain CDR3 amino acid sequence of the PD-1 inhibitor nivolumab.

[00290] SEQ ID NO:165 is the light chain CDR1 amino acid sequence of the PD-1 inhibitor nivolumab.

[00291] SEQ ID NO:166 is the light chain CDR2 amino acid sequence of the PD-1 inhibitor nivolumab.

[00292] SEQ ID NO:167 is the light chain CDR3 amino acid sequence of the PD-1 inhibitor nivolumab.

[00293] SEQ ID NO:168 is the heavy chain amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00294] SEQ ID NO:169 is the light chain amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00295] SEQ ID NO:170 is the heavy chain variable region (V_H) amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00296] SEQ ID NO:171 is the light chain variable region (V_L) amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00297] SEQ ID NO:172 is the heavy chain CDR1 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00298] SEQ ID NO:173 is the heavy chain CDR2 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00299] SEQ ID NO:174 is the heavy chain CDR3 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00300] SEQ ID NO:175 is the light chain CDR1 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00301] SEQ ID NO:176 is the light chain CDR2 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00302] SEQ ID NO:177 is the light chain CDR3 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00303] SEQ ID NO:178 is the heavy chain amino acid sequence of the PD-L1 inhibitor durvalumab.

[00304] SEQ ID NO:179 is the light chain amino acid sequence of the PD-L1 inhibitor durvalumab.

[00305] SEQ ID NO:180 is the heavy chain variable region (V_H) amino acid sequence of the PD-L1 inhibitor durvalumab.

[00306] SEQ ID NO:181 is the light chain variable region (V_L) amino acid sequence of the PD-L1 inhibitor durvalumab.

[00307] SEQ ID NO:182 is the heavy chain CDR1 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00308] SEQ ID NO:183 is the heavy chain CDR2 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00309] SEQ ID NO:184 is the heavy chain CDR3 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00310] SEQ ID NO:185 is the light chain CDR1 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00311] SEQ ID NO:186 is the light chain CDR2 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00312] SEQ ID NO:187 is the light chain CDR3 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00313] SEQ ID NO:188 is the heavy chain amino acid sequence of the PD-L1 inhibitor avelumab.

[00314] SEQ ID NO:189 is the light chain amino acid sequence of the PD-L1 inhibitor avelumab.

[00315] SEQ ID NO:190 is the heavy chain variable region (V_H) amino acid sequence of the PD-L1 inhibitor avelumab.

[00316] SEQ ID NO:191 is the light chain variable region (V_L) amino acid sequence of the PD-L1 inhibitor avelumab.

[00317] SEQ ID NO:192 is the heavy chain CDR1 amino acid sequence of the PD-L1 inhibitor avelumab.

[00318] SEQ ID NO:193 is the heavy chain CDR2 amino acid sequence of the PD-L1 inhibitor avelumab.

[00319] SEQ ID NO:194 is the heavy chain CDR3 amino acid sequence of the PD-L1 inhibitor avelumab.

[00320] SEQ ID NO:195 is the light chain CDR1 amino acid sequence of the PD-L1 inhibitor avelumab.

[00321] SEQ ID NO:196 is the light chain CDR2 amino acid sequence of the PD-L1 inhibitor avelumab.

[00322] SEQ ID NO:197 is the light chain CDR3 amino acid sequence of the PD-L1 inhibitor avelumab.

[00323] SEQ ID NO:198 is the heavy chain amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00324] SEQ ID NO:199 is the light chain amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00325] SEQ ID NO:200 is the heavy chain variable region (V_H) amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00326] SEQ ID NO:201 is the light chain variable region (V_L) amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00327] SEQ ID NO:202 is the heavy chain CDR1 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00328] SEQ ID NO:203 is the heavy chain CDR2 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00329] SEQ ID NO:204 is the heavy chain CDR3 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00330] SEQ ID NO:205 is the light chain CDR1 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00331] SEQ ID NO:206 is the light chain CDR2 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00332] SEQ ID NO:207 is the light chain CDR3 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00333] SEQ ID NO:208 is the heavy chain amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00334] SEQ ID NO:209 is the light chain amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00335] SEQ ID NO:210 is the heavy chain variable region (V_H) amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00336] SEQ ID NO:211 is the light chain variable region (V_L) amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00337] SEQ ID NO:212 is the heavy chain CDR1 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00338] SEQ ID NO:213 is the heavy chain CDR2 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00339] SEQ ID NO:214 is the heavy chain CDR3 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00340] SEQ ID NO:215 is the light chain CDR1 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00341] SEQ ID NO:216 is the light chain CDR2 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00342] SEQ ID NO:217 is the light chain CDR3 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00343] SEQ ID NO:218 is the heavy chain amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00344] SEQ ID NO:219 is the light chain amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00345] SEQ ID NO:220 is the heavy chain variable region (V_H) amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00346] SEQ ID NO:221 is the light chain variable region (V_L) amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00347] SEQ ID NO:222 is the heavy chain CDR1 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00348] SEQ ID NO:223 is the heavy chain CDR2 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00349] SEQ ID NO:224 is the heavy chain CDR3 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00350] SEQ ID NO:225 is the light chain CDR1 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00351] SEQ ID NO:226 is the light chain CDR2 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00352] SEQ ID NO:227 is the light chain CDR3 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00353] SEQ ID NO:228 is the heavy chain amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00354] SEQ ID NO:229 is the light chain amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00355] SEQ ID NO:230 is the heavy chain variable region (V_H) amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00356] SEQ ID NO:231 is the light chain variable region (V_L) amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00357] SEQ ID NO:232 is the heavy chain CDR1 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00358] SEQ ID NO:233 is the heavy chain CDR2 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00359] SEQ ID NO:234 is the heavy chain CDR3 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00360] SEQ ID NO:235 is the light chain CDR1 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00361] SEQ ID NO:236 is the light chain CDR2 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00362] SEQ ID NO:237 is the light chain CDR3 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

[00363] Adoptive cell therapy utilizing TILs cultured *ex vivo* by the Rapid Expansion Protocol (REP) has produced successful adoptive cell therapy following host immunosuppression in patients with cancer such as melanoma. Current infusion acceptance parameters rely on readouts of the composition of TILs (*e.g.*, CD28, CD8, or CD4 positivity) and on the numerical folds of expansion and viability of the REP product. While TIL can be reactivated and expanded ex vivo, their epigenetic programming in suppressive tumor microenvironment once the expanded TILs are administered could be keeping TIL in a more differentiated and less functional state.

[00364] The present invention relates to use of epigenetic reprogramming agents in the cell culture medium during *ex vivo* expansion of TILs to counter the effects of the suppressive tumor microenvironment and improve the quality of expanded TILs for persistence, functionality and antitumor potential.

II. Definitions

[00365] 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.

[00366] 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 some embodiments of the present invention, for example, 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.

[00367] The term "*in vivo*" refers to an event that takes place in a subject's body.

[00368] 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.

[00369] 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.

[00370] 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 described herein.

[00371] 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 harvested"), 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 expanded TILs ("REP TILs" or "post-REP TILs"). TIL cell populations can include genetically modified TILs.

[00372] By "population of cells" (including TILs) herein is meant a number of cells that share common traits. In general, populations generally range from $1 \text{ X } 10^6$ to $1 \text{ X } 10^{10}$ 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.

[00373] 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.

[00374] 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.

[00375] 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.

[00376] 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.

[00377] 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.

[00378] 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.

[00379] 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 no opened to the outside environment until the TILs are ready to be administered to the patient.

[00380] The terms "fragmenting," "fragment," and "fragmented," as used herein to describe processes for disrupting a tumor, includes mechanical fragmentation methods such as crushing, slicing, dividing, and morcellating tumor tissue as well as any other method for disrupting the physical structure of tumor tissue.

[00381] 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 an antigen presenting cell (PBMCs are a type of antigen-presenting cell), the peripheral blood mononuclear cells are preferably irradiated allogeneic peripheral blood mononuclear cells.

[00382] 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+.

[00383] 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.

[00384] 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

[00385] 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.

[00386] 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 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 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-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 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:5.

[00387] In some embodiments, an IL-2 form suitable for use in the invention is nemvaleukin alfa, also known as ALKS-4230 (SEQ ID NO:6), 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 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:6. 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: 6), and glycosylation sites at positions: N187, N206, T212 using the numbering in SEQ ID NO:6. 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: 6. In some embodiments, an IL-2 form suitable for use in the invention has the amino acid sequence given in SEQ ID NO: 6 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:7, 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:7, 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:8 or an amino acid sequence having at least 90% sequence identity to SEQ ID NO:8 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.

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 IL-2 form	APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR WITFCQSIIS TLT	60 120 133
SEQ ID NO:6 Nemvaleukin alfa	SKNFHLRPRD LISNINVIVL ELKGSETTFM CEYADETATI VEFLNRWITF SQSIISTLTG GSSSTKKTQL QLEHLLLDLQ MILNGINNYK NPKLTRMLTF KFYMPKKATE LKHLQCLEEE LKPLEEVLNL AQGSGGGSEL CDDDPPEIPH ATFKAMAYKE GTMLNCECKR GFRRIKSGSL YMLCTGNSSH SSWDNQCQCT SSATRNTTKQ VTPQPEEQKE RKTTEMQSPM QPVDQASLPG HCREPPPWEN EATERIYHFV VGQMVYYQCV QGYRALHRGP AESVCKMTHG KTRWTQPQLI CTG	60 120 180 240 300 303
SEQ ID NO:7 IL-2 form	MDAMKRGLCC VLLLCGAVFV SARRPSGRKS SKMQAFRIWD VNQKTFYLRN NQLVAGYLQG PNVNLEEKID VVPIEPHALF LGIHGGKMCL SCVKSGDETR LQLEAVNITD LSENRKQDKR FAFIRSDSGP TTSFESAACP GWFLCTAMEA DQPVSLTNMP DEGVMVTKFY FQEDESGSGG ASSESSASSD GPHPVITESR ASSESSASSD GPHPVITESR EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK	60 120 180 240 300 360 420 453
SEQ ID NO:8 mucin domain polypeptide	SESSASSDGP HPVITP	16
SEQ ID NO:9 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:10	MDCDIEGKDG KQYESVLMVS IDQLLDSMKE IGSNCLNNEF NFFKRHICDA NKEGMFLFRA ARKLRQFLKM NSTGDFDLHL LKVSEGTTIL LNCTGQVKGR KPAALGEAQP TKSLEENKSL	60 120

recombinant human IL-7 (rhIL-7)	KEQKKLNDLC FLKRLLQEIK TCWNKILMGT KEH	153
SEQ ID NO:11 recombinant human IL-15 (rhIL-15)	MNWVNVISDL KKIEDLIQSM HIDATLYTES DVHPSCKVTA MKCFLLELQV ISLESGDASI HDTVENLIIL ANNSLSSNGN VTESGCKECE ELEEKNIKEF LQSFVHIVQM FINTS	60 115
SEQ ID NO:12 recombinant human IL-21 (rhIL-21)	MQDRHMIRMR QLIDIVDQLK NYVNDLVPEF LPAPEDVETN CEWSAFSCFQ KAQLKSANTG NNERIINVSI KKLKRKPPST NAGRRQKHRL TCPSCDSYEK KPPKEFLERF KSLLQKMIHQ HLSSRTHGSE DS	60 120 132

[00388] 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 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:39 and a IgG class heavy chain comprising SEQ ID NO:38; a IgG class light chain comprising SEQ ID NO:37 and a IgG class heavy chain comprising SEQ ID NO:29; a IgG class light chain comprising SEQ ID NO:39 and a IgG class heavy chain comprising SEQ ID NO:29; and a IgG class light chain comprising SEQ ID NO:37 and a IgG class heavy chain comprising SEQ ID NO:38.

[00389] 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.

[00390] 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 IL-2 molecule can be as few as one or two amino acids of a CDR sequence, or the entire CDR sequences.

[00391] 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.

[00392] 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:14 or SEQ ID NO:15. 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.

[00393] In some embodiments, the antibody cytokine engrafted protein comprises an HCDR1 selected from the group consisting of SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:22 and SEQ ID NO:25. 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:13 and SEQ ID NO:16. 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:17, SEQ ID NO:20, SEQ ID NO:23, and SEQ ID NO:26. In some embodiments, the antibody cytokine engrafted protein comprises an HCDR3 selected from the group consisting of SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:24, and SEQ ID NO:27. In some embodiments, the antibody cytokine engrafted protein comprises a V_H region comprising the amino acid sequence of SEQ ID NO:28. In some embodiments, the antibody cytokine engrafted protein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:29. In some embodiments, the antibody cytokine engrafted protein comprises a V_L region comprising the amino acid sequence of SEQ ID NO:36. In some embodiments, the antibody cytokine engrafted protein comprises a light chain comprising the amino acid sequence of SEQ ID NO:37. 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:36. In some embodiments, the antibody cytokine engrafted protein comprises a heavy chain region comprising the amino acid sequence of SEQ ID NO:29 and a light chain region comprising the amino acid sequence of SEQ ID NO:37. In some embodiments, the antibody cytokine engrafted protein comprises a heavy chain region comprising the amino acid sequence of SEQ ID NO:29 and a light chain region comprising the amino acid sequence of SEQ ID NO:39. In some embodiments, the antibody cytokine engrafted protein comprises a heavy chain region comprising the amino acid sequence of SEQ ID NO:38 and a light chain region comprising the amino acid sequence of SEQ ID NO:37. In some embodiments, the antibody cytokine engrafted protein comprises a heavy chain region comprising the amino acid sequence of SEQ ID NO:38 and a light chain region comprising the amino acid sequence of SEQ ID NO:39. 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. In some embodiments, the antibody cytokine engrafted protein described herein has a sequence as set forth in Table 3.

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

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:13 IL-2	MYRMQLLSCI ALSLALVTNS APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR WITFCQSIIS TLT	60 120 153
SEQ ID NO:14 IL-2 mutein	APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTAML TFKFYMPKKA TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR WITFCQSIIS TLT	60 120 133
SEQ ID NO:15 IL-2 mutein	APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TAKFYMPKKA TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR WITFCQSIIS TLT	60 120 133
SEQ ID NO:16 HCDR1_IL-2	GFSLAPTSSS TKKTQLQLEH LLLDLQMILN GINNYKNPKL TAMLTFKFYM PKKATELKHL QCLEEELKPL EEVLNLAQSK NFHLRPRDLI SNINVIVLEL KGSETTFMCE YADETATIVE FLNRWITFCQ SIISTLTSTS GMSVG	60 120 145
SEQ ID NO:17 HCDR2	DIWWDDKKDY NPSLKS	16
SEQ ID NO:18 HCDR3	SMITNWYFDV	10
SEQ ID NO:19 HCDR1_IL-2 kabat	APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTAML TFKFYMPKKA TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR WITFCQSIIS TLTSTSGMSV G	60 120 141
SEQ ID NO:20 HCDR2 kabat	DIWWDDKKDY NPSLKS	16
SEQ ID NO:21 HCDR3 kabat	SMITNWYFDV 10	
SEQ ID NO:22 HCDR1_IL-2 clothia	GFSLAPTSSS TKKTQLQLEH LLLDLQMILN GINNYKNPKL TAMLTFKFYM PKKATELKHL QCLEEELKPL EEVLNLAQSK NFHLRPRDLI SNINVIVLEL KGSETTFMCE YADETATIVE FLNRWITFCQ SIISTLTSTS GM	60 120 142
SEQ ID NO:23 HCDR2 clothia	WWDDK	5
SEQ ID NO:24 HCDR3 clothia	SMITNWYFDV	10
SEQ ID NO:25 HCDR1_IL-2 IMGT	GFSLAPTSSS TKKTQLQLEH LLLDLQMILN GINNYKNPKL TAMLTFKFYM PKKATELKHL QCLEEELKPL EEVLNLAQSK NFHLRPRDLI SNINVIVLEL KGSETTFMCE YADETATIVE FLNRWITFCQ SIISTLTSTS GMS	60 120 143
SEQ ID NO:26 HCDR2 IMGT	IWWDDKK	7
SEQ ID NO:27 HCDR3 IMGT	ARSMITNWYF DV	12
SEQ ID NO:28 V_H	QVTLRESGPA LVKPTQTLTL TCTFSGFSLA PTSSSTKKTQ LQLEHLLLDL QMILNGINNY KNPKLTAMLT FKFYMPKKAT ELKHLQCLEE ELKPLEEVLN LAQSKNFHLR PRDLISNINV IVLELKGSET TFMCEYADET ATIVEFLNRW ITFCQSIIST LTSTSGMSVG WIRQPPGKAL EWLADIWWDD KKDYNPSLKS RLTISKDTSK NQVVLKVTNM DPADTATYYC ARSMITNWYF DVWGAGTTVT VSS	60 120 180 240 253
SEQ ID NO:29 Heavy chain	QMILNGINNY KNPKLTAMLT FKFYMPKKAT ELKHLQCLEE ELKPLEEVLN LAQSKNFHLR PRDLISNINV IVLELKGSET TFMCEYADET ATIVEFLNRW ITFCQSIIST LTSTSGMSVG WIRQPPGKAL EWLADIWWDD KKDYNPSLKS RLTISKDTSK NQVVLKVTNM DPADTATYYC ARSMITNWYF DVWGAGTTVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKR VEPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV AVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALAAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK	60 120 180 240 300 360 420 480 533
SEQ ID NO:30 LCDR1 kabat	KAQLSVGYMH	10
SEQ ID NO:31 LCDR2 kabat	DTSKLAS	7
SEQ ID NO:32 LCDR3 kabat	FQGSGYPFT	9
SEQ ID NO:33 LCDR1 chothia	QLSVGY	6
SEQ ID NO:34 LCDR2 chothia	DTS	3
SEQ ID NO:35 LCDR3 chothia	GSGYPF	6
SEQ ID NO:36 V_L	DIQMTQSPST LSASVGDRVT ITCKAQLSVG YMHWYQQKPG KAPKLLIYDT SKLASGVPSR FSGSGSGTEF TLTISSLQPD DFATYYCFQG SGYPFTFGGG TKLEIK	60 106
SEQ ID NO:37	DIQMTQSPST LSASVGDRVT ITCKAQLSVG YMHWYQQKPG KAPKLLIYDT SKLASGVPSR	60

Light chain	FSGSGSGTEF TLTISSLQPD DFATYYCFQG SGYPFTFGGG TKLEIKRTVA APSVFIFPPS	120
	DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL	180
	SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC	213
SEQ ID NO:38 Light chain	QVTLRESGPA LVKPTQTLTL TCTFSGFSLA PTSSSTKKTQ LQLEHLLLDL QMILNGINNY	60
	KNPKLTRMLT AKFYMPKKAT ELKHLQCLEE ELKPLEEVLN LAQSKNFHLR PRDLISNINV	120
	IVLELKGSET TFMCEYADET ATIVEFLNRW ITFCQSIIST LTSTSGMSVG WIRQPPGKAL	180
	EWLADIWWDD KKDYNPSLKS RLTISKDTSK NQVVLKVTNM DPADTATYYC ARSMITNWYF	240
	DVWGAGTTVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV TVSWNSGALT	300
	SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKR VEPKSCDKTH	360
	TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV AVSHEDPEVK FNWYVDGVEV	420
	HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALAAPIEK TISKAKGQPR	480
	EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF	540
	FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK	583
SEQ ID NO:39 Light chain	DIQMTQSPST LSASVGDRVT ITCKAQLSVG YMHWYQQKPG KAPKLLIYDT SKLASGVPSR	60
	FSGSGSGTEF TLTISSLQPD DFATYYCFQG SGYPFTFGGG TKLEIKRTVA APSVFIFPPS	120
	DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL	180
	SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC	213

[00394] 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:9).

[00395] 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:10).

[00396] 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 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:11).

[00397] 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:21).

[00398] 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. TILs (including in some cases, genetically modified cytotoxic lymphocytes) compositions may also be administered multiple times at these dosages. The TILs (including, in some cases, genetically engineered TILs) can be administered by using infusion techniques that are commonly known in immunotherapy (*see, e.g.*, Rosenberg, *et al.*, *New Eng. J. of Med.* ***1988,*** 319, 1676). 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.

[00399] The term "hematological malignancy", "hematologic malignancy" or terms of correlative meaning refer to mammalian cancers and tumors of the hematopoietic and 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), multiple myeloma, 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.

[00400] 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.

[00401] 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.

[00402] 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, 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.

[00403] 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.

[00404] 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.

[00405] 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.

[00406] 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).

[00407] 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.

[00408] As used herein, the term "variant" encompasses but is not limited to antibodies or fusion proteins which comprise an amino acid sequence which differs from the amino acid sequence of a reference antibody 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. 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. The term variant also includes pegylated antibodies or proteins.

[00409] 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 harvested"), 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 8, including TILs referred to as reREP TILs).

[00410] 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.

[00411] The term "deoxyribonucleotide" encompasses natural and synthetic, unmodified and modified deoxyribonucleotides. Modifications include changes to the sugar moiety, to the base moiety and/or to the linkages between deoxyribonucleotide in the oligonucleotide.

[00412] The term "RNA" defines a molecule comprising at least one ribonucleotide residue. The term "ribonucleotide" defines a nucleotide with a hydroxyl group at the 2' position of a b-D-ribofuranose moiety. The term RNA includes double-stranded RNA, single-stranded RNA, isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Nucleotides of the RNA molecules described herein may also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs or analogs of naturally-occurring RNA.

[00413] 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 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.

[00414] 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 "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.

[00415] 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."

[00416] The terms "antibody" and its plural form "antibodies" refer to whole immunoglobulins and any antigen-binding fragment ("antigen-binding portion") or single chains thereof. An "antibody" further refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen-binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light

chain constant region is comprised of one domain, C_L. The V_H and V_L regions of an antibody may be further subdivided into regions of hypervariability, which are referred to as complementarity determining regions (CDR) or hypervariable regions (HVR), and which can be interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen epitope or epitopes. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system.

[00417] The term "antigen" refers to a substance that induces an immune response. In some embodiments, an antigen is a molecule capable of being bound by an antibody or a TCR if presented by major histocompatibility complex (MHC) molecules. The term "antigen", as used herein, also encompasses T cell epitopes. An antigen is additionally capable of being recognized by the immune system. In some embodiments, an antigen is capable of inducing a humoral immune response or a cellular immune response leading to the activation of B lymphocytes and/or T lymphocytes. In some cases, this may require that the antigen contains or is linked to a Th cell epitope. An antigen can also have one or more epitopes (*e.g.*, B- and T-epitopes). In some embodiments, an antigen will preferably react, typically in a highly specific and selective manner, with its corresponding antibody or TCR and not with the multitude of other antibodies or TCRs which may be induced by other antigens.

[00418] The terms "monoclonal antibody," "mAb," "monoclonal antibody composition," or their plural forms refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. Monoclonal antibodies specific to certain receptors can be made using knowledge and skill in the art of injecting test subjects with suitable antigen and then isolating hybridomas expressing antibodies having the desired sequence or functional characteristics. DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host

cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Recombinant production of antibodies will be described in more detail below.

[00419] The terms "antigen-binding portion" or "antigen-binding fragment" of an antibody (or simply "antibody portion" or "fragment"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and CH1 domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and CH1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a domain antibody (dAb) fragment (Ward, *et al., Nature,* **1989,** *341,* 544-546), which may consist of a V_H or a V_L domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules known as single chain Fv (scFv); see, *e.g.*, Bird, *et al.*, *Science* **1988,** *242,* 423-426; and Huston, *et al.*, *Proc. Natl. Acad. Sci. USA* **1988,** *85,* 5879-5883). Such scFv antibodies are also intended to be encompassed within the terms "antigen-binding portion" or "antigen-binding fragment" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies. In some embodiments, a scFv protein domain comprises a VH portion and a VL portion. A scFv molecule is denoted as either VL-L-VH if the VL domain is the N-terminal part of the scFv molecule, or as VH-L-VL if the VH domain is the N-terminal part of the scFv molecule. Methods for making scFv molecules and designing suitable peptide linkers are described in U.S. Pat. No. 4,704,692, U.S. Pat. No. 4,946,778, R. Raag and M. Whitlow, "Single Chain Fvs." FASEB Vol 9:73-80 (1995) and R. E. Bird and B. W. Walker, Single Chain Antibody Variable Regions, TIBTECH, Vol 9: 132-137 (1991), the disclosures of which are incorporated by reference herein.

[00420] The term "human antibody," as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). The term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[00421] The term "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. In some embodiments, the human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

[00422] The term "recombinant human antibody", as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (such as a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom (described further below), (b) antibodies isolated from a host cell transformed to express the human antibody, *e.g.*, from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial human antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable regions in which the framework and CDR regions are derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[00423] As used herein, "isotype" refers to the antibody class (*e.g.*, IgM or IgG1) that is encoded by the heavy chain constant region genes.

[00424] The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen."

[00425] The term "human antibody derivatives" refers to any modified form of the human antibody, including a conjugate of the antibody and another active pharmaceutical ingredient or antibody. The terms "conjugate," "antibody-drug conjugate", "ADC," or "immunoconjugate" refers to an antibody, or a fragment thereof, conjugated to another therapeutic moiety, which can be conjugated to antibodies described herein using methods available in the art.

[00426] The terms "humanized antibody," "humanized antibodies," and "humanized" are intended to refer to antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Additional framework region modifications may be made within the human framework sequences. Humanized forms of non-human (for example, murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a 15 hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones, *et al.*, *Nature* **1986,** *321,* 522-525; Riechmann, *et al.*, *Nature* **1988,** *332,* 323-329; and Presta, *Curr. Op. Struct. Biol.* **1992,** *2,* 593-596. The antibodies described herein may also be modified to employ any Fc variant

which is known to impart an improvement (*e.g.*, reduction) in effector function and/or FcR binding. The Fc variants may include, for example, any one of the amino acid substitutions disclosed in International Patent Application Publication Nos. WO 1988/07089 A1, WO 1996/14339 A1, WO 1998/05787 A1, WO 1998/23289 A1, WO 1999/51642 A1, WO 99/58572 A1, WO 2000/09560 A2, WO 2000/32767 A1, WO 2000/42072 A2, WO 2002/44215 A2, WO 2002/060919 A2, WO 2003/074569 A2, WO 2004/016750 A2, WO 2004/029207 A2, WO 2004/035752 A2, WO 2004/063351 A2, WO 2004/074455 A2, WO 2004/099249 A2, WO 2005/040217 A2, WO 2005/070963 A1, WO 2005/077981 A2, WO 2005/092925 A2, WO 2005/123780 A2, WO 2006/019447 A1, WO 2006/047350 A2, and WO 2006/085967 A2; and U.S. Patent Nos. 5,648,260; 5,739,277; 5,834,250; 5,869,046; 6,096,871; 6,121,022; 6,194,551; 6,242,195; 6,277,375; 6,528,624; 6,538,124; 6,737,056; 6,821,505; 6,998,253; and 7,083,784; the disclosures of which are incorporated by reference herein.

[00427] The term "chimeric antibody" is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

[00428] A "diabody" is a small antibody fragment with two antigen-binding sites. The fragments comprises a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H-V_L or V_L-V_H). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, *e.g.*, European Patent No. EP 404,097, International Patent Publication No. WO 93/11161; and Bolliger, *et al.*, *Proc. Natl. Acad. Sci. USA* **1993,** *90,* 6444-6448.

[00429] The term "glycosylation" refers to a modified derivative of an antibody. An aglycoslated antibody lacks glycosylation. Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Aglycosylation may increase the affinity of the antibody for

antigen, as described in U.S. Patent Nos. 5,714,350 and 6,350,861. Additionally or alternatively, an antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNac structures. Such altered glycosylation patterns have been demonstrated to increase the ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. For example, the cell lines Ms704, Ms705, and Ms709 lack the fucosyltransferase gene, FUT8 (alpha (1,6) fucosyltransferase), such that antibodies expressed in the Ms704, Ms705, and Ms709 cell lines lack fucose on their carbohydrates. The Ms704, Ms705, and Ms709 FUT8−/− cell lines were created by the targeted disruption of the FUT8 gene in CHO/DG44 cells using two replacement vectors (see *e.g.* U.S. Patent Publication No. 2004/0110704 or Yamane-Ohnuki, *et al., Biotechnol. Bioeng.*, **2004,** *87,* 614-622). As another example, European Patent No. EP 1,176,195 describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit hypofucosylation by reducing or eliminating the alpha 1,6 bond-related enzyme, and also describes cell lines which have a low enzyme activity for adding fucose to the N-acetylglucosamine that binds to the Fc region of the antibody or does not have the enzyme activity, for example the rat myeloma cell line YB2/0 (ATCC CRL 1662). International Patent Publication WO 03/035835 describes a variant CHO cell line, Lec 13 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields, *et al., J. Biol. Chem.* **2002,** *277,* 26733-26740. International Patent Publication WO 99/54342 describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (*e.g.*, beta(1,4)-N-acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies (see also Umana, *et al.*, *Nat. Biotech.* **1999,** *17,* 176-180). Alternatively, the fucose residues of the antibody may be cleaved off using a fucosidase enzyme. For example, the fucosidase alpha-L-fucosidase removes fucosyl residues from antibodies as described in Tarentino, *et al.*, *Biochem.* **1975,** *14,* 5516-5523.

[00430] "Pegylation" refers to a modified antibody, or a fragment thereof, that typically is reacted with polyethylene glycol (PEG), such as a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. Pegylation may, for example, increase the biological (*e.g.*, serum) half life of the antibody. Preferably, the pegylation is carried out via an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer). As used herein, the term "polyethylene glycol" is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (C_1-C_{10})alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. The antibody to be pegylated may be an aglycosylated antibody. Methods for pegylation are known in the art and can be applied to the antibodies of the invention, as described for example in European Patent Nos. EP 0154316 and EP 0401384 and U.S. Patent No. 5,824,778, the disclosures of each of which are incorporated by reference herein.

[00431] The term "biosimilar" means a biological product, including a monoclonal antibody or protein, that is highly similar to a U.S. licensed reference biological product notwithstanding minor differences in clinically inactive components, and for which there are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency of the product. Furthermore, a similar biological or "biosimilar" medicine is a biological medicine that is similar to another biological medicine that has already been authorized for use by the European Medicines Agency. The term "biosimilar" is also used synonymously by other national and regional regulatory agencies. Biological products or biological medicines are medicines that are made by or derived from a biological source, such as a bacterium or yeast. They can consist of relatively small molecules such as human insulin or erythropoietin, or complex molecules such as monoclonal antibodies. For example, if the reference IL-2 protein is aldesleukin (PROLEUKIN), a protein approved by drug regulatory authorities with reference to aldesleukin is a "biosimilar to" aldesleukin or is a "biosimilar thereof" of aldesleukin. In Europe, a similar biological or "biosimilar" medicine is a biological medicine that is similar to another biological medicine that has already been authorized for use by the European Medicines Agency (EMA). The relevant legal basis for similar biological applications in Europe is Article 6 of Regulation (EC) No 726/2004 and Article 10(4) of Directive 2001/83/EC, as amended and therefore in Europe, the biosimilar may be authorized, approved for authorization or subject of an application for authorization under Article 6 of

Regulation (EC) No 726/2004 and Article 10(4) of Directive 2001/83/EC. The already authorized original biological medicinal product may be referred to as a "reference medicinal product" in Europe. Some of the requirements for a product to be considered a biosimilar are outlined in the CHMP Guideline on Similar Biological Medicinal Products. In addition, product specific guidelines, including guidelines relating to monoclonal antibody biosimilars, are provided on a product-by-product basis by the EMA and published on its website. A biosimilar as described herein may be similar to the reference medicinal product by way of quality characteristics, biological activity, mechanism of action, safety profiles and/or efficacy. In addition, the biosimilar may be used or be intended for use to treat the same conditions as the reference medicinal product. Thus, a biosimilar as described herein may be deemed to have similar or highly similar quality characteristics to a reference medicinal product. Alternatively, or in addition, a biosimilar as described herein may be deemed to have similar or highly similar biological activity to a reference medicinal product. Alternatively, or in addition, a biosimilar as described herein may be deemed to have a similar or highly similar safety profile to a reference medicinal product. Alternatively, or in addition, a biosimilar as described herein may be deemed to have similar or highly similar efficacy to a reference medicinal product. As described herein, a biosimilar in Europe is compared to a reference medicinal product which has been authorized by the EMA. However, in some instances, the biosimilar may be compared to a biological medicinal product which has been authorized outside the European Economic Area (a non-EEA authorized "comparator") in certain studies. Such studies include for example certain clinical and in vivo non-clinical studies. As used herein, the term "biosimilar" also relates to a biological medicinal product which has been or may be compared to a non-EEA authorized comparator. Certain biosimilars are proteins such as antibodies, antibody fragments (for example, antigen binding portions) and fusion proteins. A protein biosimilar may have an amino acid sequence that has minor modifications in the amino acid structure (including for example deletions, additions, and/or substitutions of amino acids) which do not significantly affect the function of the polypeptide. The biosimilar may comprise an amino acid sequence having a sequence identity of 97% or greater to the amino acid sequence of its reference medicinal product, *e.g.*, 97%, 98%, 99% or 100%. The biosimilar may comprise one or more post-translational modifications, for example, although not limited to, glycosylation, oxidation, deamidation, and/or truncation which is/are different to the post-translational modifications of the reference medicinal product, provided that the differences do not result in a change in safety and/or efficacy of the medicinal product. The biosimilar may have an identical or different

glycosylation pattern to the reference medicinal product. Particularly, although not exclusively, the biosimilar may have a different glycosylation pattern if the differences address or are intended to address safety concerns associated with the reference medicinal product. Additionally, the biosimilar may deviate from the reference medicinal product in for example its strength, pharmaceutical form, formulation, excipients and/or presentation, providing safety and efficacy of the medicinal product is not compromised. The biosimilar may comprise differences in for example pharmacokinetic (PK) and/or pharmacodynamic (PD) profiles as compared to the reference medicinal product but is still deemed sufficiently similar to the reference medicinal product as to be authorized or considered suitable for authorization. In certain circumstances, the biosimilar exhibits different binding characteristics as compared to the reference medicinal product, wherein the different binding characteristics are considered by a Regulatory Authority such as the EMA not to be a barrier for authorization as a similar biological product. The term "biosimilar" is also used synonymously by other national and regional regulatory agencies.

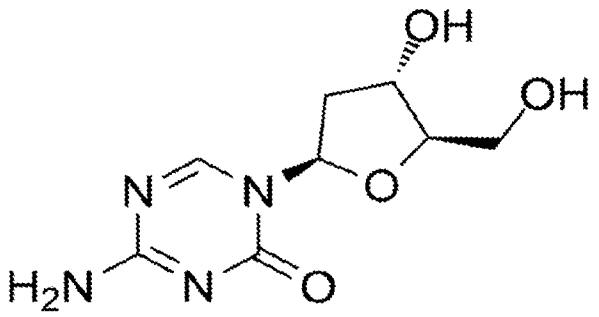
[00432] The term "epigenetic reprogramming" as used herein refers to remodeling of epigenetic marks during development of a cell. Epigenetic reprogramming affects cellular function through successive generation of cells without altering the underlying DNA sequence. Epigenetic reprogramming involves modulation of DNA and/or histone methylation to effect reconfiguration of transcription in the cells. Reprogramming can be induced artificially through the introduction of exogenous factors, usually transcription factors in cell culture media, for example, during ex vivo expansion of TILs. Various drug compounds can be used for epigenetic reprogramming, and may involve modulation of one or more pathways and/or activity of proteins involved in transcription in cells. Compounds that can reconfigure transcription in cells include, but are not limited to, DNA hypomethylating agents, mitogen-activated protein kinase (MEK) inhibitors, histone deacetylase (HDAC) inhibitors, enhancer of zeste homolog 2 (EZH2) inhibitors, bromodomain inhibitors, protein kinase B (AKT) inhibitors, and/or Ten-eleven translocation protein (TET) inhibitors.

[00433] The term "DNA hypomethylating agent" refers to a drug that inhibits, or otherwise diminishes DNA methylation. Most DNA hypomethylating agents block the activity of DNA methyltransferase, and thus function as DNA methyltransferase inhibitors. The activity of DNA methyltransferase could decrease by a statistically significant amount including, for example, a decrease of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 95% or 100% of the activity

compared to an appropriate control. Examples of DNA hypomethylating agents include decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof, cocrystals and solvates thereof. In some embodiments, the DNA hypomethylating agent may be decitabine (*see, e.g.,* Aribi A, *et al.* Cancer (2007) 109(4):713–7) including for example, a cocrystal solvate, or pharmaceutically acceptable salt thereof.

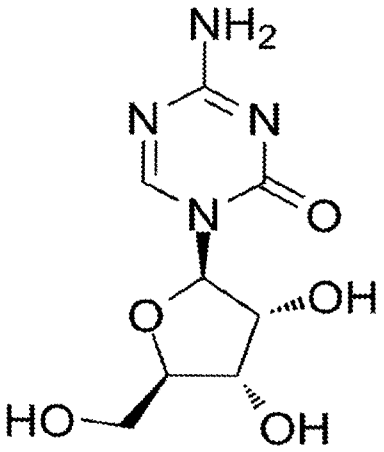
i. Decitabine

[00434] In an embodiment, the DNA hypomethylating agent is decitabine. Decitabine has the chemical structure and name shown as: 4-amino-1-[(2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,3,5-triazin-2-one



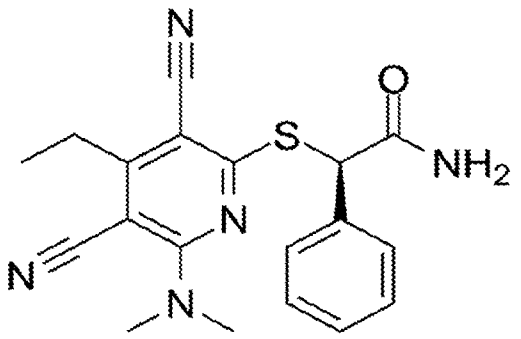
ii. Azacitidine

[00435] In an embodiment, the DNA hypomethylating agent is azacitidine. Azacitidine has the chemical structure and name shown as: 4-amino-1-[(2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,3,5-triazin-2-one



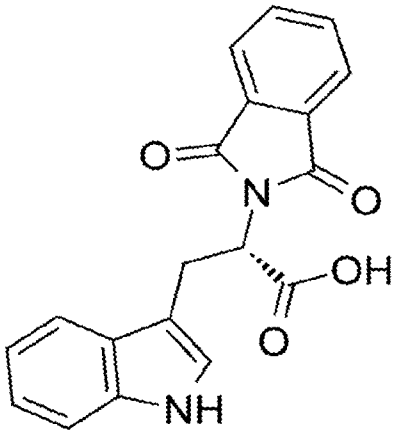
iii. GSK-3484862

[00436] In an embodiment, the DNA hypomethylating agent is GSK-3484862. GSK-3484862 has the chemical structure and name shown as: (2R)-2-[3,5-dicyano-6-(dimethylamino)-4-ethylpyridin-2-yl]sulfanyl-2-phenylacetamide



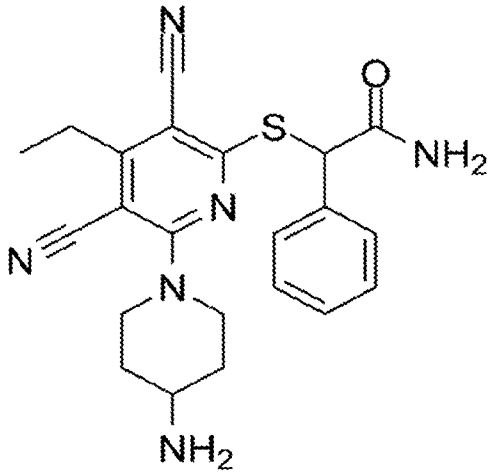
iv. RG-108

[00437] In an embodiment, the DNA hypomethylating agent is RG-108. RG-108 has the chemical structure and name shown as: (2S)-2-(1,3-dioxoisoindol-2-yl)-3-(1H-indol-3-yl)propanoic acid



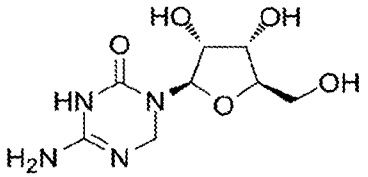
v. GSK-3685032

[00438] In an embodiment, the DNA hypomethylating agent is GSK-3685032. GSK-3685032 has the chemical structure and name shown as: 2-[6-(4-aminopiperidin-1-yl)-3,5-dicyano-4-ethylpyridin-2-yl]sulfanyl-2-phenylacetamide



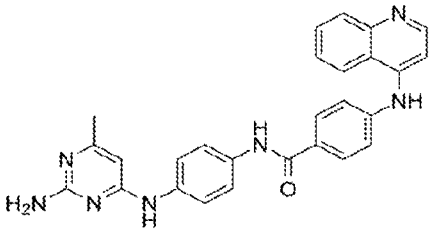
vi. DHAC

[00439] In an embodiment, the DNA hypomethylating agent is DHAC. DHAC has the chemical structure and name shown as: 6-amino-3-[3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,4-dihydro-1,3,5-triazin-2-one



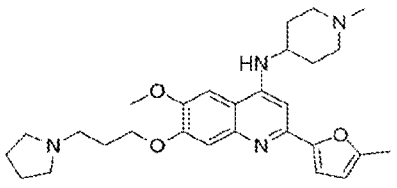
vii. SGI-1027

[00440] In an embodiment, the DNA hypomethylating agent is SGI-1027. SGI-1027 has the chemical structure and name shown as: N-[4-[(2-amino-6-methylpyrimidin-4-yl)amino]phenyl]-4-(quinolin-4-ylamino)benzamide



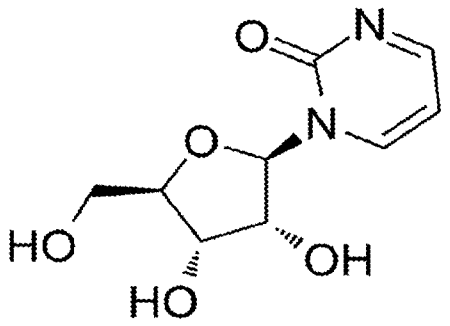
viii. CM-272

[00441] In an embodiment, the DNA hypomethylating agent is CM-272. CM-272 has the chemical structure and name shown as: 6-methoxy-2-(5-methylfuran-2-yl)-N-(1-methylpiperidin-4-yl)-7-(3-pyrrolidin-1-ylpropoxy)quinolin-4-amine



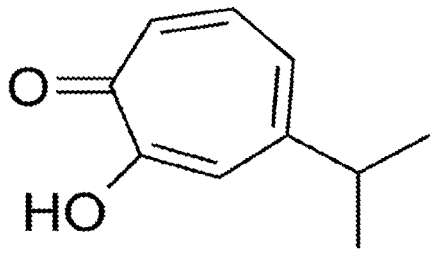
ix. Zebularine

[00442] In an embodiment, the DNA hypomethylating agent is Zebularine. Zebularine has the chemical structure and name shown as: 1-[(2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]pyrimidin-2-one



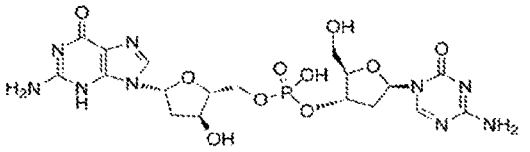
x. Hinokitiol

[00443] In an embodiment, the DNA hypomethylating agent is hinokitiol. Hinokitiol has the chemical structure and name shown as: 2-hydroxy-6-propan-2-ylcyclohepta-2,4,6-trien-1-one



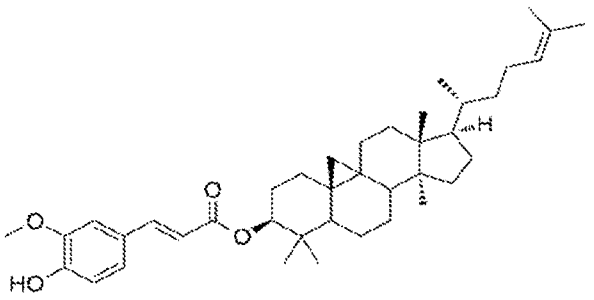
xi. Guadecitabine

[00444] In an embodiment, the DNA hypomethylating agent is guadecitabine. Guadecitabine has the chemical structure and name shown as: [(2R,3S,5R)-5-(2-amino-6-oxo-1H-purin-9-yl)-3-hydroxyoxolan-2-yl]methyl [(2R,3S,5R)-5-(4-amino-2-oxo-1,3,5-triazin-1-yl)-2-(hydroxymethyl)oxolan-3-yl] phosphate



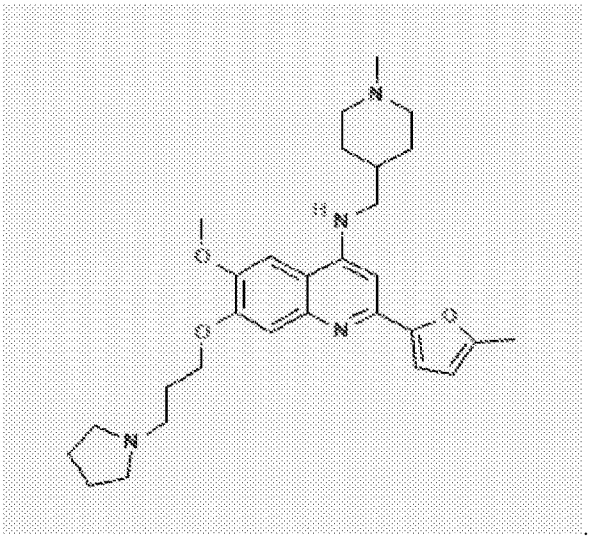
xii. Gamma-Oryzanol

[00445] In an embodiment, the DNA hypomethylating agent is gamma-Oryzanol. Gamma-Oryzanol has the chemical structure and name shown as: [(1S,3R,6S,8R,11S,12S,15R,16R)-7,7,12,16-tetramethyl-15-[(2R)-6-methylhept-5-en-2-yl]-6-pentacyclo[9.7.0.$0^{1,3}$.$0^{3,8}$.$0^{12,16}$]octadecanyl] (E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate



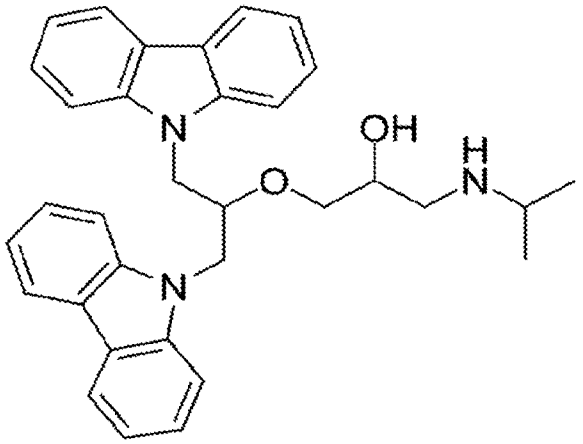
xiii. CM-579

[00446] In an embodiment, the DNA hypomethylating agent is CM-579. CM-579 has the chemical structure and name shown as: 6-methoxy-2-(5-methylfuran-2-yl)-N-[(1-methylpiperidin-4-yl)methyl]-7-(3-pyrrolidin-1-ylpropoxy)quinolin-4-amine



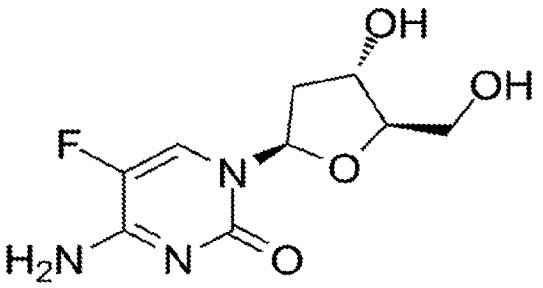
xiv. DC-517

[00447] In an embodiment, the DNA hypomethylating agent is DC-517. DC-517 has the chemical structure and name shown as: 1-[1,3-di(carbazol-9-yl)propan-2-yloxy]-3-(propan-2-ylamino)propan-2-ol



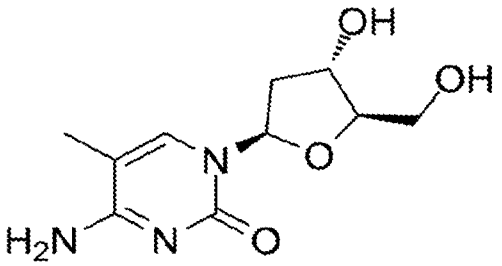
xv. 5-Fluoro-2'-deoxycytidine

[00448] In an embodiment, the DNA hypomethylating agent is 5-fluoro-2'-deoxycytidine. 5-Fluoro-2'-deoxycytidine has the chemical structure and name shown as: 4-amino-5-fluoro-1-[(2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]pyrimidin-2-one



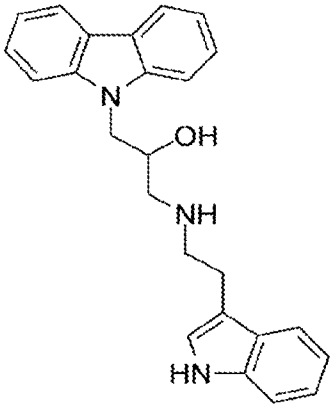
xvi. 5-Methyldeoxycytidine

[00449] In an embodiment, the DNA hypomethylating agent is 5-methyldeoxycytidine. 5-Methyldeoxycytidine has the chemical structure and name shown as: 4-amino-1-[(2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidin-2-one



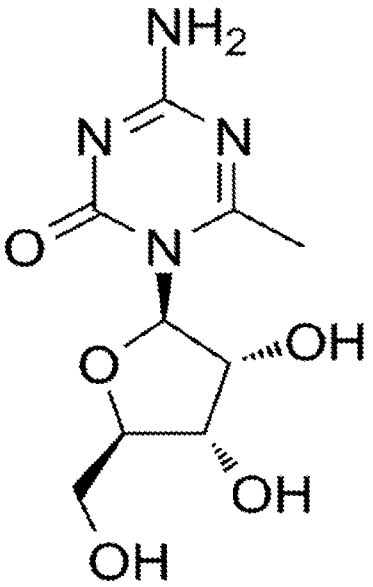
xvii. DC-05

[00450] In an embodiment, the DNA hypomethylating agent is DC-05. DC-05 has the chemical structure and name shown as: 1-carbazol-9-yl-3-[2-(1H-indol-3-yl)ethylamino]propan-2-ol



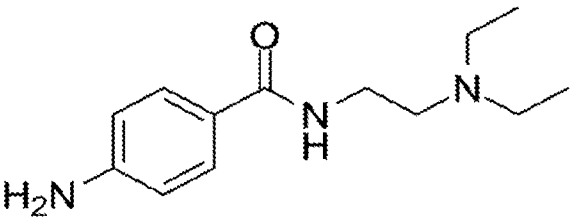
xviii. 6-Methyl-5-azacytidine

[00451] In an embodiment, the DNA hypomethylating agent is 6-methyl-5-azacytidine. 6-Methyl-5-azacytidine has the chemical structure and name shown as: 4-amino-1-[(2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-methyl-1,3,5-triazin-2-one



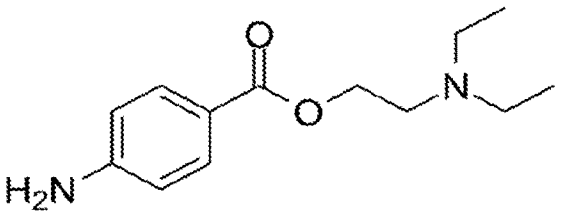
xix. Procainamide

[00452] In an embodiment, the DNA hypomethylating agent is procainamide. Procainamide has the chemical structure and name shown as: 4-amino-N-[2-(diethylamino)ethyl]benzamide



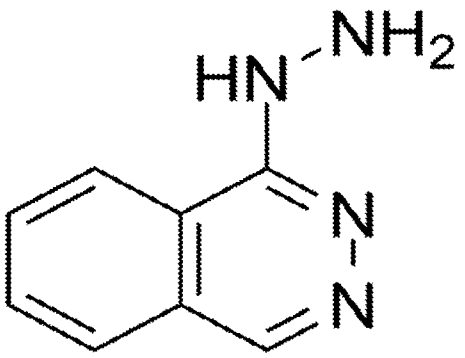
xx. Procaine

[00453] In an embodiment, the DNA hypomethylating agent is procaine. Procaine has the chemical structure and name shown as: 2-(diethylamino)ethyl 4-aminobenzoate



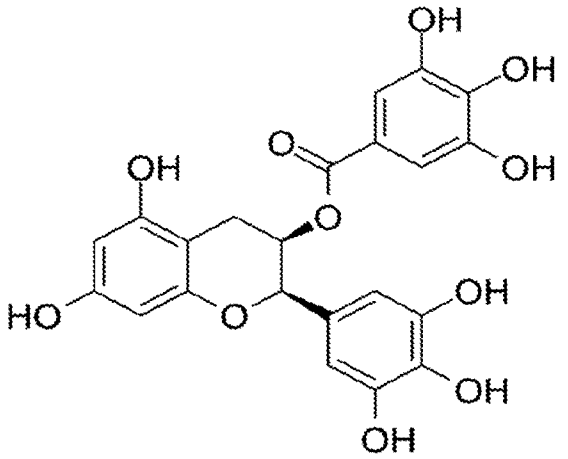
xxi. Hydralazine

[00454] In an embodiment, the DNA hypomethylating agent is hydralazine. Hydralazine has the chemical structure and name shown as: phthalazin-1-ylhydrazine



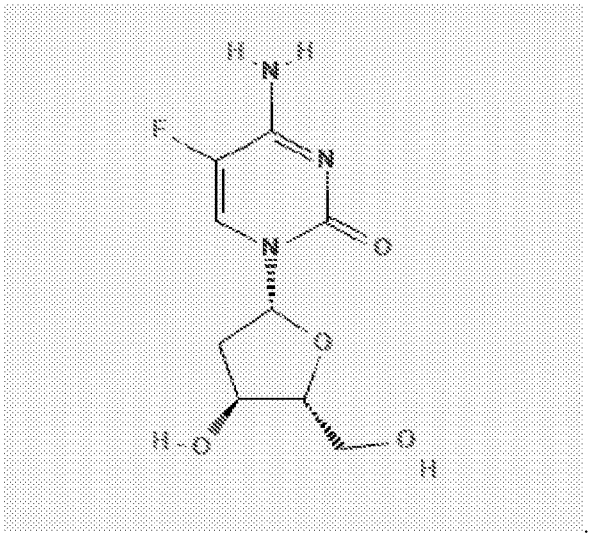
xxii. EGCG

[00455] In an embodiment, the DNA hypomethylating agent is EGCG. EGCG has the chemical structure and name shown as: [(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl] 3,4,5-trihydroxybenzoate



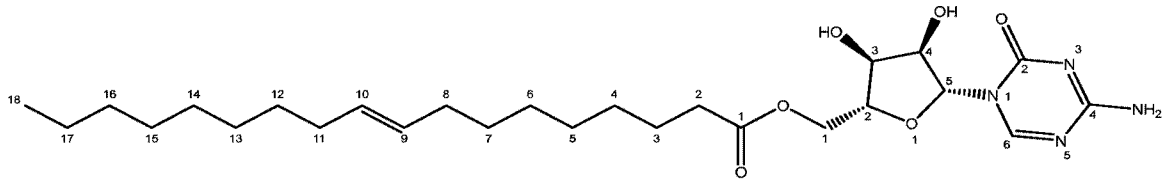
xxiii. FdCyd

In an embodiment, the DNA hypomethylating agent is FdCyD. FdCyD has the chemical structure and name shown as: 4-amino-5-fluoro-1-[(2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]pyrimidin-2-one



xxiv. CP-4200

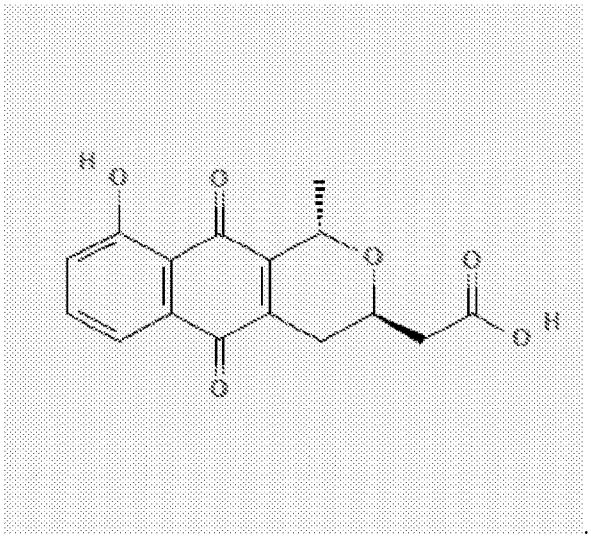
[00456] In an embodiment, the DNA hypomethylating agent is CP-4200. CP-4200 has the chemical structure and name shown as: 5-azacytidine-5'-elaidate



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xxv. Nanomycin A

[00457] In an embodiment, the DNA hypomethylating agent is Nanomycin A. Nanomycin A has the chemical structure and name shown as: 2-[(1S,3R)-9-hydroxy-1-methyl-5,10-dioxo-3,4-dihydro-1H-benzo[g]isochromen-3-yl]acetic acid



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[00458] As used herein, the term "MEK inhibitor" refers to a compound that reduces, inhibits, or otherwise diminishes one or more of the biological activities of mitogen-activated protein kinase (MEK) (MEK1 and/or MEK2). The activity could decrease by a statistically significant amount including, for example, a decrease of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 95% or 100% of the activity of MEK compared to an appropriate control. MEK is a dual-specificity kinase that phosphorylates the tyrosine and threonine residues on ERKs 1 and 2 required for activation. Two related genes encode MEK1 and MEK2 which differ in their binding to

ERKs and, possibly, in their activation profiles. MEKs are substrates for several protein kinases including B-Raf in the MAPK/ERK pathway. MEK inhibitors include, but are not limited to, trametinib (Mekinist®, GSK1120212), cobimetinib (Cotellic®), binimetinib (Mektovi®, MEK162, ARRY-162, ARRY-438162), selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325 and pharmaceutically acceptable salts, cocrystals and solvates thereof. In some embodiments, the MEK inhibitor is trametinib (*see, e.g.*, Flaherty *et al.*, N. Engl. J. Med. **2012,** 367:1694-1703) including for example, a cocrystal solvate, or pharmaceutically acceptable salt thereof. In some embodiments, the MEK inhibitor is trametinib as a DMSO solvate or cocrystal.

[00459] As used herein, the term "HDAC" refers to a compound that reduces, inhibits, or otherwise diminishes one or more of the biological activity of histone deacetylase (HDAC). The activity could decrease by a statistically significant amount including, for example, a decrease of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 95% or 100% of the activity of HDAC compared to an appropriate control. Examples of HDAC inhibitors include, but are not limited to, rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and cocrystal solvate, or pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is ricolinistat (*see, e.g.,* Dan T. Vogl, *et al.* Clin Cancer Res. 2017, 23(13) 3307-15) including for example, a cocrystal solvate, or pharmaceutically acceptable salt thereof.

[00460] As used herein, the term "EZH2 inhibitor" refers to a compound that reduces, inhibits, or otherwise diminishes one or more of the biological activity of enhancer of zeste homolog 2 (EZH2). The activity could decrease by a statistically significant amount including, for example, a decrease of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 95% or 100% of the activity of EZH2 compared to an appropriate control. Examples of EZH2 inhibitors include, but are not limited to, 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and cocrystal solvates or pharmaceutically acceptable salts thereof.

[00461] As used herein, the term "AKT inhibitor" refers to a compound that reduces, inhibits, or otherwise diminishes one or more of the biological activity of protein kinase B

(AKT). The activity could decrease by a statistically significant amount including, for example, a decrease of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 95% or 100% of the activity of AKT compared to an appropriate control. Examples of AKT inhibitors include, but are not limited to, ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib (*see, e.g.,* Lin *et. al*, Clin. Cancer Res. (2013) 19 (7): 1760–72) and cocrystal solvates or pharmaceutically acceptable salts thereof.

[00462] As used herein, the term "bromodomain inhibitor" refers to a compound that reduces, inhibits, or otherwise diminishes interaction between a bromodomain containing protein and the acetyl group during DNA transcription. The activity could decrease by a statistically significant amount including, for example, a decrease of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 95% or 100% of the activity of bromodomains compared to an appropriate control. Bromodomains bind the acetylated lysines in histone tails, the recognition of the acetyl group being decisive for the recruitment of other chromatin factors and transcriptional machinery, and thereby the regulation of gene transcription. *See e.g.,* Perez-Salvia and Esteller, Epigenetics: 2017, 12(5), 323-329. Examples of bromodomain inhibitors include, but are not limited to, JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof.

[00463] As used herein, the term "TET inhibitor" refers to a compound that reduces, inhibits, or otherwise diminishes one or more of the biological activity of Ten-eleven translocation protein (TET). The activity could decrease by a statistically significant amount including, for example, a decrease of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 95% or 100% of the activity of TET compared to an appropriate control. Examples of TET inhibitors include, but are not limited to, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), L-2-Hydroxyglutarate (L2HG) and C35 (*see, e.g.,* Singh, *et al.*, PNAS February 18, 2020 117 (7) 3621-3626); and pharmaceutically acceptable salts thereof.

III. Gen 2 TIL Manufacturing Processes

[00464] An exemplary family of TIL processes known as Gen 2 (also known as process 2A) containing some of these features is depicted in Figures 1 and 2. An embodiment of Gen 2 is shown in Figure 2.

[00465] As discussed herein, the present invention can include a step relating to the restimulation of cryopreserved TILs to increase their metabolic activity and thus relative health prior to transplant into a patient, and methods of testing said metabolic health. As generally outlined herein, TILs are generally taken from a patient sample and manipulated to expand their number prior to transplant into a patient. In some embodiments, the TILs may be optionally genetically manipulated as discussed below.

[00466] In some embodiments, the TILs may be cryopreserved. Once thawed, they may also be restimulated to increase their metabolism prior to infusion into a patient.

[00467] In some embodiments, the first expansion (including processes referred to as the pre-REP as well as processes shown in Figure 1 as Step A) is shortened to 3 to 14 days and the second expansion (including processes referred to as the REP as well as processes shown in Figure 1 as Step B) is shorted to 7 to 14 days, as discussed in detail below as well as in the examples and figures. In some embodiments, the first expansion (for example, an expansion described as Step B in Figure 1) is shortened to 11 days and the second expansion (for example, an expansion as described in Step D in Figure 1) is shortened to 11 days. In some embodiments, the combination of the first expansion and second expansion (for example, expansions described as Step B and Step D in Figure 1) is shortened to 22 days, as discussed in detail below and in the examples and figures.

[00468] The "Step" Designations A, B, C, *etc.*, below are in reference to Figure 1 and in reference to certain embodiments described herein. The ordering of the Steps below and in Figure 1 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

[00469] In general, TILs are initially obtained from a patient tumor sample ("primary TILs") and then expanded into a larger population for further manipulation as described

herein, optionally cryopreserved, restimulated as outlined herein and optionally evaluated for phenotype and metabolic parameters as an indication of TIL health.

[00470] A patient tumor sample may be obtained using methods known in the art, generally via surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells. In some embodiments, multilesional sampling is used. In some embodiments, surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells includes multilesional sampling (*i.e.*, obtaining samples from one or more tumor sites and/or locations in the patient, as well as one or more tumors in the same location or in close proximity). 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 lung tissue. In some embodiments, useful TILs are obtained from non-small cell lung carcinoma (NSCLC). The solid tumor may be of skin tissue. In some embodiments, useful TILs are obtained from a melanoma.

[00471] 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. In some embodiments, 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.

[00472] 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...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. In some instances, collagenase (such as animal free- type 1 collagenase) is reconstituted 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...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. 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...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...In some embodiments, the stock of enzymes is variable and the concentrations may need to be determined. In some embodiments, the concentration of the lyophilized stock can be verified.

In some embodiments, the final amount of enzyme added to the digest cocktail is adjusted based on the determined stock concentration...In some embodiment, the enzyme mixture includes about 10.2-ul of neutral protease (0.36 DMC U/mL), 21.3 μL of collagenase (1.2 PZ/mL) and 250-ul of DNAse I (200 U/mL) in about 4.7 mL of sterile HBSS.

[00473] 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 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.

[00474] In some embodiments, the tumor is reconstituted with the lyophilized enzymes in a sterile buffer. In some embodiments, the buffer is sterile HBSS.

[00475] In some embodiments, the enzyme 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 10× working stock.

[00476] In some embodiments, the enzyme mixture comprises DNAse. In some embodiments, the working stock for the DNAse is a 10,000 IU/mL 10× working stock.

[00477] In some embodiments, the enzyme mixture comprises hyaluronidase. In some embodiments, the working stock for the hyaluronidase is a 10 mg/mL 10× working stock.

[00478] In some embodiments, the enzyme mixture comprises 10 mg/mL collagenase, 1000 IU/mL DNAse, and 1 mg/mL hyaluronidase.

[00479] In some embodiments, the enzyme mixture comprises 10 mg/mL collagenase, 500 IU/mL DNAse, and 1 mg/mL hyaluronidase.

[00480] In general, the harvested cell suspension is called a "primary cell population" or a "freshly harvested" cell population.

[00481] 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 cultured from enzymatic tumor digests and tumor fragments obtained from digesting or fragmenting a tumor sample obtained from a patient.

[00482] 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 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 tumor is fragmented and 10, 20, 30, 40 or more fragments or pieces are placed in each container for the first expansion. In some embodiments, the tumor is fragmented and 30 or 40 fragments or pieces are placed in each container for the first expansion. In some embodiments, the tumor is fragmented and 40 fragments or pieces are placed in each container for the 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.

[00483] 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 tumors are 1-4 mm × 1-4 mm × 1-4 mm. In some embodiments, the tumors are 1 mm × 1 mm × 1 mm. In some embodiments, the tumors are 2 mm × 2 mm × 2 mm. In some embodiments, the tumors are 3 mm × 3 mm × 3 mm. In some embodiments, the tumors are 4 mm × 4 mm × 4 mm.

[00484] In some embodiments, the tumors are resected in order to minimize the amount of hemorrhagic, necrotic, and/or fatty tissues on each piece. In some embodiments, the tumors are resected in order to minimize the amount of hemorrhagic tissue on each piece. In some embodiments, the tumors are resected in order to minimize the amount of necrotic tissue on each piece. In some embodiments, the tumors are resected in order to minimize the amount of fatty tissue on each piece.

[00485] 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 performing 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 contains a large number of red blood cells or dead cells, a density gradient separation using Ficoll can be performed to remove these cells.

[00486] In some embodiments, the harvested cell suspension prior to the first expansion step is called a "primary cell population" or a "freshly harvested" cell population.

[00487] In some embodiments, cells can be optionally frozen after sample harvest 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, as well as Figure 8

1. Pleural effusion T-cells and TILs

[00488] In some embodiments, the sample is a pleural fluid sample. In some embodiments, the source of the T-cells or 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 T-cells or 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.

[00489] 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 derived from 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 disclosed methods utilize pleural fluid, the same methods may be performed with similar results using ascites or other cyst fluids containing TILs.

[00490] 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 further processing steps. In some embodiments, the unprocessed pleural fluid is placed in a standard CellSave® tube (Veridex) prior to further processing steps. In some embodiments, the sample is placed in the CellSave tube 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.

[00491] In some embodiments, the pleural fluid sample from the chosen subject may be diluted. In some embodiments, 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.

[00492] In still other embodiments, pleural fluid samples are concentrated by conventional means prior to 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.

[00493] 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 used in further processing steps 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 further processing steps of the method.

[00494] 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.

[00495] 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.

B. STEP B: First Expansion

[00496] In some embodiments, the present methods provide for obtaining young TILs, which are capable of increased replication cycles upon administration to a subject/patient and as such 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 in Donia, et al.,

Scand. J. Immunol. 2012, 75, 157–167; Dudley, et al., Clin. Cancer Res. 2010, 16, 6122-6131; Huang, et al., J. Immunother. 2005, 28, 258–267; Besser, et al., Clin. Cancer Res. 2013, 19, OF1-OF9; Besser, et al., J. Immunother. 2009, 32:415–423; Robbins, et al., J. Immunol. 2004, 173, 7125-7130; Shen, et al., J. Immunother., 2007, 30, 123–129; Zhou, et al., J. Immunother. 2005, 28, 53–62; and Tran, et al., J. Immunother., 2008, 31, 742–751, each of which is incorporated herein by reference.

[00497] 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 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 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 1C, as exemplified in Figure 5 and/or Figure 6. 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 TCRab (*i.e.*, TCRα/β).

[00498] After dissection or digestion of tumor fragments, for example such as described in Step A of Figure 1, the resulting cells are cultured in serum containing IL-2

under conditions that favor the growth of TILs over tumor and other cells. In some embodiments, the tumor digests are incubated in 2 mL wells in media comprising inactivated human AB serum with 6000 IU/mL of IL-2. This primary cell population is cultured for a period of days, generally from 3 to 14 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this primary cell population is cultured for a period of 7 to 14 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this primary cell population is cultured for a period of 10 to 14 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this primary cell population is cultured for a period of about 11 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells.

[00499] In some embodiments, expansion of TILs may be performed using an initial bulk TIL expansion step (for example such as those described in Step B of Figure 1, which can include processes referred to as pre-REP) as described below and herein, followed by a 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.

[00500] In embodiments where TIL cultures are initiated in 24-well plates, for example, using Costar 24-well cell culture cluster, flat bottom (Corning Incorporated, Corning, NY, each well can be seeded with 1×10^6 tumor digest cells or one tumor fragment in 2 mL of complete medium (CM) with IL-2 (6000 IU/mL; Chiron Corp., Emeryville, CA). In some embodiments, the tumor fragment is between about 1 mm^3 and 10 mm^3.

[00501] 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. In embodiments where cultures are initiated in gas-permeable flasks with a 40 mL capacity and a 10 cm^2 gas-permeable silicon bottom (for example, G-REX10; Wilson Wolf Manufacturing, New Brighton, MN), each flask was loaded with $10–40 \times 10^6$ viable tumor digest cells or 5–30 tumor fragments in 10–40 mL of CM with IL-2. Both the G-REX10 and 24-well plates were incubated in a humidified incubator at 37°C in 5% CO_2 and 5 days after culture initiation, half the media was removed and replaced with fresh CM and IL-2 and after day 5, half the media was changed every 2–3 days.

[00502] After preparation of the tumor fragments, the resulting cells (*i.e.*, fragments) are cultured in serum containing IL-2 under conditions that favor the growth of TILs over tumor and other cells. In some embodiments, the tumor digests are incubated in 2 mL wells in media comprising inactivated human AB serum (or, in some cases, as outlined herein, in the presence of an APC cell population) with 6000 IU/mL of IL-2. This primary cell population is cultured for a period of days, generally from 10 to 14 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, the growth media during the first expansion comprises IL-2 or a variant thereof. In some embodiments, the IL is recombinant human IL-2 (rhIL-2). In some embodiments the IL-2 stock solution has a specific activity of 20-30$\times10^6$ 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 4-8$\times10^6$ IU/mg of IL-2. In some embodiments, the IL- 2 stock solution has a final concentration of 5-7$\times10^6$ 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 5. In some embodiments, the 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 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 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 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 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 cell culture medium comprises about 3000 IU/mL of IL-2. 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 about 8000 IU/mL of IL-2.

[00503] In some embodiments, 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 first expansion culture media comprises about 500 IU/mL of IL-15 to about 100 IU/mL of IL-15. In some embodiments, the first expansion culture media comprises about 400 IU/mL of IL-15 to about 100 IU/mL of IL-15. In some embodiments, the first expansion culture media comprises about 300 IU/mL of IL-15 to about 100 IU/mL of IL-15. In some embodiments, the first 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.

[00504] In some embodiments, 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 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 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 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 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 first expansion culture media comprises about 5 IU/mL of IL-21 to about 1 IU/mL of IL-21. In some embodiments, the first 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.

[00505] In some embodiments, the cell culture medium comprises an anti-CD3 agonist antibody, e.g., 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, 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 does not comprise OKT-3 antibody. In some embodiments, the OKT-3 antibody is muromonab. See, for example, Table 1.

[00506] 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.

[00507] 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.

[00508] In some embodiments, the cell culture medium comprises one or more epigenetic reprogramming agents in a cell culture medium. In some embodiments, the epigenetic reprogramming agent is a DNA hypomethylating agent. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is decitabine.

[00509] In some embodiments, the epigenetic reprogramming agent is a MEK inhibitor. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is trametinib.

[00510] In some embodiments, the epigenetic reprogramming agent is an HDAC inhibitor. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is ricolinistat.

[00511] In some embodiments, the epigenetic reprogramming agent is a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is JQ1.

[00512] In some embodiments, the epigenetic reprogramming agent is an EZH2 inhibitor. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

[00513] In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is ipatasertib.

[00514] In some embodiments, the epigenetic reprogramming agent is a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-

Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35.

[00515] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and a MEK inhibitor. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00516] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and an HDAC inhibitor. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00517] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and an EZH2 inhibitor. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof. In

some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00518] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00519] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00520] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00521] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and an HDAC inhibitor. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[00522] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and an EZH2 inhibitor. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[00523] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and a bromodomain inhibitor. In some embodiments, the bromodomain

inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[00524] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[00525] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[00526] In some embodiments, the epigenetic reprogramming agent is a combination of an HDAC inhibitor and an EZH2 inhibitor. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric

acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat.

[00527] In some embodiments, the epigenetic reprogramming agent is a combination of an HDAC inhibitor and a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat.

[00528] In some embodiments, the epigenetic reprogramming agent is a combination of an HDAC inhibitor and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat.

[00529] In some embodiments, the epigenetic reprogramming agent is a combination of an HDAC inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some

embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat.

[00530] In some embodiments, the epigenetic reprogramming agent is a combination of an EZH2 inhibitor and a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

[00531] In some embodiments, the epigenetic reprogramming agent is a combination of EZH2 inhibitor and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

[00532] In some embodiments, the epigenetic reprogramming agent is a combination of EZH2 inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

[00533] In some embodiments, the epigenetic reprogramming agent is a combination of bromodomain inhibitor and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1.

[00534] In some embodiments, the epigenetic reprogramming agent is a combination of bromodomain inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1.

[00535] In some embodiments, the epigenetic reprogramming agent is a combination of an AKT inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib.

[00536] In some embodiments, the epigenetic reprogramming agent is a combination of three or more epigenetic reprogramming agents described herein.

[00537] In some embodiments, the epigenetic reprogramming agent may be added at a concentration in a range from about 5 nM to about 5 μM. For example, the concentration of the epigenetic reprogramming agent in the first cell culture medium may be about 5 nM, about 10 nM, about 15 nM, about 20 nM, about 25 nM, about 30 nM, about 35 nM, about 40 nM, about 45 nM, about 50 nM, about 60 nM, about 70 nM, about 80 nM, about 90 nM, about 100 nM, about 110 nM, about 120 nM, about 130 nM, about 140 nM, about 150 nM, about 160 nM, about 170 nM, about 180 nM, about 190 nM, about 200 nM, about 220 nM, about 240 nM, about 260 nM, about 280 nM, about 300 nM, about 320 nM, about 340 nM, about 360 nM, about 380 nM, about 400 nM, about 420 nM, about 440 nM, about 460 nM, about 480 nM, about 500 nM, about 550 nM, about 600 nM, about 650 nM, about 700 nM, about 750 nM, about 800 nM, about 850 nM, about 900 nM, about 950 nM, about 1 μM, about 1.5μM, about 2 μM, about 2.5 μM, about 3 μM, about 3.5 μM, about 4 μM, about 4.5 μM, about 5 μM or any other concentration between any two of these values.

[00538] In some embodiments, the epigenetic reprogramming agent may be a combination of two or more epigenetic reprogramming agents. In such embodiments, the two or more epigenetic reprogramming agents may be added at different concentrations. For example, a first epigenetic reprogramming agent may be added at a concentration of 5 nM and a second epigenetic reprogramming agent may be added at a concentration of 50 nM. In another example, a first epigenetic reprogramming agent may be added at a concentration of 5 nM, a second epigenetic reprogramming agent may be added at a concentration of 50 nM and a third epigenetic reprogramming agent may be added at a concentration of 100 nM. Other combinations of concentrations of one or more epigenetic reprogramming agents within the concentration ranges described herein are contemplated.

[00539] In some embodiments, the 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 embodiments where cultures are initiated in gas-permeable flasks with a 40 mL capacity and a 10cm^2 gas-permeable silicon bottom (for example, G-REX10; Wilson Wolf Manufacturing, New Brighton, MN), each flask was loaded with 10–40x10^6 viable tumor digest cells or 5–30 tumor fragments in 10–40mL of CM with IL-2. Both the G-REX10 and 24-well plates were incubated in a humidified incubator at 37°C in 5% CO_2 and 5 days after culture initiation, half the media was removed and replaced with fresh CM and IL-2 and after day 5, half the

media was changed every 2–3 days. In some embodiments, the CM is the CM1 described in the Examples, *see*, Example 1. In some embodiments, the first expansion occurs in an initial cell culture medium or a first cell culture medium. In some embodiments, the initial cell culture medium or the first cell culture medium comprises IL-2.

[00540] 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.

[00541] 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.

[00542] 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.

[00543] 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.

[00544] 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.

[00545] 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 1 L 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), along with 2-mercaptoethanol at 55μM.

[00546] 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 1 L 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 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), along with 2-mercaptoethanol at 55μM.

[00547] In some embodiments, the serum-free medium or defined medium is supplemented with glutamine (*i.e.*, GlutaMAX®) at a concentration of from about 0.1 mM to about 10 mM, 0.5 mM to about 9 mM, 1 mM to about 8 mM, 2 mM to about 7 mM, 3 mM to about 6 mM, or 4 mM 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.

[00548] In some embodiments, the serum-free medium or defined medium is supplemented with 2-mercaptoethanol at a concentration of from about 5 mM to about 150 mM, 10 mM to about 140 mM, 15 mM to about 130 mM, 20 mM to about 12 0mM, 25 mM to about 110 mM, 30 mM to about 100 mM, 35 mM to about 95 mM, 40 mM to about 90 mM, 45 mM to about 85 mM, 50 mM to about 80 mM, 55 mM to about 75 mM, 60 mM to about 70 mM, or about 65 mM. In some embodiments, the serum-free medium or defined medium is supplemented with 2-mercaptoethanol at a concentration of about 55 mM.

[00549] In some embodiments, the defined media described in International Patent Application Publication No. WO 1998/030679 and U.S. Patent Application Publication No. US 2002/0076747 A1, which are 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^{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.

[00550] 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.

[00551] 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 below. 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 "Some embodiments of the 1X Medium" in Table 4 below. 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 "Some embodiments in Supplement" in Table 4 below.

TABLE 4. Concentrations of Non-Trace Element Moiety Ingredients

Ingredient	Some embodiments in supplement (mg/L) (About)	Concentration range in 1X medium (mg/L) (About)	Some embodiments 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

[00552] 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).

[00553] In some embodiments, the defined media described in Smith, *et al.*, *Clin. Transl. Immunology*, **2015**, 4(1), e31, the disclosures of which are incorporated by reference herein, 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.

[00554] In some embodiments, the first expansion (including processes such as for example those described in Step B of Figure 1, which can include those sometimes referred to

as the pre-REP) process is shortened to 3-14 days, as discussed in the examples and figures. In some embodiments, the first expansion (including processes such as for example those described in Step B of Figure 1, which can include those sometimes referred to as the pre-REP) is shortened to 7 to 14 days, as discussed in the Examples and shown in Figures 4 and 5, as well as including for example, an expansion as described in Step B of Figure 1. In some embodiments, the first expansion of Step B is shortened to 10-14 days. In some embodiments, the first expansion is shortened to 11 days, as discussed in, for example, an expansion as described in Step B of Figure 1.

[00555] In some embodiments, the first TIL expansion can proceed for 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days. In some embodiments, the first TIL expansion can proceed for 1 day to 14 days. In some embodiments, the first TIL expansion can proceed for 2 days to 14 days. In some embodiments, the first TIL expansion can proceed for 3 days to 14 days. In some embodiments, the first TIL expansion can proceed for 4 days to 14 days. In some embodiments, the first TIL expansion can proceed for 5 days to 14 days. In some embodiments, the first TIL expansion can proceed for 6 days to 14 days. In some embodiments, the first TIL expansion can proceed for 7 days to 14 days. In some embodiments, the first TIL expansion can proceed for 8 days to 14 days. In some embodiments, the first TIL expansion can proceed for 9 days to 14 days. In some embodiments, the first TIL expansion can proceed for 10 days to 14 days. In some embodiments, the first TIL expansion can proceed for 11 days to 14 days. In some embodiments, the first TIL expansion can proceed for 12 days to 14 days. In some embodiments, the first TIL expansion can proceed for 13 days to 14 days. In some embodiments, the first TIL expansion can proceed for 14 days. In some embodiments, the first TIL expansion can proceed for 1 day to 11 days. In some embodiments, the first TIL expansion can proceed for 2 days to 11 days. In some embodiments, the first TIL expansion can proceed for 3 days to 11 days. In some embodiments, the first TIL expansion can proceed for 4 days to 11 days. In some embodiments, the first TIL expansion can proceed for 5 days to 11 days. In some embodiments, the first TIL expansion can proceed for 6 days to 11 days. In some embodiments, the first TIL expansion can proceed for 7 days to 11 days. In some embodiments, the first TIL expansion can proceed for 8 days to 11 days. In some embodiments, the first TIL expansion can proceed for 9 days to 11 days. In some

embodiments, the first TIL expansion can proceed for 10 days to 11 days. In some embodiments, the first TIL expansion can proceed for 11 days.

[00556] In some embodiments, a combination of IL-2, IL-7, IL-15, and/or IL-21 are employed as a combination during the 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 first expansion, including for example during a Step B processes according to Figure 1, 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 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 and as described herein.

[00557] In some embodiments, the first expansion (including processes referred to as the pre-REP; for example, Step B according to Figure 1) process is shortened to 3 to 14 days, as discussed in the examples and figures. In some embodiments, the first expansion of Step B is shortened to 7 to 14 days. In some embodiments, the first expansion of Step B is shortened to 10 to 14 days. In some embodiments, the first expansion is shortened to 11 days.

[00558] In some embodiments, the first expansion, for example, Step B according to Figure 1, 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 G-REX -10 or a G-REX -100. In some embodiments, the closed system bioreactor is a single bioreactor.

1. Cytokines and Other Additives

[00559] 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.

[00560] Alternatively, using combinations of cytokines for the rapid expansion and or second expansion of TILs is additionally possible, with combinations of two or more of IL-2, IL-15 and IL-21 as is described in U.S. Patent Application Publication No. US 2017/0107490 A1, the disclosure of which is incorporated by reference herein. Thus, possible combinations include IL-2 and IL-15, IL-2 and IL-21, IL-15 and IL-21 and IL-2, or 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.

[00561] In some embodiments, Step B may also include the addition of OKT-3 antibody or muromonab to the culture media, as described elsewhere herein. In some embodiments, Step B may also include the addition of a 4-1BB agonist to the culture media, as described elsewhere herein. In some embodiments, Step B may also include the addition of an OX-40 agonist to the culture media, as described elsewhere herein. In other embodiments, additives such as peroxisome proliferator-activated receptor gamma coactivator I-alpha agonists, including proliferator-activated receptor (PPAR)-gamma agonists such as a thiazolidinedione compound, may be used in the culture media during Step B, as described in U.S. Patent Application Publication No. US 2019/0307796 A1, the disclosure of which is incorporated by reference herein.

C. STEP C: First Expansion to Second Expansion Transition

[00562] In some cases, the bulk TIL population obtained from the first expansion, including for example the TIL population obtained from for example, Step B as indicated in Figure 1, can be cryopreserved immediately, using the protocols discussed herein below. Alternatively, the TIL population obtained from the first expansion, referred to as the second TIL population, can be subjected to a second expansion (which can include expansions sometimes referred to as REP) and then cryopreserved as discussed below. Similarly, in the case where genetically modified TILs will be used in therapy, the first TIL population (sometimes referred to as the bulk TIL population) or the second TIL population (which can in some embodiments include populations referred to as the REP TIL populations) can be subjected to genetic modifications for suitable treatments prior to expansion or after the first expansion and prior to the second expansion.

[00563] In some embodiments, the TILs obtained from the first expansion (for example, from Step B as indicated in Figure 1) are stored until phenotyped for selection. In some embodiments, the TILs obtained from the first expansion (for example, from Step B as indicated in Figure 1) are not stored and proceed directly to the second expansion. In some embodiments, the TILs obtained from the first expansion are not cryopreserved after the first expansion and prior to the second expansion. In some embodiments, the transition from the first expansion to the second expansion occurs at about 3 days, 4, days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs at about 3 days to 14 days from when fragmentation occurs. In some embodiments,

the transition from the first expansion to the second expansion occurs at about 4 days to 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs at about 4 days to 10 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs at about 7 days to 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs at about 14 days from when fragmentation occurs.

[00564] In some embodiments, the transition from the first expansion to the second expansion occurs at 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 1 day to 14 days from when fragmentation occurs. In some embodiments, the first TIL expansion can proceed for 2 days to 14 days. In some embodiments, the transition from the first expansion to the second expansion occurs 3 days to 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 4 days to 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 5 days to 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 6 days to 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 7 days to 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 8 days to 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 9 days to 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 10 days to 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 11 days to 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 12 days to 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 13 days to 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 1 day to 11 days from when fragmentation occurs.

In some embodiments, the transition from the first expansion to the second expansion occurs 2 days to 11 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 3 days to 11 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 4 days to 11 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 5 days to 11 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 6 days to 11 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 7 days to 11 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 8 days to 11 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 9 days to 11 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 10 days to 11 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 11 days from when fragmentation occurs.

[00565] In some embodiments, the second population of cells is at least 5-fold greater in number than the first population of TILs, wherein the first cell culture medium comprises IL-2. For example, the second population of cells may be about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 11-fold, about 12-fold, about 13-fold, about 14-fold, about 15-fold, about 16-fold, about 17-fold, about 18-fold, about 19-fold, about 20-fold, or even greater in number than the first population of TILs.

[00566] In some embodiments, the second population of TILs has an increased frequency of CD8 TILs when compared to a corresponding population of TILs expanded in a cell culture medium without the epigenetic reprogramming agent. Additionally, or alternatively, the second population of TILs has an increased ratio of CD4 TILs to CD8 TILs when compared to a corresponding population of TILs expanded in a cell culture medium without the epigenetic reprogramming agent.

[00567] In some embodiments, the TILs are not stored after the first expansion and prior to the second expansion, and the TILs proceed directly to the 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 some embodiments, the transition occurs in closed system, as

described herein. In some embodiments, the TILs from the first expansion, the second population of TILs, proceeds directly into the second expansion with no transition period.

[00568] In some embodiments, the transition from the first expansion to the second expansion, for example, Step C according to Figure 1, 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 G-REX -10 or a G-REX -100. In some embodiments, the closed system bioreactor is a single bioreactor.

D. STEP D: Second Expansion

[00569] In some embodiments, the TIL cell population is expanded in number after harvest and initial bulk processing for example, after Step A and Step B, and the transition referred to as Step C, as indicated in Figure 1). This further expansion is referred to herein as the second expansion, which can include expansion processes generally referred to in the art as a rapid expansion process (REP); as well as processes as indicated in Step D of Figure 1. The second expansion is generally accomplished using a culture media comprising a number of components, including feeder cells, a cytokine source, and an anti-CD3 antibody, in a gas-permeable container.

[00570] In some embodiments, the second expansion or second TIL expansion (which can include expansions sometimes referred to as REP; as well as processes as indicated in Step D of Figure 1) 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 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days. In some embodiments, the second TIL expansion can proceed for about 7 days to about 14 days. In some embodiments, the second TIL expansion can proceed for about 8 days to about 14 days. In some embodiments, the second TIL expansion can proceed for about 9 days to about 14 days. In some embodiments, the second TIL expansion can proceed for about 10 days to about 14 days. In some embodiments, the second TIL expansion can proceed for about 11 days to about 14 days. In some embodiments, the second TIL expansion can proceed for about 12 days to about 14 days. In some embodiments, the second TIL expansion can proceed for about 13 days to about 14 days. In some embodiments, the second TIL expansion can proceed for about 14 days.

[00571] In some embodiments, the 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). For example, TILs can be rapidly expanded using non-specific T-cell receptor stimulation in the presence of interleukin-2 (IL-2) or interleukin-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.

[00572] 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.

[00573] 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, 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 does not comprise OKT-3 antibody. In some embodiments, the OKT-3 antibody is muromonab.

[00574] 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.

[00575] 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.

[00576] 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, 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 and as described herein.

[00577] In some embodiments, the second cell culture medium may include one or more epigenetic reprogramming agents. In some embodiments, the epigenetic reprogramming agent is a DNA hypomethylating agent. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is decitabine.

[00578] In some embodiments, the epigenetic reprogramming agent is a MEK inhibitor. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is trametinib.

[00579] In some embodiments, the epigenetic reprogramming agent is an HDAC inhibitor. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is ricolinistat.

[00580] In some embodiments, the epigenetic reprogramming agent is a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is JQ1.

[00581] In some embodiments, the epigenetic reprogramming agent is an EZH2 inhibitor. In some embodiments, the EZH2 inhibitor is selected from the group consisting of

3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

[00582] In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is ipatasertib.

[00583] In some embodiments, the epigenetic reprogramming agent is a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35.

[00584] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and a MEK inhibitor. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00585] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and an HDAC inhibitor. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat. In some

embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00586] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and an EZH2 inhibitor. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00587] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00588] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and an AKT inhibitor. In some embodiments, the AKT

inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00589] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00590] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and an HDAC inhibitor. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623,

pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[00591] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and an EZH2 inhibitor. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[00592] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[00593] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[00594] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected

from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[00595] In some embodiments, the epigenetic reprogramming agent is a combination of an HDAC inhibitor and an EZH2 inhibitor. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat.

[00596] In some embodiments, the epigenetic reprogramming agent is a combination of an HDAC inhibitor and a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat.

[00597] In some embodiments, the epigenetic reprogramming agent is a combination of an HDAC inhibitor and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine,

Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat.

[00598] In some embodiments, the epigenetic reprogramming agent is a combination of an HDAC inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat.

[00599] In some embodiments, the epigenetic reprogramming agent is a combination of an EZH2 inhibitor and a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

[00600] In some embodiments, the epigenetic reprogramming agent is a combination of EZH2 inhibitor and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and

pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

[00601] In some embodiments, the epigenetic reprogramming agent is a combination of EZH2 inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

[00602] In some embodiments, the epigenetic reprogramming agent is a combination of bromodomain inhibitor and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1.

[00603] In some embodiments, the epigenetic reprogramming agent is a combination of bromodomain inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1.

[00604] In some embodiments, the epigenetic reprogramming agent is a combination of an AKT inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib.

[00605] In some embodiments, the epigenetic reprogramming agent is a combination of three or more epigenetic reprogramming agents described herein.

[00606] In some embodiments, the epigenetic reprogramming agent may be added at a concentration in a range from about 5 nM to about 5 μM. For example, the concentration of the epigenetic reprogramming agent in the second cell culture medium may be about 5 nM, about 10 nM, about 15 nM, about 20 nM, about 25 nM, about 30 nM, about 35 nM, about 40 nM, about 45 nM, about 50 nM, about 60 nM, about 70 nM, about 80 nM, about 90 nM, about 100 nM, about 110 nM, about 120 nM, about 130 nM, about 140 nM, about 150 nM, about 160 nM, about 170 nM, about 180 nM, about 190 nM, about 200 nM, about 220 nM, about 240 nM, about 260 nM, about 280 nM, about 300 nM, about 320 nM, about 340 nM, about 360 nM, about 380 nM, about 400 nM, about 420 nM, about 440 nM, about 460 nM, about 480 nM, about 500 nM, about 550 nM, about 600 nM, about 650 nM, about 700 nM, about 750 nM, about 800 nM, about 850 nM, about 900 nM, about 950 nM, about 1 μM, about 1.5μM, about 2 μM, about 2.5 μM, about 3 μM, about 3.5 μM, about 4 μM, about 4.5 μM, about 5 μM, or any other concentration between any two of these values.

[00607] In some embodiments, the epigenetic reprogramming agent may be a combination of two or more epigenetic reprogramming agents. In such embodiments, the two or more epigenetic reprogramming agents may be added at different concentrations. For example, a first epigenetic reprogramming agent may be added at a concentration of 5 nM and a second epigenetic reprogramming agent may be added at a concentration of 50 nM. In another example, a first epigenetic reprogramming agent may be added at a concentration of 5 nM, a second epigenetic reprogramming agent may be added at a concentration of 50 nM and a third epigenetic reprogramming agent may be added at a concentration of 100 nM.

Other combinations of concentrations of one or more epigenetic reprogramming agents within the concentration ranges described herein are contemplated.

[00608] 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). 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).

[00609] 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.

[00610] 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.

[00611] 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 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 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.

[00612] In some embodiments, REP and/or the second expansion is performed in flasks with the bulk TILs being mixed with a 100- or 200-fold excess of inactivated feeder cells, 30 mg/mL OKT3 anti-CD3 antibody and 3000 IU/mL IL-2 in 150 ml media. Media replacement is done (generally 2/3 media replacement via respiration with 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.

[00613] In some embodiments, the second expansion (which can include processes referred to as the REP process) is shortened to 7-14 days, as discussed in the examples and figures. In some embodiments, the second expansion is shortened to 11 days.

[00614] In some embodiments, REP and/or the second expansion may be performed using T-175 flasks and gas permeable bags as previously described (Tran, *et al.*, *J. Immunother.* **2008,** *31,* 742-51; Dudley, *et al.*, *J. Immunother.* **2003,** *26,* 332-42) or gas permeable cultureware (G-REX flasks). In some embodiments, the second expansion (including expansions referred to as rapid expansions) is performed in T-175 flasks, and about 1 x 10^6 TILs suspended in 150 mL of media may be added to each T-175 flask. The TILs may be cultured in a 1 to 1 mixture of CM and AIM-V medium, supplemented with 3000 IU per mL of IL-2 and 30 ng per ml of anti-CD3. The T-175 flasks may be incubated at

37° C in 5% CO_2. Half the media may be exchanged on day 5 using 50/50 medium with 3000 IU per mL of IL-2. In some embodiments, on day 7 cells from two T-175 flasks may be combined in a 3 L bag and 300 mL of AIM V with 5% human AB serum and 3000 IU per mL of IL-2 was added to the 300 ml of TIL suspension. The number of cells in each bag was counted every day or two and fresh media was added to keep the cell count between 0.5 and 2.0 x 10^6 cells/mL.

[00615] 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) 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, 3000 IU per mL of IL-2, and added back to the original G-REX 100 flasks. When TIL are expanded serially in G-REX 100 flasks, on day 7 the TIL in each G-REX 100 may be suspended in the 300 mL of media present in each flask and the cell suspension may be divided into 3 100 mL aliquots that may be used to seed 3 G-REX 100 flasks. Then 150 mL of AIM-V with 5% human AB serum and 3000 IU per mL of IL-2 may be added to each flask. The G-REX 100 flasks may be incubated at 37° C in 5% CO_2 and after 4 days 150 mL of AIM-V with 3000 IU per mL of IL-2 may be added to each G-REX 100 flask. The cells may be harvested on day 14 of culture.

[00616] In some embodiments, the second expansion (including expansions referred to as REP) is performed in flasks with the bulk TILs being mixed with a 100- or 200-fold excess of inactivated feeder cells, 30 mg/mL OKT3 anti-CD3 antibody and 3000 IU/mL IL-2 in 150 ml media. 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 respiration with fresh media. In some embodiments, alternative growth chambers include G-REX flasks and gas permeable containers as more fully discussed below.

[00617] In some embodiments, the 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.

[00618] For example, in some embodiments, the third population of TILs has an increased frequency of CD8 TILs when compared to a corresponding population of TILs expanded in a cell culture medium without the epigenetic reprogramming agent. Additionally, or alternatively, the third population of TILs has an increased ratio of CD4 TILs to CD8 TILs when compared to a corresponding population of TILs expanded in a cell culture medium without the epigenetic reprogramming agent.

[00619] Optionally, a cell viability assay can be performed after the 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, 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.

[00620] In some embodiments, the second expansion (including expansions referred to as REP) of TIL can be performed using T-175 flasks and gas-permeable bags as previously described (Tran KQ, Zhou J, Durflinger KH, et al., **2008**, *J Immunother.*, 31:742–751, and Dudley ME, Wunderlich JR, Shelton TE, et al. 2003, *J Immunother.*, 26:332–342) or gas-permeable G-REX flasks. In some embodiments, the second expansion is performed using flasks. In some embodiments, the second expansion is performed using gas-permeable G-REX flasks. In some embodiments, the second expansion is performed in T-175 flasks, and about 1 x 10^6 TILs are suspended in about 150 mL of media and this is added to each T-175 flask. The TILs are cultured with irradiated (50 Gy) allogeneic PBMC as "feeder" cells at a ratio of 1 to 100 and the cells were cultured in a 1 to 1 mixture of CM and AIM-V medium (50/50 medium), supplemented with 3000 IU/mL of IL-2 and 30 ng/mL of anti-CD3. The T-175 flasks are incubated at 37°C in 5% CO_2. In some embodiments, half the media is changed on day 5 using 50/50 medium with 3000 IU/mL of IL-2. In some embodiments, on day 7, cells from 2 T-175 flasks are combined in a 3 L bag and 300 mL of AIM-V with 5% human AB serum and 3000 IU/mL of IL-2 is added to the 300 mL of TIL suspension.

The number of cells in each bag can be counted every day or two and fresh media can be added to keep the cell count between about 0.5 and about 2.0 x 10^6 cells/mL.

[00621] In some embodiments, the second expansion (including expansions referred to as REP) are performed in 500 mL capacity flasks with 100 cm^2 gas-permeable silicon bottoms (G-REX 100, Wilson Wolf), about 5x10^6 or 10x10^6 TIL are cultured with irradiated allogeneic PBMC at a ratio of 1 to 100 in 400 mL of 50/50 medium, supplemented with 3000 IU/mL of IL-2 and 30 ng/ mL of anti-CD3. The G-REX 100 flasks are incubated at 37°C in 5% CO_2. In some embodiments, on day 5, 250mL of supernatant is removed and placed into centrifuge bottles and centrifuged at 1500 rpm (491g) for 10 minutes. The TIL pellets can then be resuspended with 150 mL of fresh 50/50 medium with 3000 IU/ mL of IL-2 and added back to the original G-REX 100 flasks. In embodiments where TILs are expanded serially in G-REX 100 flasks, on day 7 the TIL in each G-REX 100 are suspended in the 300 mL of media present in each flask and the cell suspension was divided into three 100 mL aliquots that are used to seed 3 G-REX 100 flasks. Then 150 mL of AIM-V with 5% human AB serum and 3000 IU/mL of IL-2 is added to each flask. The G-REX 100 flasks are incubated at 37°C in 5% CO_2 and after 4 days 150 mL of AIM-V with 3000 IU/mL of IL-2 is added to each G-REX 100 flask. The cells are harvested on day 14 of culture.

[00622] 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.

[00623] 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.

[00624] 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.

[00625] 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.

[00626] 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.

[00627] 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 1 L 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.

[00628] 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 1 L 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 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.

[00629] In some embodiments, the serum-free medium or defined medium is supplemented with glutamine (*i.e.*, GlutaMAX®) at a concentration of from about 0.1 mM to about 10 mM, 0.5 mM to about 9 mM, 1 mM to about 8 mM, 2 mM to about 7 mM, 3 mM to about 6 mM, or 4 mM 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.

[00630] In some embodiments, the serum-free medium or defined medium is supplemented with 2-mercaptoethanol at a concentration of from about 5 mM to about 150 mM, 10 mM to about 140 mM, 15 mM to about 130 mM, 20 mM to about 12 0mM, 25 mM to about 110 mM, 30 mM to about 100 mM, 35 mM to about 95 mM, 40 mM to about 90 mM, 45 mM to about 85 mM, 50 mM to about 80 mM, 55 mM to about 75 mM, 60 mM to about 70 mM, or about 65 mM. In some embodiments, the serum-free medium or defined medium is supplemented with 2-mercaptoethanol at a concentration of about 55 mM.

[00631] In some embodiments, the defined media described in International Patent Application Publication No. WO 1998/030679 and U.S. Patent Application Publication No. US 2002/0076747 A1, which are 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^{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.

[00632] 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.

[00633] 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 below. 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 "Some embodiments of the 1X Medium" in Table 4 below. 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 "Some embodiments in Supplement" in Table 4.

[00634] 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).

[00635] In some embodiments, the defined media described in Smith, *et al.*, *Clin. Transl. Immunology*, **2015**, 4(1), e31, the disclosures of which are incorporated by reference herein, 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.

[00636] 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).

[00637] In some embodiments, the 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.

[00638] In some embodiments, the second expansion, for example, Step D according to Figure 1, 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 G-REX -10 or a G-REX -100. In some embodiments, the closed system bioreactor is a single bioreactor.

[00639] In some embodiments, the third population of cells is at least 50-fold greater in number than the second population of TILs, wherein the first cell culture medium comprises IL-2. For example, the second population of cells may be about 50-fold, about 55-fold, about 60-fold, about 65-fold, about 70-fold, about 75-fold, about 80-fold, about 85-fold, about 90-fold, about 100-fold, about 150-fold, about 200-fold, about 250-fold, about 300-fold, about 350-fold, about 400-fold, or even greater in number than the first population of TILs.

[00640] In some embodiments, the step of rapid or second expansion is split into a plurality of steps to achieve a scaling up of the culture by: (a) performing the rapid or second expansion by culturing TILs in a small scale culture in a first container, *e.g.*, a G-REX-100 MCS container, for a period of about 3 to 7 days, and then (b) effecting the transfer of the TILs in the small scale culture to a second container larger than the first container, *e.g.*, a G-REX-500-MCS container, and culturing the TILs from the small scale culture in a larger scale culture in the second container for a period of about 4 to 7 days.

[00641] In some embodiments, the step of rapid or second expansion is split into a plurality of steps to achieve a scaling out of the culture by: (a) performing the rapid or second expansion by culturing TILs in a first small scale culture in a first container, *e.g.*, a G-REX-100 MCS container, for a period of about 3 to 7 days, and then (b) effecting the transfer and

apportioning of the 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 TILs 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.

[00642] In some embodiments, the first small scale TIL culture is apportioned into a plurality of about 2 to 5 subpopulations of TILs.

[00643] In some embodiments, the step of rapid or second expansion is split into a plurality of steps to achieve a scaling out and scaling up of the culture by: (a) performing the rapid or second expansion by culturing TILs in a small scale culture in a first container, *e.g.*, a G-REX-100 MCS container, for a period of about 3 to 7 days, and then (b) effecting the transfer and apportioning of the TILs 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 TILs 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.

[00644] In some embodiments, the step of rapid or second expansion is split into a plurality of steps to achieve a scaling out and scaling up of the culture by: (a) performing the rapid or second expansion by culturing TILs in a small scale culture in a first container, *e.g.*, a G-REX-100 MCS container, for a period of about 5 days, and then (b) effecting the transfer and apportioning of the TILs 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-500 MCS containers, wherein in each second container the portion of the TILs from the small scale culture transferred to such second container is cultured in a larger scale culture for a period of about 6 days.

[00645] In some embodiments, upon the splitting of the rapid or second expansion, each second container comprises at least 10^8 TILs. In some embodiments, upon the splitting of the rapid or second expansion, each second container comprises at least 10^8 TILs, at least 10^9 TILs, or at least 10^{10} TILs. In one exemplary embodiment, each second container comprises at least 10^{10} TILs.

[00646] In some embodiments, the first small scale TIL culture is apportioned into a plurality of subpopulations. In some embodiments, the first small scale TIL culture is

apportioned into a plurality of about 2 to 5 subpopulations. In some embodiments, the first small scale TIL culture is apportioned into a plurality of about 2, 3, 4, or 5 subpopulations.

[00647] In some embodiments, after the completion of the rapid or second expansion, the plurality of subpopulations comprises a therapeutically effective amount of TILs. In some embodiments, after the completion of the rapid or second expansion, one or more subpopulations of TILs are pooled together to produce a therapeutically effective amount of TILs. In some embodiments, after the completion of the rapid expansion, each subpopulation of TILs comprises a therapeutically effective amount of TILs.

[00648] In some embodiments, the rapid or second expansion is performed for a period of about 3 to 7 days before being split into a plurality of steps. In some embodiments, the splitting of the rapid or second expansion occurs at about day 3, day 4, day 5, day 6, or day 7 after the initiation of the rapid or second expansion.

[00649] In some embodiments, the splitting of the rapid or second expansion occurs at about day 7, day 8, day 9, day 10, day 11, day 12, day 13, day 14, day 15, or day 16 day 17, or day 18 after the initiation of the first expansion (*i.e.*, pre-REP expansion). In one exemplary embodiment, the splitting of the rapid or second expansion occurs at about day 16 after the initiation of the first expansion.

[00650] In some embodiments, the rapid or second expansion is further performed for a period of about 7 to 11 days after the splitting. In some embodiments, the rapid or second expansion is further performed for a period of about 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, or 11 days after the splitting.

[00651] In some embodiments, the cell culture medium used for the rapid or second expansion before the splitting comprises the same components as the cell culture medium used for the rapid or second expansion after the splitting. In some embodiments, the cell culture medium used for the rapid or second expansion before the splitting comprises different components from the cell culture medium used for the rapid or second expansion after the splitting.

[00652] In some embodiments, the cell culture medium used for the rapid or second expansion before the splitting comprises IL-2, optionally OKT-3 and further optionally APCs. In some embodiments, the cell culture medium used for the rapid or second expansion before the splitting comprises IL-2, OKT-3, and further optionally APCs. In some

embodiments, the cell culture medium used for the rapid or second expansion before the splitting comprises IL-2, OKT-3 and APCs.

[00653] In some embodiments, the cell culture medium used for the rapid or second expansion before the splitting is generated by supplementing the cell culture medium in the first expansion with fresh culture medium comprising IL-2, optionally OKT-3 and further optionally APCs. In some embodiments, the cell culture medium used for the rapid or second expansion before the splitting is generated by supplementing the cell culture medium in the first expansion with fresh culture medium comprising IL-2, OKT-3 and APCs. In some embodiments, the cell culture medium used for the rapid or second expansion before the splitting is generated by replacing the cell culture medium in the first expansion with fresh cell culture medium comprising IL-2, optionally OKT-3 and further optionally APCs. In some embodiments, the cell culture medium used for the rapid or second expansion before the splitting is generated by replacing the cell culture medium in the first expansion with fresh cell culture medium comprising IL-2, OKT-3 and APCs.

[00654] In some embodiments, the cell culture medium used for the rapid or second expansion after the splitting comprises IL-2, and optionally OKT-3. In some embodiments, the cell culture medium used for the rapid or second expansion after the splitting comprises IL-2, and OKT-3. In some embodiments, the cell culture medium used for the rapid or second expansion after the splitting is generated by replacing the cell culture medium used for the rapid or second expansion before the splitting with fresh culture medium comprising IL-2 and optionally OKT-3. In some embodiments, the cell culture medium used for the rapid or second expansion after the splitting is generated by replacing the cell culture medium used for the rapid or second expansion before the splitting with fresh culture medium comprising IL-2 and OKT-3.

[00655] In some embodiments, the splitting of the rapid expansion occurs in a closed system.

[00656] In some embodiments, the scaling up of the TIL culture during the rapid or second expansion comprises adding fresh cell culture medium to the TIL culture (also referred to as feeding the TILs). In some embodiments, the feeding comprises adding fresh cell culture medium to the TIL culture frequently. In some embodiments, the feeding comprises adding fresh cell culture medium to the TIL culture at a regular interval. In some embodiments, the fresh cell culture medium is supplied to the TILs via a constant flow. In

some embodiments, an automated cell expansion system such as Xuri W25 is used for the rapid expansion and feeding.

1. Feeder Cells and Antigen Presenting Cells

[00657] In some embodiments, the second expansion procedures described herein (for example including expansion such as those described in Step D from Figure 1, as well as those referred to as REP) require an excess of feeder cells during REP TIL expansion and/or during the second expansion. 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.

[00658] In general, the allogeneic 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.

[00659] In some embodiments, PBMCs are considered replication incompetent and accepted 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 REP and/or day 0 of the second expansion (*i.e.*, the start day of the second expansion).

[00660] In some embodiments, PBMCs are considered replication incompetent and accepted 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 ng/ml OKT3 antibody and 3000 IU/ml IL-2.

[00661] In some embodiments, PBMCs are considered replication incompetent and accepted 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 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.

[00662] 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.

[00663] In some embodiments, the second expansion procedures described herein require a ratio of about $2.5x10^9$ feeder cells to about $100x10^6$ TILs. In other embodiments, the second expansion procedures described herein require a ratio of about $2.5x10^9$ feeder cells to about $50x10^6$ TILs. In yet other embodiments, the second expansion procedures described herein require about $2.5x10^9$ feeder cells to about $25x10^6$ TILs.

[00664] In some embodiments, the second expansion procedures described herein require an excess of feeder cells during the second expansion. 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. In some embodiments, artificial antigen-presenting (aAPC) cells are used in place of PBMCs.

[00665] In general, the allogeneic 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.

[00666] In some embodiments, artificial antigen presenting cells are used in the second expansion as a replacement for, or in combination with, PBMCs.

2. Cytokines and Other Additives

[00667] 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.

[00668] Alternatively, using combinations of cytokines for the rapid expansion and or second expansion of TILs is additionally possible, with combinations of two or more of IL-2, IL-15 and IL-21 as is described in U.S. Patent Application Publication No. US 2017/0107490 A1, the disclosure of which is incorporated by reference herein. 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.

[00669] In some embodiments, Step D may also include the addition of OKT-3 antibody or muromonab to the culture media, as described elsewhere herein. In some embodiments, Step D may also include the addition of a 4-1BB agonist to the culture media, as described elsewhere herein. In some embodiments, Step D may also include the addition of an OX-40 agonist to the culture media, as described elsewhere herein. In addition, additives such as peroxisome proliferator-activated receptor gamma coactivator I-alpha agonists, including proliferator-activated receptor (PPAR)-gamma agonists such as a thiazolidinedione compound, may be used in the culture media during Step D, as described in U.S. Patent Application Publication No. US 2019/0307796 A1, the disclosure of which is incorporated by reference herein.

E. STEP E: Harvest TILs

[00670] After the 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 some embodiments the TILs are harvested after two expansion steps, for example as provided in Figure 1.

[00671] 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 know methods can be employed with the present process. In some embodiments, TILs are harvested using an automated system.

[00672] 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 systems 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 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.

[00673] In some embodiments, the harvest, for example, Step E according to Figure 1, is performed from 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 G-REX 10 or a G-REX 100. In some embodiments, the closed system bioreactor is a single bioreactor.

[00674] In some embodiments, Step E according to Figure 1, 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 in the Examples is employed.

[00675] In some embodiments, TILs are harvested according to the methods described in the Examples. In some embodiments, TILs between days 1 and 11 are harvested using the methods as described in the steps referred herein, such as in the day 11 TIL harvest in the Examples. In some embodiments, TILs between days 12 and 24 are harvested using the methods as described in the steps referred herein, such as in the Day 22 TIL harvest in the Examples.

[00676] In some embodiments, TILs between days 12 and 22 are harvested using the methods as described in the steps referred herein, such as in the Day 22 TIL harvest in the Examples.

F. STEP F: Final Formulation and Transfer to Infusion Container

[00677] After Steps A through E as provided in an exemplary order in Figure 1, and as outlined in detail above and herein are complete, cells are transferred to a container for use in

administration to a patient such as an infusion bag or sterile vial. 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.

[00678] In some embodiments, TILs expanded using APCs 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 routes of administration include intraperitoneal, intrathecal, and intralymphatic administration.

IV. Gen 3 TIL Manufacturing Processes

[00679] 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 primed by exposure to an anti-CD3 antibody (*e.g.* OKT-3), IL-2 and optionally antigen-presenting cells (APCs) and then boosted by subsequent exposure to additional anti-CD-3 antibody (*e.g.* OKT-3), IL-2 and APCs 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 100 MCS 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 500 MCS 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 100 MCS 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 100 MCS 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 500 MCS 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 cells in a small scale culture in a first container, *e.g.*, a G-REX 100 MCS 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 500 MCS 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.

[00680] In some embodiments, upon the splitting of the rapid expansion, each second container comprises at least 10^8 TILs. In some embodiments, upon the splitting of the rapid expansion, each second container comprises at least 10^8 TILs, at least 10^9 TILs, or at least 10^{10} TILs. In one exemplary embodiment, each second container comprises at least 10^{10} TILs.

[00681] In some embodiments, the first small scale TIL culture is apportioned into a plurality of subpopulations. In some embodiments, the first small scale TIL culture is apportioned into a plurality of about 2 to 5 subpopulations. In some embodiments, the first small scale TIL culture is apportioned into a plurality of about 2, 3, 4, or 5 subpopulations.

[00682] In some embodiments, after the completion of the rapid expansion, the plurality of subpopulations comprises a therapeutically effective amount of TILs. In some embodiments, after the completion of the rapid expansion, one or more subpopulations of TILs are pooled together to produce a therapeutically effective amount of TILs. In some embodiments, after the completion of the rapid expansion, each subpopulation of TILs comprises a therapeutically effective amount of TILs.

[00683] In some embodiments, the rapid expansion is performed for a period of about 1 to 5 days before being split into a plurality of steps. In some embodiments, the splitting of the rapid expansion occurs at about day 1, day 2, day 3, day 4, or day 5 after the initiation of the rapid expansion.

[00684] In some embodiments, the splitting of the rapid expansion occurs at about day 8, day 9, day 10, day 11, day 12, or day 13 after the initiation of the first expansion (*i.e.*, pre-REP expansion). In one exemplary embodiment, the splitting of the rapid expansion occurs at about day 10 after the initiation of the priming first expansion. In another exemplary embodiment, the splitting of the rapid expansion occurs at about day 11 after the initiation of the priming first expansion.

[00685] In some embodiments, the rapid expansion is further performed for a period of about 4 to 11 days after the splitting. In some embodiments, the rapid expansion is further performed for a period of about 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, or 11 days after the splitting.

[00686] In some embodiments, the cell culture medium used for the rapid expansion before the splitting comprises the same components as the cell culture medium used for the rapid expansion after the splitting. In some embodiments, the cell culture medium used for the rapid expansion before the splitting comprises different components from the cell culture medium used for the rapid expansion after the splitting.

[00687] In some embodiments, the cell culture medium used for the rapid expansion before the splitting comprises IL-2, optionally OKT-3 and further optionally APCs. In some embodiments, the cell culture medium used for the rapid expansion before the splitting comprises IL-2, OKT-3, and further optionally APCs. In some embodiments, the cell culture medium used for the rapid expansion before the splitting comprises IL-2, OKT-3 and APCs.

[00688] In some embodiments, the cell culture medium used for the rapid expansion before the splitting is generated by supplementing the cell culture medium in the first

expansion with fresh culture medium comprising IL-2, optionally OKT-3 and further optionally APCs. In some embodiments, the cell culture medium used for the rapid expansion before the splitting is generated by supplementing the cell culture medium in the first expansion with fresh culture medium comprising IL-2, OKT-3 and APCs. In some embodiments, the cell culture medium used for the rapid expansion before the splitting is generated by replacing the cell culture medium in the first expansion with fresh cell culture medium comprising IL-2, optionally OKT-3 and further optionally APCs. In some embodiments, the cell culture medium used for the rapid expansion before the splitting is generated by replacing the cell culture medium in the first expansion with fresh cell culture medium comprising IL-2, OKT-3 and APCs.

[00689] In some embodiments, the cell culture medium used for the rapid expansion after the splitting comprises IL-2, and optionally OKT-3. In some embodiments, the cell culture medium used for the rapid expansion after the splitting comprises IL-2, and OKT-3. In some embodiments, the cell culture medium used for the rapid expansion after the splitting is generated by replacing the cell culture medium used for the rapid expansion before the splitting with fresh culture medium comprising IL-2 and optionally OKT-3. In some embodiments, the cell culture medium used for the rapid expansion after the splitting is generated by replacing the cell culture medium used for the rapid expansion before the splitting with fresh culture medium comprising IL-2 and OKT-3.

[00690] In some embodiments, the splitting of the rapid expansion occurs in a closed system.

[00691] In some embodiments, the scaling up of the TIL culture during the rapid expansion comprises adding fresh cell culture medium to the TIL culture (also referred to as feeding the TILs). In some embodiments, the feeding comprises adding fresh cell culture medium to the TIL culture frequently. In some embodiments, the feeding comprises adding fresh cell culture medium to the TIL culture at a regular interval. In some embodiments, the fresh cell culture medium is supplied to the TILs via a constant flow. In some embodiments, an automated cell expansion system such as Xuri W25 is used for the rapid expansion and feeding.

[00692] 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.

[00693] 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%.

[00694] 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%.

[00695] 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%.

[00696] 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%.

[00697] 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, 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%.

[00698] 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.

[00699] 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.

[00700] 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.

[00701] 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.

[00702] In some embodiments, the rapid second expansion of T cells is performed during a period of up to at or about 11 days.

[00703] 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.

[00704] 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.

[00705] 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.

[00706] 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.

[00707] 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.

[00708] 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.

[00709] 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.

[00710] 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.

[00711] In some embodiments, the T cells are tumor infiltrating lymphocytes (TILs).

[00712] In some embodiments, the T cells are marrow infiltrating lymphocytes (MILs).

[00713] In some embodiments, the T cells are peripheral blood lymphocytes (PBLs).

[00714] In some embodiments, the T cells are obtained from a donor suffering from a cancer.

[00715] In some embodiments, the T cells are TILs obtained from a tumor excised from a patient suffering from a cancer.

[00716] In some embodiments, the T cells are MILs obtained from bone marrow of a patient suffering from a hematologic malignancy.

[00717] 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 selected from the group consisting of melanoma, ovarian cancer, endometrial cancer, thyroid 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, 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 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.

[00718] 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 some embodiments, 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.

[00719] 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, 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, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, cancer caused by human papilloma virus, head and neck cancer (including head and neck squamous cell carcinoma (HNSCC)), glioblastoma (including GBM), gastrointestinal cancer, renal cancer, and renal cell carcinoma. In some embodiments, 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 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.

[00720] An exemplary TIL process known as process 3 (also referred to herein as Gen 3) containing some of these features is depicted in Figure 8 (in particular, *e.g.*, Figure 8B and/or Figure 8C and/or Figure 8D), and some of the advantages of this embodiment of the present invention over process 2A are described in Figures 1, 2, 8, 30, and 31 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D). Embodiments of Gen 3 are shown in Figures 1, 8 and 30 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D). Process 2A or Gen 2 or Gen 2A is also described in U.S. Patent Publication No. 2018/0280436, incorporated by reference herein in its entirety. The Gen 3 process is also described in International Patent Publication WO 2020/096988.

[00721] 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.

[00722] 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 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) 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 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) 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 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) 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 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) 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 8 (in particular, *e.g.*, Figure 1B and/or Figure 8C) 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 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) 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 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) 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 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) 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 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is shortened to 8 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is 7 to 9 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is 8 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is 8 to 9 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is shortened to 7 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is 7 to 8 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is shortened to 8 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is 8 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is 8 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is 9 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is 8 days and the rapid second expansion (for example, an expansion as

described in Step D in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is 10 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is 7 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is 7 to 10 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is 7 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is 8 to 10 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is 7 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is 9 to 10 days. In some embodiments, the priming first expansion (for example, an expansion described as Step B in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) is shortened to 7 days and the rapid second expansion (for example, an expansion as described in Step D in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) 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 8 (in particular, *e.g.*, Figure 1B and/or Figure 8C) is 14-16 days, as discussed 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.

[00723] The "Step" Designations A, B, C, *etc.*, below are in reference to the non-limiting example in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) and in reference to certain non-limiting embodiments described herein. The ordering of the Steps below and in Figure 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D) 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

[00724] In general, TILs are initially obtained from a patient tumor sample ("primary TILs") or from circulating lymphocytes, such as peripheral blood lymphocytes, including peripheral 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.

[00725] 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, the cancer is melanoma. In some embodiments, useful TILs are obtained from malignant melanoma tumors, as these have been reported to have particularly high levels of TILs.

[00726] 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.

[00727] 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.

[00728] 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.

[00729] 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.

[00730] 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.

[00731] 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.

[00732] 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.

[00733] In some embodiments, the enzyme mixture includes neutral protease, DNase, and collagenase.

[00734] 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.

[00735] 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 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 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 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.

[00736] In some embodiments, the tumor is reconstituted with the lyophilized enzymes in a sterile buffer. In some embodiments, the buffer is sterile HBSS.

[00737] In some embodiments, the enzyme 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.

[00738] 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.

[00739] 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.

[00740] In some embodiments, the enzyme mixture comprises 10 mg/mL collagenase, 1000 IU/mL DNAse, and 1 mg/mL hyaluronidase.

[00741] In some embodiments, the enzyme mixture comprises 10 mg/mL collagenase, 500 IU/mL DNAse, and 1 mg/mL hyaluronidase.

[00742] 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-2 and OKT-3.

[00743] 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 cultured from enzymatic tumor digests and tumor fragments obtained from patients.

[00744] 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 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D)). 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.

[00745] 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 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.

[00746] 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.

[00747] 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.

[00748] 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.

[00749] 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 8 (in particular, *e.g.*, Figure 8B).

1. Core/Small Biopsy Derived TILs

[00750] 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.

[00751] In some embodiments, 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 is melanoma. The solid tumor may be of lung and/or non-small cell lung carcinoma (NSCLC).

[00752] 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 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.

[00753] 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, the tumor is a melanoma. In some embodiments, the small biopsy for a melanoma comprises a mole or portion thereof.

[00754] 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.

[00755] 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.

[00756] 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.

[00757] 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, 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 via 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.

[00758] 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.

[00759] 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.

[00760] 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 cancers of the lung. In some embodiments, the cancer is melanoma. In some embodiments, the cancer is non-small cell lung carcinoma (NSCLC). 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.

[00761] In some embodiments, the sample from the tumor is obtained as a fine needle aspirate (FNA), a core biopsy, or 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.

[00762] The FNA can be obtained from a skin tumor, including, for example, a melanoma. In some embodiments, the FNA is obtained from a skin tumor, such as a skin tumor from a patient with metastatic melanoma. In some cases, the patient with melanoma has previously undergone a surgical treatment.

[00763] The FNA can be obtained from a lung tumor, including, for example, an NSCLC. 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.

[00764] 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.

[00765] 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.*, 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.

[00766] In general, the harvested cell suspension is called a "primary cell population" or a "freshly harvested" cell population.

[00767] In some embodiments, the TILs are not obtained from tumor digests. In some embodiments, the solid tumor cores are not fragmented.

[00768] 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.

[00769] In some embodiments, obtaining the first population of TILs comprises a multilesional sampling method.

[00770] 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.

[00771] 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 Hank's balance salt solution (HBSS).

[00772] In some instances, collagenase (such as animal free type 1 collagenase) is reconstituted 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 embodiments, 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.

[00773] 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. 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.

[00774] 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 to 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.

[00775] 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.

[00776] 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 T-cells and TILs

[00777] In some embodiments, the sample is a pleural fluid sample. In some embodiments, the source of the T-cells and/or 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 T-cells and/or 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.

[00778] 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.

[00779] 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 further processing steps. In some embodiments, the unprocessed pleural fluid is placed in a standard CellSave® tube (Veridex) prior to further processing steps. In some embodiments, the sample is placed in the CellSave tube 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.

[00780] In some embodiments, the pleural fluid sample from the chosen subject may be diluted. In some embodiments, 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.

[00781] In still other 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.

[00782] 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 used in further processing steps 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 further processing steps of the method.

[00783] 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.

[00784] 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

[00785] 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 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.

[00786] 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).

[00787] 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.

[00788] 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.

[00789] 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.

[00790] 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).

[00791] 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 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.

[00792] 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.

[00793] 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.

[00794] 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.

[00795] 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.

[00796] In some embodiments 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 binding beads. In some embodiments of the invention, pure T-cells are isolated on Day 0 from the PBMCs.

[00797] 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.

[00798] 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.

[00799] 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.

[00800] In some embodiments, PBLs are prepared using the methods described in U.S. Patent Application Publication No. US 2020/0347350 A1, the disclosures of which are incorporated by reference herein.

4. Methods of Expanding Marrow Infiltrating Lymphocytes (MILs) from PBMCs Derived from Bone Marrow

[00801] 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.

[00802] 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+).

[00803] 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.

[00804] 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 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.

[00805] In some embodiments of the invention, the number of PBMCs yielded from about 10-50 ml 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.

[00806] 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.

[00807] In some embodiments of the invention, 12×10^6 PBMC derived from bone marrow aspirate yields approximately 1.4×10^5 MILs.

[00808] 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.

[00809] In any of the foregoing embodiments, the MILs may be genetically modified to express the CCRs described herein. In some embodiments, MILs are prepared using the methods described in U.S. Patent Application Publication No. US 2020/0347350 A1, the disclosures of which are incorporated by reference herein.

B. STEP B: Priming First Expansion

[00810] 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 in Donia, *et al.*, *Scand. J. Immunol.* **2012,** *75,* 157–167; Dudley, *et al.*, *Clin. Cancer Res.* **2010,** *16,* 6122-6131; Huang, *et al.*, *J. Immunother.* **2005,** *28*, 258–267; Besser, *et al.*, *Clin. Cancer Res.* **2013,** *19*, OF1-OF9; Besser, *et al.*, *J. Immunother.* **2009**, *32,* 415–423; Robbins, *et al., J. Immunol.* **2004**, *173,* 7125-7130; Shen, *et al.*, *J. Immunother.*, **2007,** *30,* 123–129; Zhou, *et al.*, *J. Immunother.* **2005,** *28,* 53–62; and Tran, *et al.*, *J. Immunother.*, **2008**, *31*, 742–751, each of which is incorporated herein by reference.

[00811] After dissection or digestion of tumor fragments and/or tumor fragments, for example such as described in Step A of Figure 8 (in particular, *e.g.,* Figure 8A and/or Figure 8B and/or Figure 8C), the resulting cells are cultured in serum containing IL-2, OKT-3, and feeder cells (*e.g.*, antigen-presenting feeder cells), under conditions that favor the growth of TILs over tumor and other cells. In some embodiments, the IL-2, 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 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 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, 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

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.

[00812] 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 8 (in particular, *e.g.*, Figure 8A and/or Figure 8B and/or Figure 8C and/or Figure 8D), which can include processes referred to as pre-REP or priming REP and which contains feeder cells 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.

[00813] 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.

[00814] 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, 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.

[00815] 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, 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.

[00816] 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.

[00817] 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.

[00818] 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 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, for example, Table 1.

[00819] 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.

[00820] 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.

[00821] In some embodiments, the priming first expansion cell culture medium comprises one or more epigenetic reprogramming agents in a cell culture medium. In some embodiments, the epigenetic reprogramming agent is a DNA hypomethylating agent. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is decitabine.

[00822] In some embodiments, the epigenetic reprogramming agent is a MEK inhibitor. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is trametinib.

[00823] In some embodiments, the epigenetic reprogramming agent is an HDAC inhibitor. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is ricolinistat.

[00824] In some embodiments, the epigenetic reprogramming agent is a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is JQ1.

[00825] In some embodiments, the epigenetic reprogramming agent is an EZH2 inhibitor. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

[00826] In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the epigenetic reprogramming agent is ipatasertib.

[00827] In some embodiments, the epigenetic reprogramming agent is a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35.

[00828] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and a MEK inhibitor. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00829] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and an HDAC inhibitor. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00830] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and an EZH2 inhibitor. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00831] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of

decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00832] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00833] In some embodiments, the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof. In some embodiments, the DNA hypomethylating agent is decitabine.

[00834] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and an HDAC inhibitor. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat,

panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[00835] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and an EZH2 inhibitor. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[00836] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[00837] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is

ipatasertib. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[00838] In some embodiments, the epigenetic reprogramming agent is a combination of a MEK inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG), D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof. In some embodiments, the MEK inhibitor is trametinib.

[00839] In some embodiments, the epigenetic reprogramming agent is a combination of an HDAC inhibitor and an EZH2 inhibitor. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat.

[00840] In some embodiments, the epigenetic reprogramming agent is a combination of an HDAC inhibitor and a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and

pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat.

[00841] In some embodiments, the epigenetic reprogramming agent is a combination of an HDAC inhibitor and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat.

[00842] In some embodiments, the epigenetic reprogramming agent is a combination of an HDAC inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof. In some embodiments, the HDAC inhibitor is rocilinostat.

[00843] In some embodiments, the epigenetic reprogramming agent is a combination of an EZH2 inhibitor and a bromodomain inhibitor. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1. In some embodiments, the

EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

[00844] In some embodiments, the epigenetic reprogramming agent is a combination of EZH2 inhibitor and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

[00845] In some embodiments, the epigenetic reprogramming agent is a combination of EZH2 inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

[00846] In some embodiments, the epigenetic reprogramming agent is a combination of bromodomain inhibitor and an AKT inhibitor. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1.

[00847] In some embodiments, the epigenetic reprogramming agent is a combination of bromodomain inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG)

and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof. In some embodiments, the bromodomain inhibitor is JQ1.

[00848] In some embodiments, the epigenetic reprogramming agent is a combination of an AKT inhibitor and a TET inhibitor. In some embodiments, the TET inhibitor is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG). In some embodiments, the epigenetic reprogramming agent is C35. In some embodiments, the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof. In some embodiments, the AKT inhibitor is ipatasertib.

[00849] In some embodiments, the epigenetic reprogramming agent is a combination of three or more epigenetic reprogramming agents described herein.

[00850] In some embodiments, the epigenetic reprogramming agent may be added at a concentration in a range from about 5 nM to about 5 μM. For example, the concentration of the epigenetic reprogramming agent in the priming first expansion cell culture medium may be about 10 nM, about 15 nM, about 20 nM, about 25 nM, about 30 nM, about 35 nM, about 40 nM, about 45 nM, about 50 nM, about 60 nM, about 70 nM, about 80 nM, about 90 nM, about 100 nM, about 110 nM, about 120 nM, about 130 nM, about 140 nM, about 150 nM, about 160 nM, about 170 nM, about 180 nM, about 190 nM, about 200 nM, about 220 nM, about 240 nM, about 260 nM, about 280 nM, about 300 nM, about 320 nM, about 340 nM, about 360 nM, about 380 nM, about 400 nM, about 420 nM, about 440 nM, about 460 nM, about 480 nM, about 500 nM, about 550 nM, about 600 nM, about 650 nM, about 700 nM, about 750 nM, about 800 nM, about 850 nM, about 900 nM, about 950 nM, about 1 μM, about 1.5μM, about 2 μM, about 2.5 μM, about 3 μM, about 3.5 μM, about 4 μM, about 4.5 μM, about 5 μM, or any other concentration between any two of these values.

[00851] In some embodiments, the epigenetic reprogramming agent may be a combination of two or more epigenetic reprogramming agents. In such embodiments, the two

or more epigenetic reprogramming agents may be added at different concentrations. For example, a first epigenetic reprogramming agent may be added at a concentration of 5 nM and a second epigenetic reprogramming agent may be added at a concentration of 50 nM. In another example, a first epigenetic reprogramming agent may be added at a concentration of 5 nM, a second epigenetic reprogramming agent may be added at a concentration of 50 nM and a third epigenetic reprogramming agent may be added at a concentration of 100 nM. Other combinations of concentrations of one or more epigenetic reprogramming agents within the concentration ranges described herein are contemplated.

[00852] 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 some embodiments, the CM is the CM1 described in the Examples. 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).

[00853] 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.

[00854] 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.

[00855] 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.

[00856] 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.

[00857] 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.

[00858] 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 1 L 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.

[00859] 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 1 L 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

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WHAT IS CLAIMED IS:

1. A method of treating a cancer in a subject in need thereof comprising administering a population of modified tumor infiltrating lymphocytes (TILs), the method comprising the steps of:
    a. obtaining and/or receiving a first population of TILs from a tumor resected from the subject or patient by processing a tumor sample obtained from the subject into multiple tumor fragments or processing a tumor sample obtained from the subject into a tumor digest;
    b. performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-14 days to obtain the second population of TILs;
    c. performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-14 days to obtain the third population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (b) to step (c) optionally occurs without opening the system;
    d. harvesting the third population of TILs obtained from step (c), wherein the transition from step (c) to step (d) optionally occurs without opening the system;
    e. administering a therapeutically effective dosage of the third population of TILs obtained in step (d) to the subject;
    f. adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).
2. A method of treating a cancer in a patient or subject in need thereof comprising administering a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

    (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 or

processing a tumor sample obtained from the subject into a tumor digest;

(b) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-11 days to obtain the second population of TILs;

(c) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-11 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 (b) to step (c) optionally occurs without opening the system;

(d) harvesting the third population of TILs obtained from step (c), wherein the transition from step (c) to step (d) optionally occurs without opening the system;

(e) transferring the harvested third TIL population from step (d) to an infusion bag, wherein the transfer from step (d) to (e) optionally occurs without opening the system;

(f) cryopreserving the infusion bag comprising the harvested TIL population from step (e) using a cryopreservation process;

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

(h) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

3. A method of treating a cancer in a patient or subject in need thereof comprising administering a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) obtaining and/or receiving a first population of TILs from surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from the cancer in the patient or subject,

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

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expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-11 days to obtain the second population of TILs;

(c) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-11 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 (b) to step (c) optionally occurs without opening the system;

(d) harvesting the third population of TILs obtained from step (c), wherein the transition from step (c) to step (d) optionally occurs without opening the system;

(e) transferring the harvested third TIL population from step (d) to an infusion bag, wherein the transfer from step (d) to (e) optionally occurs without opening the system;

(f) cryopreserving the infusion bag comprising the harvested TIL population from step (e) using a cryopreservation process;

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

(h) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

4. A method of treating a cancer in a patient or subject in need thereof comprising administering a population of modified tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) resecting a tumor from the subject or patient, the tumor comprising a first population of TILs, optionally from surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from the cancer;

(b) processing the tumor into multiple tumor fragments;

(c) enzymatically digesting the multiple tumor fragments to obtain the first population of TILs;

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(d) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-11 days to obtain the second population of TILs;

(e) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-11 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 (d) to step (e) optionally occurs without opening the system;

(f) harvesting the third population of TILs obtained from step (e), wherein the transition from step (e) to step (f) optionally occurs without opening the system;

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

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

(i) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (h) to the subject or patient with the cancer; and

(j) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (d) and/or step (e).

5. A method of treating a cancer in a patient or subject in need thereof comprising administering a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) obtaining and/or receiving a first population of TILs from surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from the subject or patient;

(b) performing an initial expansion (or priming first expansion) of the first population of TILs in the first cell culture medium to obtain a second population of TILs, optionally OKT-3 (anti-CD3 antibody), and optionally antigen presenting cells (APCs), where

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the priming first expansion occurs for a period of 1 to 8 days;

(c) performing a rapid second expansion of the second population of TILs in a second cell culture medium to obtain a third population of TILs, wherein the second cell culture medium comprises IL-2, OKT-3 (anti-CD3 antibody), and APCs; and wherein the rapid expansion is performed over a period of 14 days or less, optionally the rapid second 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;

(d) harvesting the third population of TILs;

(e) administering a therapeutically effective portion of the third population of TILs to the subject or patient with the cancer; and

(h) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

6. A method of treating a cancer in a patient or subject in need thereof comprising administering a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) resecting a tumor from the cancer in the subject or patient, the tumor comprising a first population of TILs, optionally from surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from the cancer;

(b) fragmenting the tumor into tumor fragments or processing the tumor into a tumor digest;

(c) contacting the tumor fragments or tumor digest with a first cell culture medium;

(d) performing an initial expansion (or priming first expansion) of the first population of TILs in the first cell culture medium to obtain a second population of TILs, optionally OKT-3 (anti-CD3 antibody), and optionally antigen presenting cells (APCs), where the priming first expansion occurs for a period of 1 to 8 days;

(e) performing a rapid second expansion of the second population of TILs in a second cell culture medium to obtain a third population of TILs wherein the second cell culture medium comprises IL-2, OKT-3 (anti-CD3 antibody), and APCs; and wherein the rapid expansion is performed over a period of 14 days or less, optionally the rapid second expansion can proceed for 1 day, 2 days, 3 days, 4, days, 5 days, 6 days, 7

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days, 8 days, 9 days or 10 days after initiation of the rapid second expansion;

(f) harvesting the third population of TILs;

(g) administering a therapeutically effective portion of the third population of TILs to the subject or patient with the cancer; and

(h) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (d) and/or step (e).

7. A method of treating a cancer in a patient or subject in need thereof comprising administering a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) obtaining and/or receiving a first population of TILs from surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from the cancer in the patient or subject,

(b) culturing the first population of TILs in a culture medium comprising IL-2 for 1 to 3 days;

(c) performing a priming first expansion by culturing the first population of TILs in a first cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for first period of about 1 to 11 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

(d) performing a rapid second expansion by culturing the modified second population of TILs in a second 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 14 days or less to obtain the therapeutic population of TILs, wherein the third population of TILs is a therapeutic population of TILs;

(e) harvesting the third population of TILs;

(f) administering a therapeutically effective portion of the third population of TILs to the subject or patient with the cancer, and

(g) adding an epigenetic reprogramming agent to the cell culture medium, and optionally

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a cell permeating agent, in step (b) and/or step (c).

8. 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 cancer in a subject by processing a tumor sample obtained from the tumor into multiple tumor fragments or processing a tumor sample obtained from the subject into a tumor digest;

(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 antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for first period of about 1 to 7/8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

(c) performing a rapid second expansion by culturing the second population of TILs in a second culture medium comprising IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the number of APCs added in the rapid second expansion is at least twice the number of APCs added in step (b), wherein the rapid second expansion is performed for a second period of about 1 to 11 days to obtain the therapeutic 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) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

9. A method of expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs, the method comprising the steps of:

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

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into a tumor digest;

(b) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-14 days to obtain the second population of TILs;

(c) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-14 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is optionally performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (b) to step (c) optionally occurs without opening the system;

(d) harvesting the third population of TILs obtained from step (c), wherein the transition from step (c) to step (d) optionally occurs without opening the system;

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

(h) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

10. A method of expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs, the method comprising the steps of:

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

(b) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-11 days to obtain the second population of TILs;

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(c) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-11 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 (b) to step (c) optionally occurs without opening the system;

(d) harvesting the third population of TILs obtained from step (c), wherein the transition from step (c) to step (d) optionally occurs without opening the system;

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

(h) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

11. A method of expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs, the method comprising the steps of:

(a) obtaining and/or receiving a first population of TILs from surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from a cancer in a patient or subject,

(b) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-11 days to obtain the second population of TILs;

(c) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-11 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 (b) to step (c) optionally occurs without opening the system;

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(d) harvesting the third population of TILs obtained from step (c), wherein the transition from step (c) to step (d) optionally occurs without opening the system;

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

(f) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

12. A method of expanding tumor infiltrating lymphocytes (TILs) to a therapeutic population of TILs, the method comprising the steps of:

(a) resecting a tumor from a cancer in subject or patient, the tumor comprising a first population of TILs, optionally from surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from the cancer;

(b) processing the tumor into multiple tumor fragments;

(c) enzymatically digesting the multiple tumor fragments to obtain the first population of TILs;

(d) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is optionally performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-11 days to obtain the second population of TILs;

(e) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-11 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 (d) to step (e) optionally occurs without opening the system;

(f) harvesting the third population of TILs obtained from step (e), wherein the transition from step (e) to step (f) optionally occurs without opening the system;

(g) transferring the harvested third TIL population from step (f) to an infusion bag,

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wherein the transfer from step (f) to (g) optionally occurs without opening the system; and

(j) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (d) and/or step (e).

13. A method of expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs, the method comprising the steps of:

(a) obtaining and/or receiving a first population of TILs from surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from a cancer in the subject or patient;

(b) performing an initial expansion (or priming first expansion) of the first population of TILs in the first cell culture medium to obtain a second population of TILs, optionally OKT-3 (anti-CD3 antibody), and optionally antigen presenting cells (APCs), where the priming first expansion occurs for a period of 1 to 8 days;

(c) performing a rapid second expansion of the second population of TILs in a second cell culture medium to obtain a third population of TILs, wherein the second cell culture medium comprises IL-2, OKT-3 (anti-CD3 antibody), and APCs; and wherein the rapid expansion is performed over a period of 14 days or less, optionally the rapid second 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;

(d) harvesting the third population of TILs; and

(e) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

14. A method of expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs, the method comprising the steps of:

(a) resecting a tumor from the cancer in the subject or patient, the tumor comprising a first population of TILs, optionally from surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from the cancer;

(b) fragmenting the tumor into tumor fragments or into a tumor digest;

(c) contacting the tumor fragments or tumor digest with a first cell culture medium;

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(d) performing an initial expansion (or priming first expansion) of the first population of TILs in the first cell culture medium to obtain a second population of TILs, wherein the first cell culture medium comprises IL-2, optionally OKT-3 (anti-CD3 antibody), and optionally antigen presenting cells (APCs), where the priming first expansion occurs for a period of 1 to 8 days;

(e) performing a rapid second expansion of the second population of TILs in a second cell culture medium to obtain a third population of TILs; wherein the second cell culture medium comprises IL-2, OKT-3 (anti-CD3 antibody), and APCs; and wherein the rapid expansion is performed over a period of 14 days or less, optionally the rapid second 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;

(f) harvesting the third population of TILs; and

(g) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (d) and/or step (e).

15. 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 cancer in a subject by processing a tumor sample obtained from the tumor into multiple tumor fragments or processing a tumor sample obtained from the subject into a tumor digest;

(b) performing a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, optionally OKT-3, and optionally comprising antigen presenting cells (APCs), to produce a second population of TILs, wherein the priming first expansion is performed for a first period of about 1 to 11 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

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

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

(g) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b) and/or step (c).

16. The method of claim 15, wherein in step (c) the cell culture medium further comprises antigen-presenting cells (APCs), and wherein the number of APCs in the culture medium in step (d) is greater than the number of APCs in the culture medium in step (c).

17. 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 surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from a cancer in a patient or subject,

(b) culturing the first population of TILs in a culture medium comprising IL-2 for 1 to 3 days;

(c) performing a priming first expansion by culturing the first population of TIL in a first cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for first period of about 1 to 11 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

(d) performing a rapid second expansion by culturing the modified second population of TILs in a second 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 14 days or less to obtain the therapeutic population of TILs;

(e) harvesting the third population of TILs; and

(f) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (b), step (c) and/or step (d).

18. The method of any one of claims 1-17, 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

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caused by human papilloma virus, head and neck cancer (including head and neck squamous cell carcinoma (HNSCC)), renal cancer, and renal cell carcinoma.

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

(a) performing a priming first expansion by culturing a first population of TILs in a cell culture medium comprising IL-2, optionally OKT-3, and optionally comprising antigen presenting cells (APCs), to produce a second population of TILs, wherein the priming first expansion is performed for a first period of about 1 to 11 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

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

(c) harvesting the third population of TILs obtained from step (b); and

(d) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (a) and/or step (b).

20. The method of claim 19, 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).

21. A method of expanding T cells comprising:

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

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

(c) harvesting the second population of T cells; and

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(d) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (a) and/or step (b).

22. A method of expanding T cells comprising:

(a) 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;

(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; and

(d) adding an epigenetic reprogramming agent to the cell culture medium, and optionally a cell permeating agent, in step (a) and/or step (b).

23. The method of any of claims 1-22, wherein the second population of TILs and/or the third population of TILs has an increased frequency of CD8 TILs and/or an increased ratio of CD4 TILs to CD8 TILs when compared to a corresponding population of TILs expanded in a cell culture medium without the epigenetic reprogramming agent.

24. The method of any of claims 5, 6, and 13, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, wherein the first cell culture medium comprises IL-2.

25. The method of any of claims 5, 6, and 13, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7-8 days from the start of the rapid expansion.

26. The method of any of claims 1-25, wherein the epigenetic reprogramming agent includes one or more of a DNA hypomethylating agent, a MEK inhibitor, a HDAC inhibitor, an EZH2 inhibitor, a bromodomain inhibitor, an AKT inhibitor, and/or a TET inhibitor.

27. The method of claim 26, wherein the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-

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5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof.

28. The method of claim 26, wherein the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof.
29. The method of claim 26, wherein the TET inhibitor includes is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG).
30. The method of claim 26, wherein the bromodomain inhibitor is one or more selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof.
31. The method of claim 26, wherein the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.
32. The method of claim 26, wherein the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof.
33. The method of claim 26, wherein the MEK inhibitor inhibits MEK1 and/or MEK2.
34. The method of claim 33, wherein the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof.
35. The method of any of claims 1-34, wherein the second population of TILs and/or the third population of TILs has an increased expression of IL-7 receptor when compared to a corresponding population of TILs expanded in a cell culture medium without the epigenetic reprogramming agent.
36. The method of any of claims 1-34, wherein the second population of TILs and/or the third population of TILs has an increased expression of at least one of CD25, CD28, ICOS, Ki-

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67 and GZMB, when compared to a corresponding population of TILs expanded in a cell culture medium without the epigenetic reprogramming agent.

37. The method of any of claims 1-34, wherein the second population of TILs and/or the third population of TILs has an increased expression of at least one of PD-I and TIGIT, when compared to a corresponding population of TILs expanded in a cell culture medium without the epigenetic reprogramming agent.
38. The method of any of claims 1-34, wherein the second population of TILs and/or the third population of TILs has an increased expression of at least one of TCFI, EOMES and KLF2, when compared to a corresponding population of TILs expanded in a cell culture medium without the epigenetic reprogramming agent.
39. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a DNA hypomethylating agent.
40. The method of claim 39, wherein the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof.
41. The method of claim 39, wherein the epigenetic reprogramming agent is decitabine.
42. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a MEK inhibitor.
43. The method of claim 42, wherein the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof.
44. The method of claim 42, wherein the epigenetic reprogramming agent is trametinib.
45. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is an HDAC inhibitor.
46. The method of claim 45, wherein the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof.

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47. The method of claim 45, wherein the epigenetic reprogramming agent is ricolinistat.
48. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a bromodomain inhibitor.
49. The method of claim 48, wherein the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof.
50. The method of claim 48, wherein the epigenetic reprogramming agent is JQ1.
51. The method of claim 45, wherein the epigenetic reprogramming agent is an EZH2 inhibitor.
52. The method of claim 41, wherein the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.
53. The method of any of claims 1-26, wherein the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof.
54. The method of claim 53, wherein the epigenetic reprogramming agent is ipatasertib.
55. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a TET inhibitor.
56. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and a MEK inhibitor.
57. The method of claim 56, wherein the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof.
58. The method of claim 57, wherein the MEK inhibitor is trametinib.
59. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and an HDAC inhibitor.
60. The method of claim 59, wherein the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat,

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romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof.

61. The method of claim 60, wherein the HDAC inhibitor is rocilinostat.
62. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and an EZH2 inhibitor.
63. The method of claim 62, wherein the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.
64. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and a bromodomain inhibitor.
65. The method of claim 64, wherein the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof.
66. The method of claim 65, wherein the bromodomain inhibitor is JQ1.
67. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and an AKT inhibitor.
68. The method of claim 67, wherein the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof.
69. The method of claim 68, wherein the AKT inhibitor is ipatasertib.
70. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of a DNA hypomethylating agent and a TET inhibitor.
71. The method of any of claims 56-70, wherein the DNA hypomethylating agent is selected from the group consisting of decitabine, azacitidine, GSK-3484862, RG-108, GSK-3685032, DHAC, SGI-1027, CM-272, zebularine, hinokitiol, guadecitabine, gamma-Oryzanol, CM-579, DC-517, 5-fluoro-2'-deoxycytidine, 5-methyldeoxycytidine, DC-05, 6-methyl-5-azacytidine, procainamide, procaine, hydralazine, EGCG, FdCyd, CP-4200, Nanomycin A, and pharmaceutically acceptable salts thereof.
72. The method of claim 71, wherein the DNA hypomethylating agent is decitabine.
73. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of a MEK inhibitor and an HDAC inhibitor.

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74. The method of claim 73, wherein the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof.
75. The method of claim 74, wherein the HDAC inhibitor is rocilinostat.
76. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of a MEK inhibitor and an EZH2 inhibitor.
77. The method of claim 76, wherein the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.
78. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of a MEK inhibitor and a bromodomain inhibitor.
79. The method of claim 75, wherein the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof.
80. The method of claim 76, wherein the bromodomain inhibitor is JQ1.
81. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of a MEK inhibitor and an AKT inhibitor.
82. The method of claim 81, wherein the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof.
83. The method of claim 79, wherein the AKT inhibitor is ipatasertib.
84. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of a MEK inhibitor and a TET inhibitor.
85. The method of any of claims 73-84, wherein the MEK inhibitor is selected from the group consisting of trametinib, cobimetinib, binimetinib, selumetinib, PD-325901, CI-1040, TAK-733, GDC-0623, pimasertinib, refametinib, BI-847325, and pharmaceutically acceptable salts thereof.
86. The method of claim 85, wherein the MEK inhibitor is trametinib.

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87. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of an HDAC inhibitor and an EZH2 inhibitor.
88. The method of claim 87, wherein the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.
89. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of an HDAC inhibitor and a bromodomain inhibitor.
90. The method of claim 869, wherein the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof.
91. The method of claim 90, wherein the bromodomain inhibitor is JQ1.
92. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of an HDAC inhibitor and an AKT inhibitor.
93. The method of claim 92, wherein the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof.
94. The method of claim 93, wherein the AKT inhibitor is ipatasertib.
95. The method of any of claims 87-94, wherein the HDAC inhibitor is selected from the group consisting of rocilinostat, vorinostat, trichostatin A, belinostat, panabiostat, panobinostat, quisinostat, givinostat, resminostat, abexinostat, quisinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entionstat, tacedinaline, mocetinostat, romidespin, nicotinamide, sirtinol, cambinol, EX-527, apicidin, depsipeptide, MS275, BML-210, splitomicin, RGFP966, and pharmaceutically acceptable salts thereof.
96. The method of claim 95, wherein the HDAC inhibitor is rocilinostat.
97. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of an HDAC inhibitor and a TET inhibitor.
98. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of an EZH2 inhibitor and a bromodomain inhibitor.
99. The method of claim 98, wherein the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744,

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SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof.

100. The method of claim 99, wherein the bromodomain inhibitor is JQ1.

101. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of EZH2 inhibitor and an AKT inhibitor.

102. The method of claim 101, wherein the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof.

103. The method of claim 102, wherein the AKT inhibitor is ipatasertib.

104. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of EZH2 inhibitor and a TET inhibitor.

105. The method of any of claims 98-104, wherein the EZH2 inhibitor is selected from the group consisting of 3-deazaneplanocin A, tazemetostat, GSK343, GSK926, GSK126, EPZ005687, and pharmaceutically acceptable salts thereof.

106. The method of any of claim 1-26, wherein the epigenetic reprogramming agent is a combination of bromodomain inhibitor and an AKT inhibitor.

107. The method of claim 106, wherein the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof.

108. The method of claim 107, wherein the AKT inhibitor is ipatasertib.

109. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of bromodomain inhibitor and a TET inhibitor.

110. The method of any of claims 106-109, wherein the bromodomain inhibitor is selected from JQ1, ZEN-3694, I-BET762, OTX015, I-BET151, RVX-208, MS417, ABBV-075, ABBV-744, SJ432, AZD5153, INCB054329, INCB054329, FT-1101, CPI-0610, RO6870810, BAY1238097, RVX000222, and pharmaceutically acceptable salts thereof.

111. The method of claim 110, wherein the bromodomain inhibitor is JQ1.

112. The method of any of claims 1-26, wherein the epigenetic reprogramming agent is a combination of an AKT inhibitor and a TET inhibitor.

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113. The method of claim 112, wherein the AKT inhibitor is selected from the group consisting of ipatasertib, GSK690693, GSK2141795, GSK2110183, AZD5363, GDC-0068, AT7867, CCT128930, MK-2206, BAY 1125976, Perifosine, Oridonin, Herbacetin, Tehranolide, Isoliquiritigenin, Scutellarin, Honokiol, and pharmaceutically acceptable salts thereof.

114. The method of any of claims 26, 55, 70, 84, 97, 104, 109 or 112, wherein the TET inhibitors is selected from the group consisting of C35, Bobcat339, D(R)-2-Hydroxyglutarate (D2HG) and L-2-Hydroxyglutarate (L2HG).

115. The method of claim 113, wherein the AKT inhibitor is ipatasertib.

116. The method of any of claims 1-22, wherein the cell permeating agent comprises an octyl ester or a disodium salt.

117. The method of claim 116, wherein the octyl ester is selected from (2S)-Octyl-a-hydroxyglutarate and (2R)-Octyl-a-hydroxyglutarate.

118. The method of claim 116, wherein the disodium salt is selected from R-2-hydroxyglutaric acid disodium salt and S-2-hydroxyglutaric acid disodium salt.

119. Use according to any of the methods described in any of the preceding claims.

120. Invention of product, process, system, kit or use, characterized by one or more elements disclosed in the application.

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Process 2A: about 22 days from Steps A - E

1. **STEP A**

Obtain Patient Tumor Sample

2. **STEP B**

Fragmentation and First Expansion

3 days to 14 days

3. **STEP C**

First Expansion to Second Expansion Transition

No Storage and Closed System

4. **STEP D**

Second Expansion

IL-2, OKT-3, and antigen-presenting feeder cells

Closed System

5. **STEP E**

Harvest TILS from Step D

Closed System

6. **STEP E**

Final Formulation and/or Transfer to Infusion Bag

(optionally cryopreserve)

Figure 1

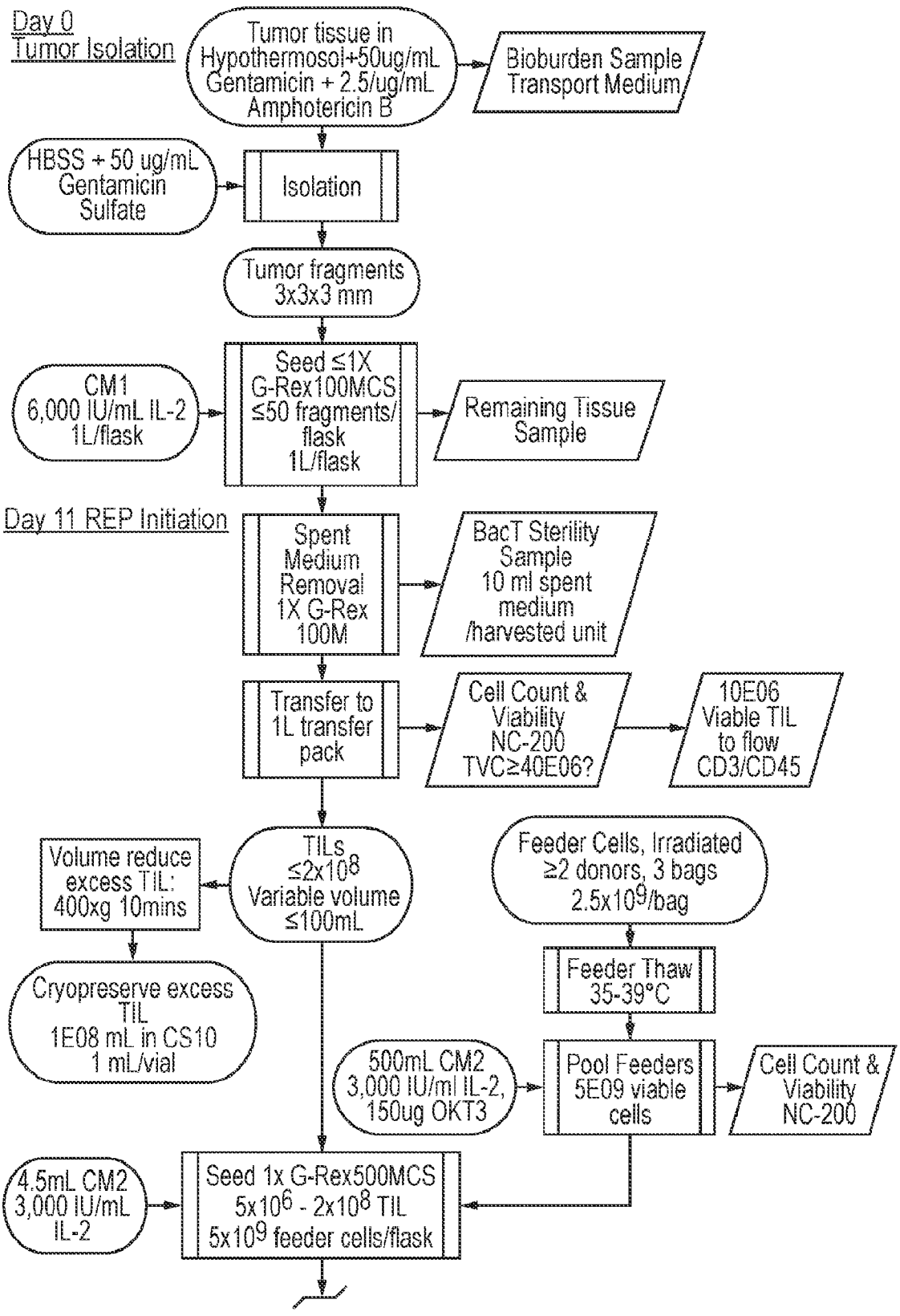


Figure 2B

Figure 2A

Figure 2A

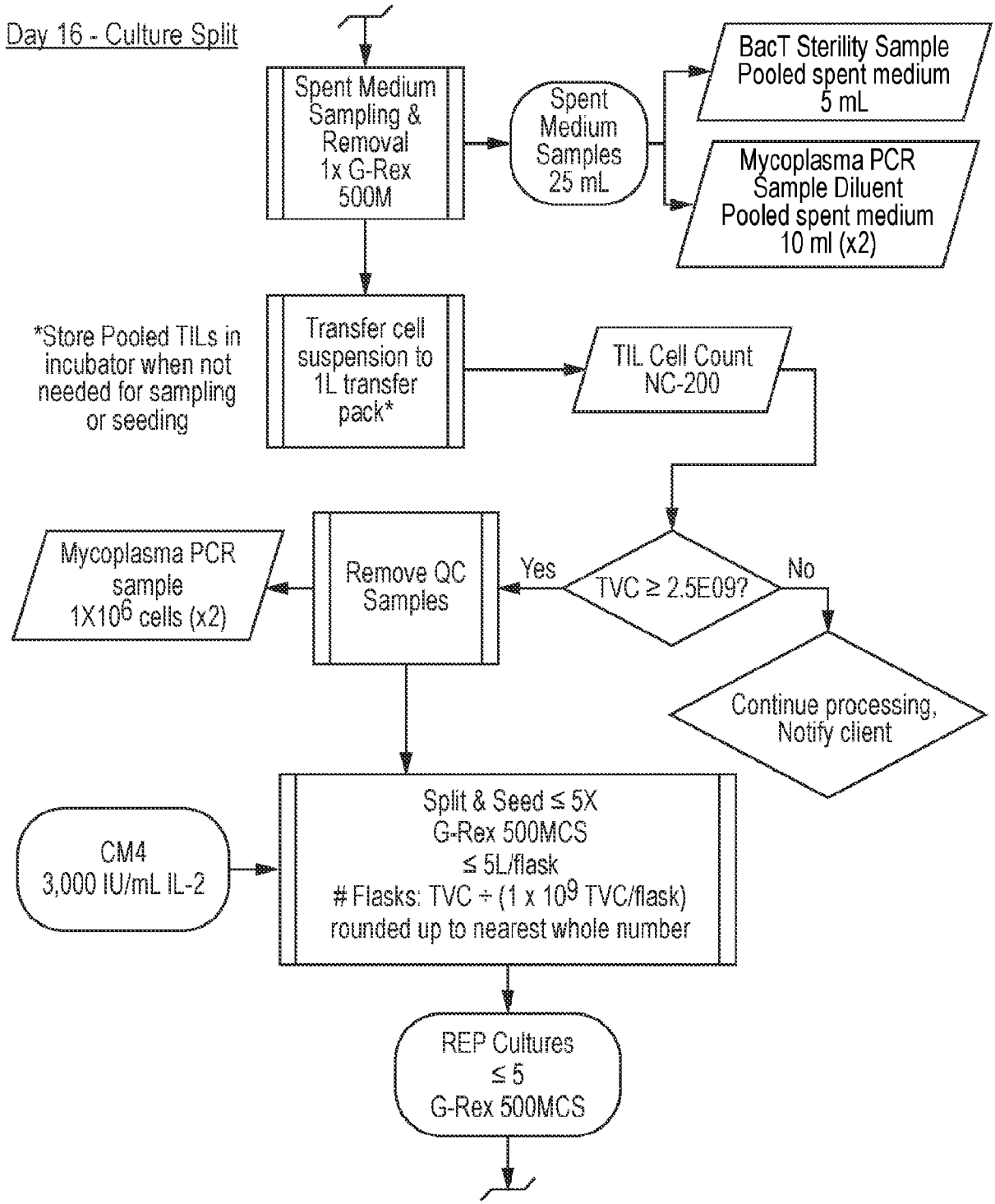


Figure 2C

Figure 2B

Figure 2B

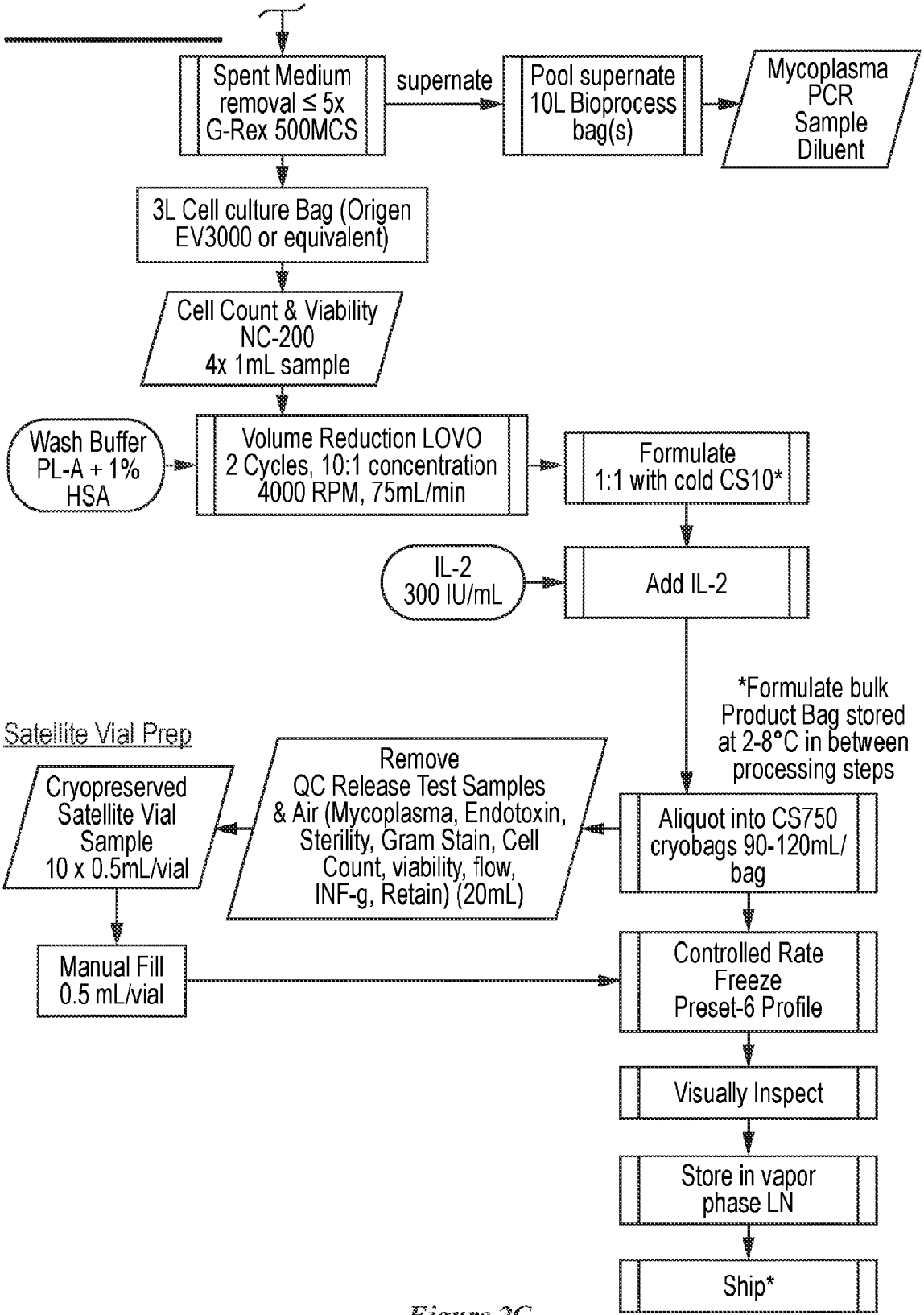


Figure 2C

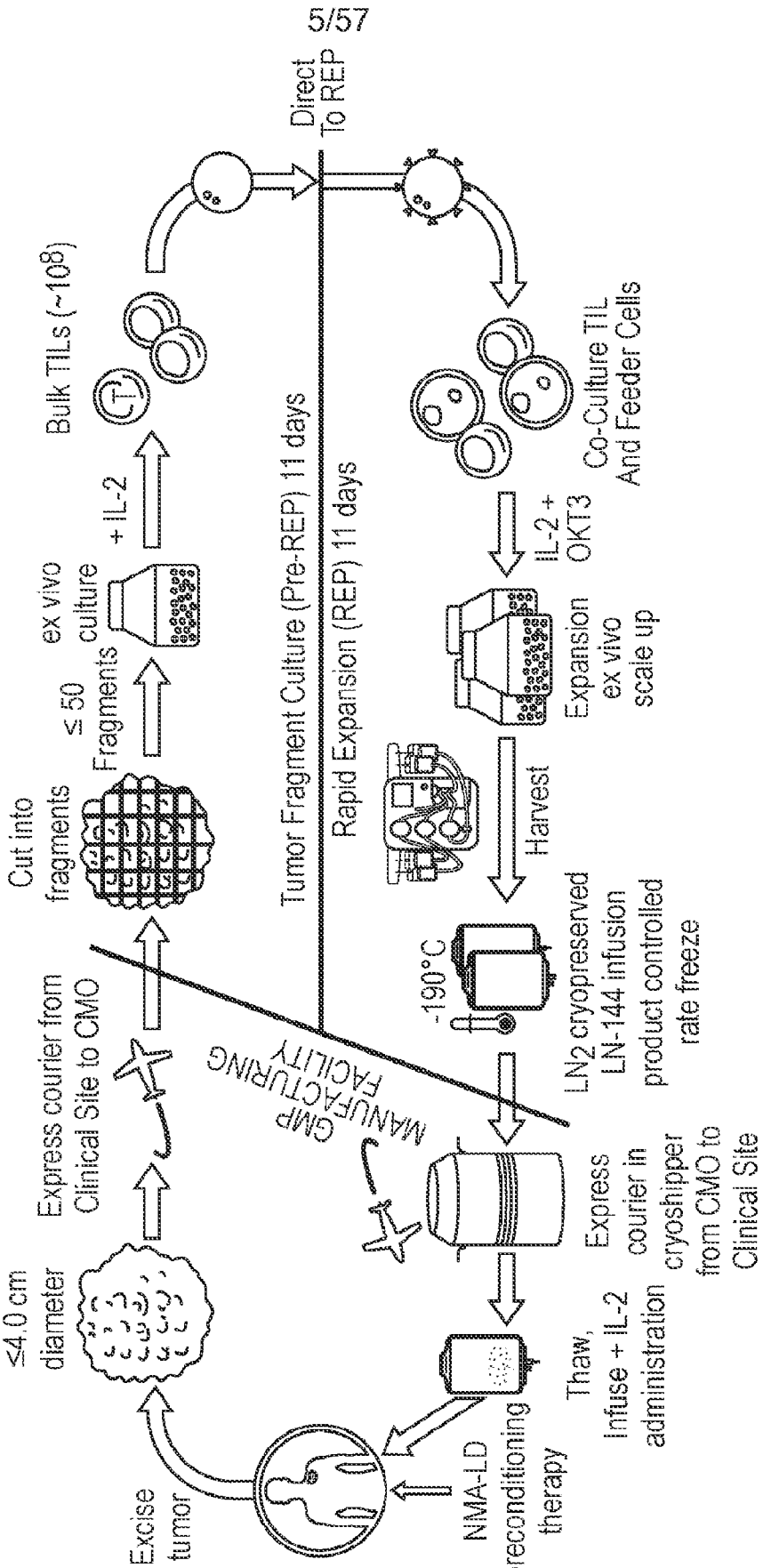

Direct To REP
Bulk TILs (~10^8)
+ IL-2
ex vivo culture
≤ 50 Fragments
Cut into fragments
Tumor Fragment Culture (Pre-REP) 11 days
Rapid Expansion (REP) 11 days
Co-Culture TIL And Feeder Cells
IL-2 + OKT3
Expansion ex vivo scale up
Harvest
-190°C
LN2 cryopreserved LN-144 infusion product controlled rate freeze
Express courier in cryoshipper from CMO to Clinical Site
Express courier from Clinical Site to CMO
MANUFACTURING FACILITY
GMP
≤4.0 cm diameter
Excise tumor
NMA-LD preconditioning therapy
Thaw, Infuse + IL-2 administration

Figure 3

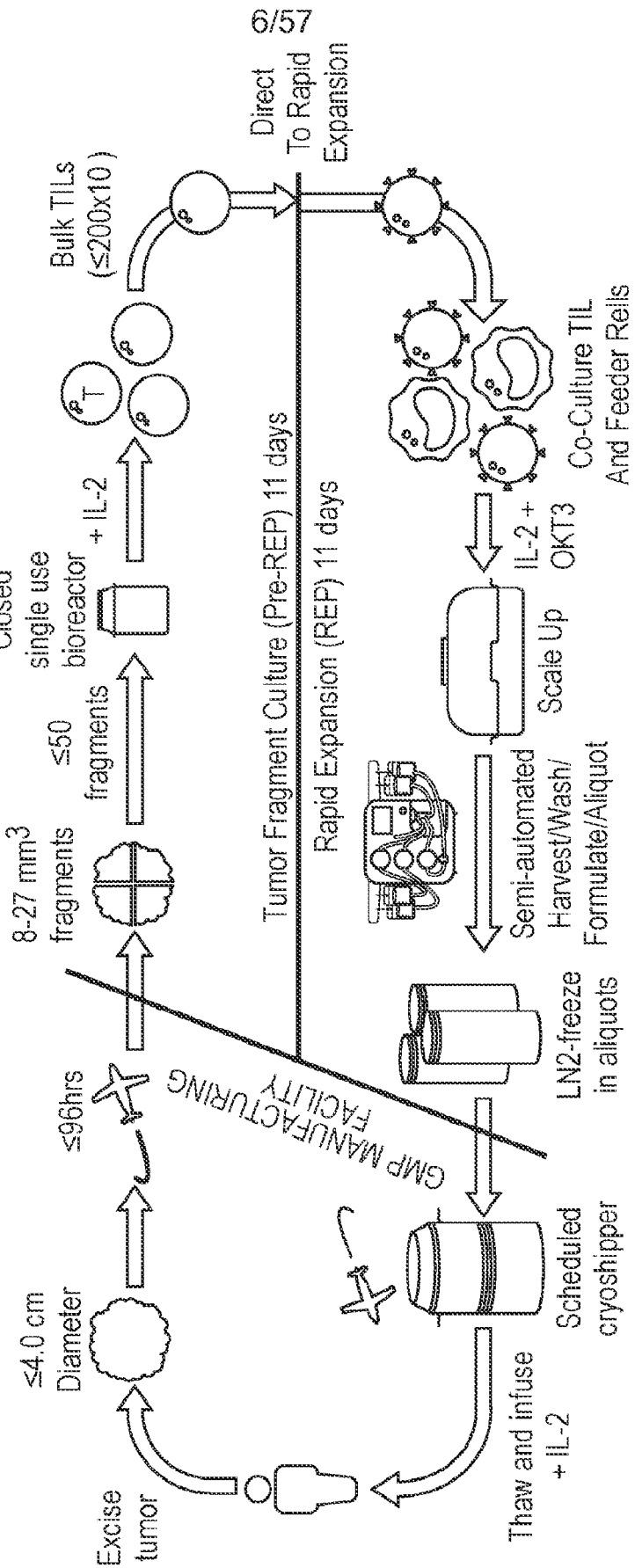

Direct To Rapid Expansion
Bulk TILs (≤200x10)
Closed single use bioreactor + IL-2
≤50 fragments
8-27 mm3 fragments
≤96hrs
≤4.0 cm Diameter
Excise tumor
Tumor Fragment Culture (Pre-REP) 11 days
Rapid Expansion (REP) 11 days
GMP MANUFACTURING FACILITY
Co-Culture TIL And Feeder Rells
IL-2 + OKT3
Scale Up
Semi-automated Harvest/Wash/ Formulate/Aliquot
LN2-freeze in aliquots
Scheduled cryoshipper
Thaw and infuse + IL-2

Figure 4

Process 1C: 43-55 Days for Steps A - E	Process 2A: about 22 days from Steps A - E
1. **STEP A** Obtain Patient Tumor Sample	1. **STEP A** Obtain Patient Tumor Sample
2. **STEP B** Fragmentation and First Expansion 11 days to 21 days	2. **STEP B** Fragmentation and First Expansion 3 days to 14 days
3. **STEP C** First Expansion to Second Expansion Transition Optional Storage until Selection	3. **STEP C** First Expansion to Second Expansion Transition No Storage and Closed System
4. **STEP D** Second Expansion IL-2, OKT-3, antigen-presenting feeder cells Optionally repeat one or more times	4. **STEP D** Second Expansion IL-2, OKT-3, antigen-presenting feeder cells Closed System
5. **STEP E** Harvest TILs from Step D	5. **STEP E** Harvest TILs from Step D Closed System
6. **STEP F** Final Formulation and/or Transfer to Infusion Bag	6. **STEP F** Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)

Figure 5

Process Step	Process 1C Embodiment	Process 2A Embodiment	Advantages
Pre-REP	• 4 fragments per 10 GREX-10 flasks • 11-21 day duration	• 40 fragments per 1 GREX-100M flask • 11 day duration	• Increased tumor fragments per flask • Shortened culture time • Reduced number of steps • Amenable to closed system
Pre-REP to REP Transition	• Pre-REP TIL are frozen until phenotyped for selection then thawed to proceed to the REP (-day 30) • REP requires >40x10^6 TIL	• Pre-REP TIL directly move to REP on day 11 • REP requires 25-200x10^6 TIL	• Shortened pre-REP-to-REP process • Reduced number of steps • Eliminated phenotyping selection • Amenable to closed system
REP	• 6 GREX-100M flasks on REP day 0 • 5x10^6 TIL and 5x10^8 PBMC feeders per flask on REP day 0 • Split to 18-36 flasks on REP day 7 • 14 day duration	• 1 GREX-500M flask on day 11 • 25-200x10^6 TIL and 5x10^9 PBMC feeders on day 11 • Split to ≤ 6 GREX-500M flasks on day 16 • 11 day duration	• Reduced number of steps • Shorter REP duration • Closed system transfer of TIL between flasks • Closed system media exchanges
Harvest	• TIL harvested via centrifugation	• TIL harvested via LOVO automated cell washing system	• Reduced number of steps • Automated cell washing • Closed system • Reduced loss of product during wash
Final Formulation	• Fresh product in Hypothermosol • Single infusion bag • Limited shipping stability	• Cryopreserved product in PlasmaLyte-A + 1% HSA and CS10 stored in LN_2 • Multiple aliquots • Longer shipping stability	• Shipping flexibility • Flexible patient scheduling • More timely release testing
Overall Estimated Process Time	• 43 -55 days	• 22 days	• Faster turnaround to patient

Figure 6

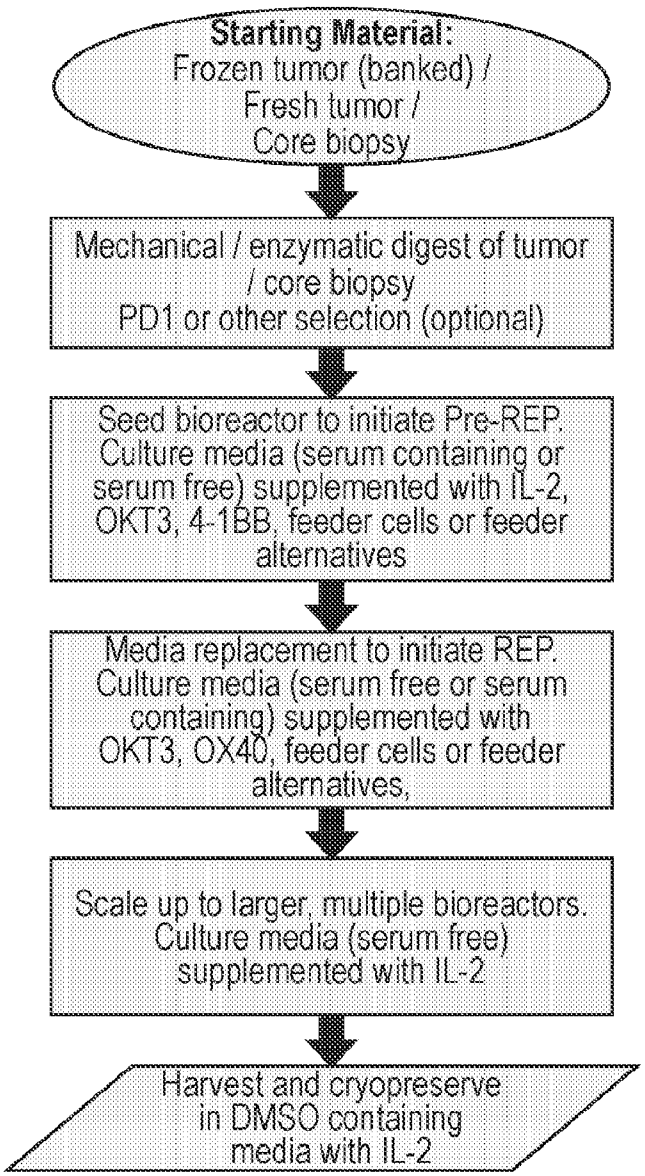

Starting Material:
Frozen tumor (banked) /
Fresh tumor /
Core biopsy
Mechanical / enzymatic digest of tumor / core biopsy
PD1 or other selection (optional)
Seed bioreactor to initiate Pre-REP. Culture media (serum containing or serum free) supplemented with IL-2, OKT3, 4-1BB, feeder cells or feeder alternatives
Media replacement to initiate REP. Culture media (serum free or serum containing) supplemented with OKT3, OX40, feeder cells or feeder alternatives,
Scale up to larger, multiple bioreactors. Culture media (serum free) supplemented with IL-2
Harvest and cryopreserve in DMSO containing media with IL-2
Day 0: Tumor / biopsy prep
Day 0: Pre-REP Initiation
Day 5-8: REP Initiation
Day 9 to 11: REP Scale Up
Day 12 to 16: Harvest

Figure 7

Process 2A/GEN 2: about 22 days from Steps A-E	Process GEN3: 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 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 days with media comprising IL-2, OKT-3, and 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)
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 8A

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 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)

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 8B

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 8C

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 8D

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 days with media comprising IL-2, OKT-3, and antigen-presenting feeder cells)
Optionally adding an epigenetic reprogramming agent

STEP C
Priming First Expansion to Rapid Second Expansion Transition
(Step B TILs directly move to Step D on day 7)

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)
Optionally adding an epigenetic reprogramming agent

STEP E
Harvest TILS from Step D

STEP F
Final Formulation and/or Transfer to Infusion Bag
(optionally cryopreserve)

Figure 8E

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 comprosing IL-2) Optionally adding an epigenetic reprogramming agent	**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) Optionally adding an epigenetic reprogramming agent	**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) Optionally adding an epigenetic reprogramming agent
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) Optionally adding an epigenetic reprogramming agent	**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) Optionally adding an epigenetic reprogramming agent	**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) Optionally adding an epigenetic reprogramming agent
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 8F

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
Optionally adding an epigenetic reprogramming agent

STEP B2

Priming First Expansion
TILs grown for 8 days in growth medium comprising IL-2, OKT-3, and antigen-presenting feeder cells)
Optionally adding an epigenetic reprogramming agent

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)
Optionally adding an epigenetic reprogramming agent

STEP E

Harvest TILS from Step D

STEP F

Final Formulation and/or Transfer to Infusion Bag
(optionally cryopreserve)

Figure 8G

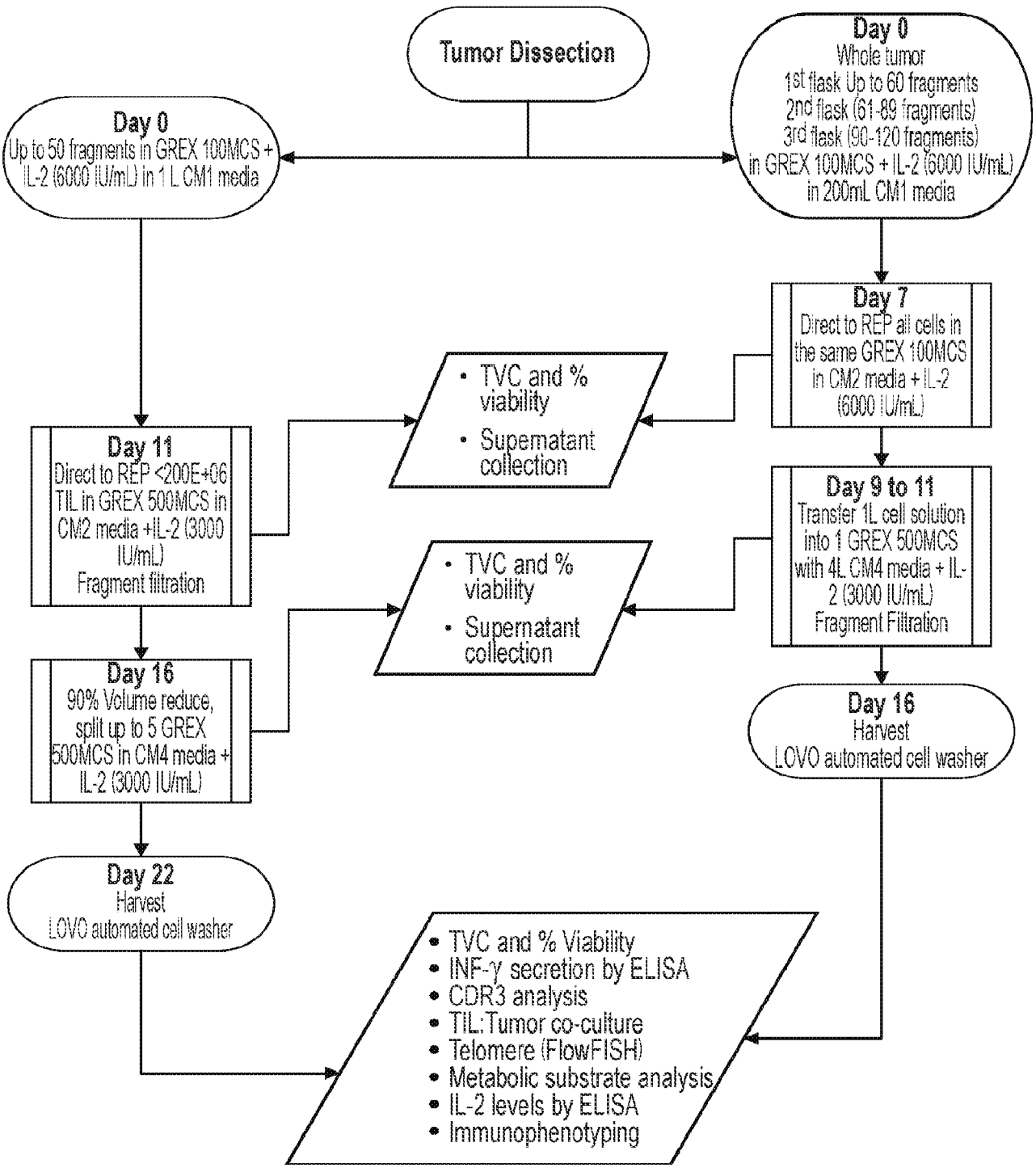

Tumor Dissection
Day 0
Whole tumor
1st flask Up to 60 fragments
2nd flask (61-89 fragments)
3rd flask (90-120 fragments)
in GREX 100MCS + IL-2 (6000 IU/mL)
in 200mL CM1 media
Day 0
Up to 50 fragments in GREX 100MCS + IL-2 (6000 IU/mL) in 1 L CM1 media
Day 7
Direct to REP all cells in the same GREX 100MCS in CM2 media + IL-2 (6000 IU/mL)
• TVC and % viability
• Supernatant collection
Day 11
Direct to REP <200E+06 TIL in GREX 500MCS in CM2 media +IL-2 (3000 IU/mL)
Fragment filtration
Day 9 to 11
Transfer 1L cell solution into 1 GREX 500MCS with 4L CM4 media + IL-2 (3000 IU/mL)
Fragment Filtration
• TVC and % viability
• Supernatant collection
Day 16
90% Volume reduce, split up to 5 GREX 500MCS in 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 9

Gen 2 Process
Gen 2.1 Process
Gen 3.1 Process
Day 0:Pre-REP
≤ 50 fragments in 1 GREX-100MCS with 1L of CM1+6,000 IU/mL IL-2
Day 11: REP-initiation
Direct to REP <200e6 TIL 1 GREX 500M in CM2+3,000 IU/mL IL-2 5E9 feeder cells 30 ng/mL OKT3 (150ul)
Day 16: TIL-Scale Up
Volume reduce and split up on 2 up to 5 GREX 500M AIMV+3,000 IU/mL of IL-2
Day 22: Harvest
Harvest-LOVO automated cell washer
≤ 120 fragments in 3 GREX-100MCS with 1L of CM1+6,000 IU/mL IL-2
Direct to REP <200e6 TIL 2 one GREX 500M in CM2+3,000 IU/mL IL-2 5E9 feeder cells 30 ng/mL OKT3 (150ul)
Volume reduce and split up on 2 up to 5 GREX 500MCS AIMV+3,000 IU/mL of IL-2
Harvest-LOVO automated cell washer
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 (30ng/mL) +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 10

Step	Gen 2	Gen 2.1	Gen 3.1 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 11

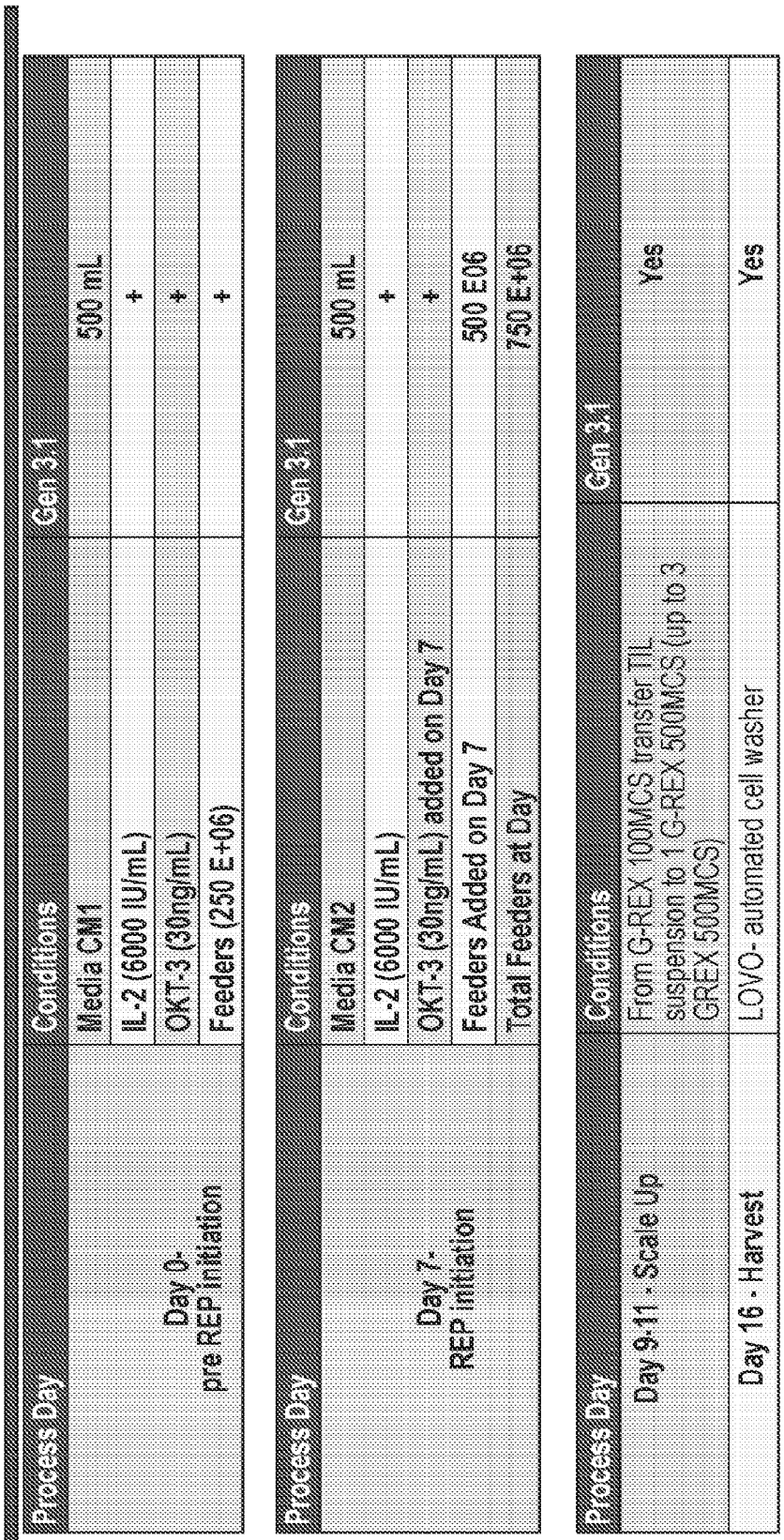

Process Day
Conditions
Gen 3.1
Day 0-
pre REP initiation
Media CM1
500 mL
IL-2 (6000 IU/mL)
+
OKT-3 (30ng/mL)
+
Feeders (250 E+06)
+
Process Day
Conditions
Gen 3.1
Day 7-
REP initiation
Media CM2
500 mL
IL-2 (6000 IU/mL)
+
OKT-3 (30ng/mL) added on Day 7
+
Feeders Added on Day 7
500 E06
Total Feeders 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 12

Process Comparison	Key Process Changes	Benefit
Gen 2 : Gen 2.1	• Initiate process with two flasks instead of one flask • Divide REP initiation feeder layer between 2 G-Rex500MCS Flasks • Pre-formulate media and warm prior to use	• Potential doubling of final cell count (dose) with increased TIL repertoire. • Process redundancy throughout process
Gen 2.1 : Gen 3.1	• Fresh or Frozen tumor • 14-16 day process (from 22 day) • Reduce total feeder layer on process • Feeder layer and OKT3 present at Day 0 • REP initiated with fragments • 100MCS scales to 500MCS • Scales to multiple pre-REP flasks • Standard Media and Defined Media (Serum Free)	• Increased potency • Improved phenotype • Decreased process time • Reduced reagent testing • Decreased process variability • Defined reagents • Increased repertoire • Reduce impurities (feeder) • Comparable or Higher Dose.

Figure 13

Process Comparison	Key Process Changes	Desired Improvement	Criteria for Success	Outcome
Gen 2 : Gen 3.0	• 14-16 days • Initiate REP with fragments up to 4 flask. • 100MCS scales to 500MCS	• Increased potency • Improved phenotype • Decreased process time	• Increase potency as measured by INF-g ✓ • Comparable phenotype ✓ • Comparable Dose ✓ • Comparable purity ✓ (feeder cell) • Maintain clonal diversity ✓	• Potency increased over Gen2 • Improved expression of CD28 on CD8 cells • Maximum capacity of flask reached by day 16 on Gen 3.1 • Reduced feeder cell usage • Increased diversity

Figure 14

Process	Gen 2	Gen 3
L4054	Standard Media	Standard Media
L4055	Standard Media	Standard Media
M1085T	Standard Media	Standard Media

Standard Media:
Pre REP: CM1
REP initiation : CM2
Split or Scale up : CM4

Process	Gen 3	Gen 3.1 control	Gen 3.1
L4063	Standard Media	Standard Media	Standard Media
L4064	Defined Media	Defined Media	Defined Media

Defined Media:
CTS Optimizer (Serum Free Media) in each day of the process

Figure 15

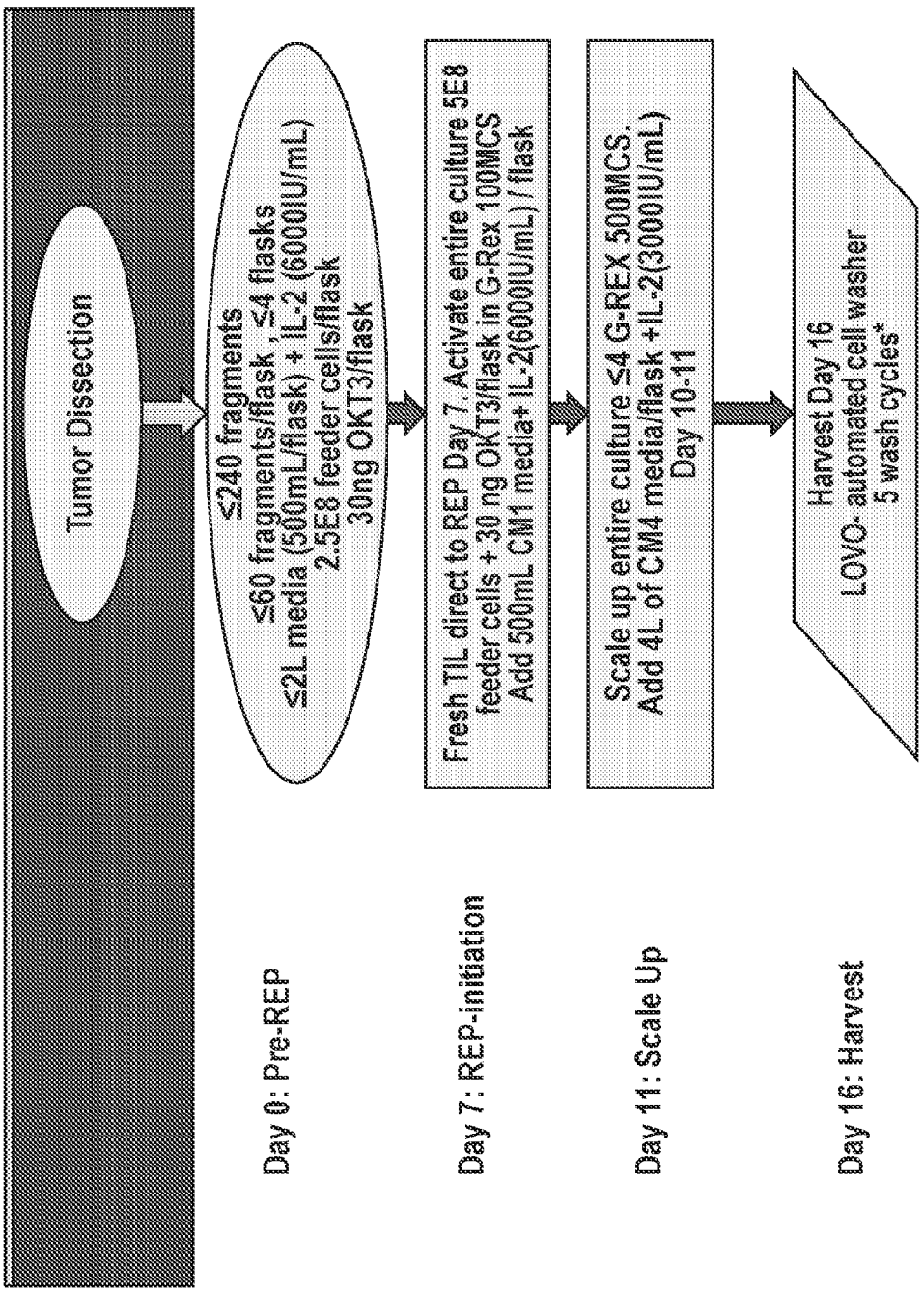

Tumor Dissection
Day 0: Pre-REP
≤240 fragments
≤60 fragments/flask , ≤4 flasks
≤2L media (500mL/flask) + IL-2 (6000IU/mL)
2.5E8 feeder cells/flask
30ng OKT3/flask
Day 7: REP-initiation
Fresh TIL direct to REP Day 7, Activate entire culture 5E8
feeder cells + 30 ng OKT3/flask in G-Rex 100MCS
Add 500mL CM1 media+ IL-2(6000IU/mL) / flask
Day 11: Scale Up
Scale up entire culture ≤4 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 16

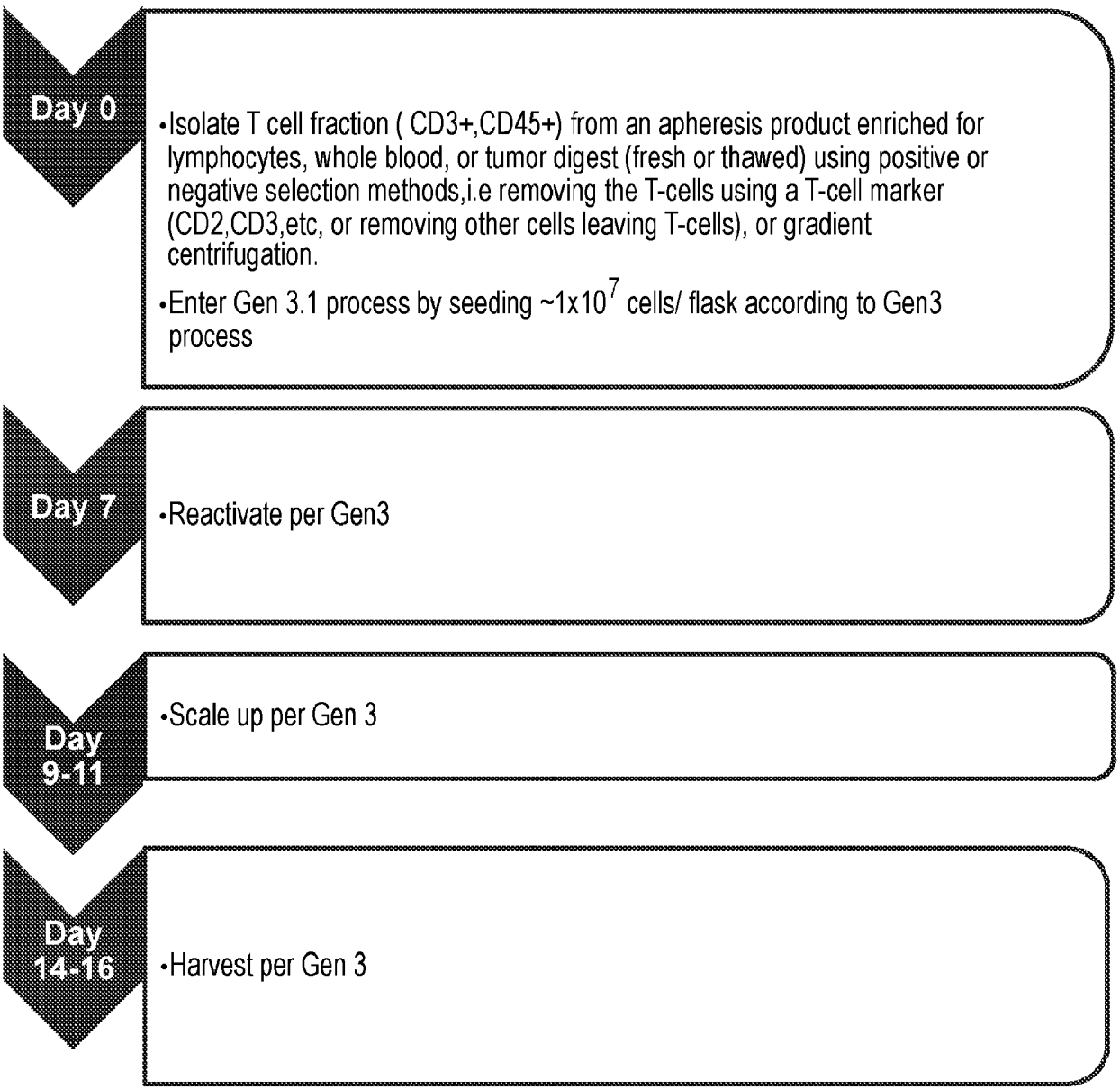

Day 0
•Isolate T cell fraction (CD3+,CD45+) from an apheresis product enriched for lymphocytes, whole blood, or tumor digest (fresh or thawed) using positive or negative selection methods,i.e removing the T-cells using a T-cell marker (CD2,CD3,etc, or removing other cells leaving T-cells), or gradient 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 17

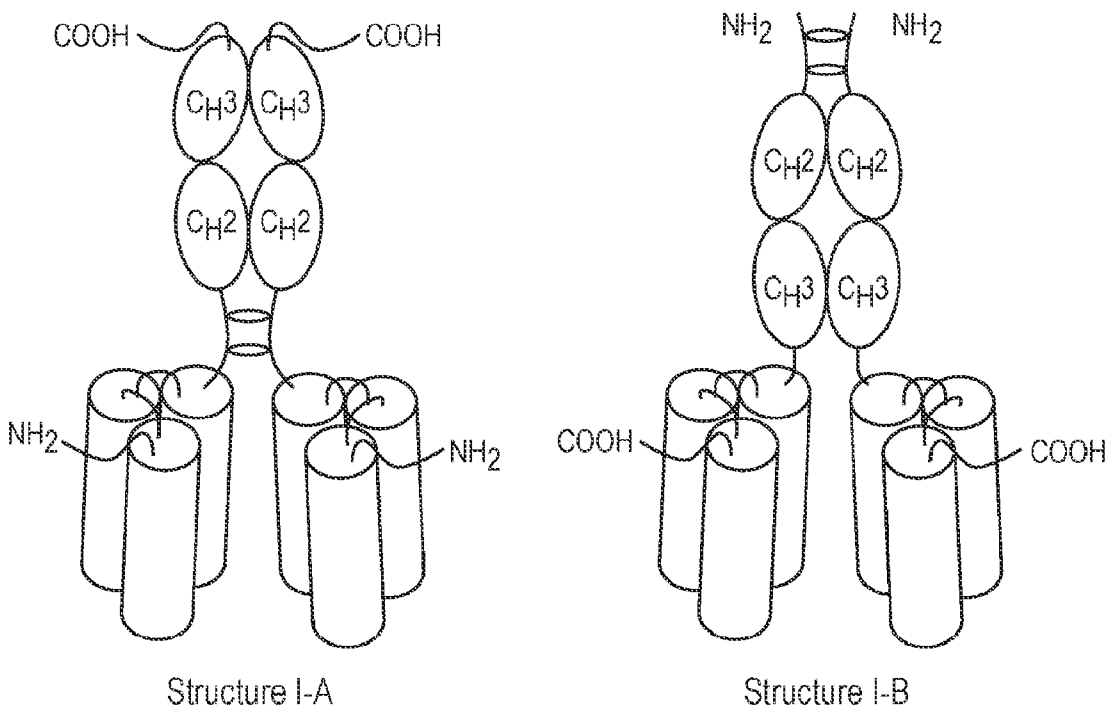

COOH
COOH
CH3
CH3
CH2
CH2
NH2
NH2
Structure I-A
NH2
NH2
CH2
CH2
CH3
CH3
COOH
COOH
Structure I-B

Figure 18

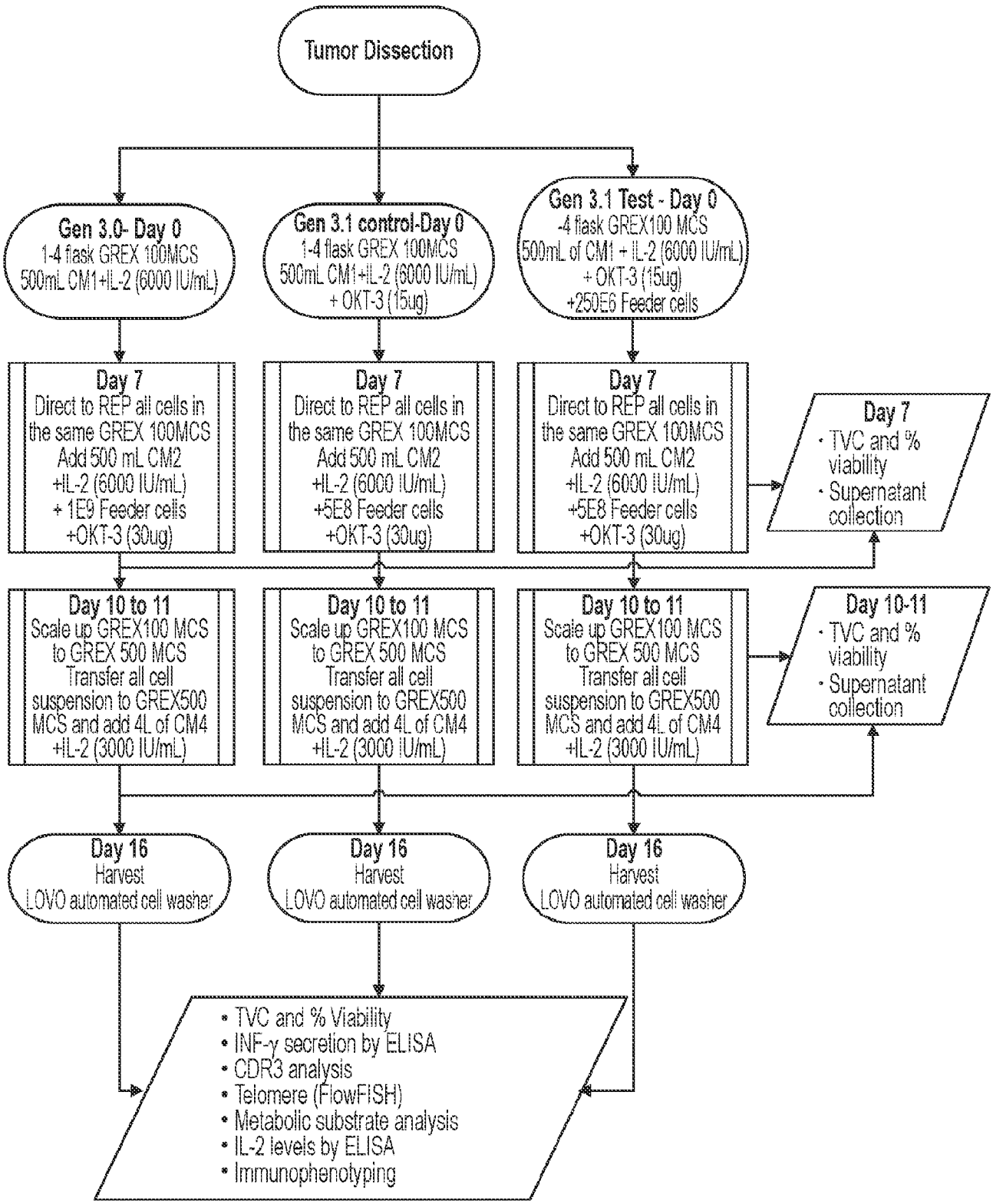

Tumor Dissection
Gen 3.0- Day 0
1-4 flask GREX 100MCS
500mL CM1+IL-2 (6000 IU/mL)
Gen 3.1 control-Day 0
1-4 flask GREX 100MCS
500mL CM1+IL-2 (6000 IU/mL)
+ OKT-3 (15ug)
Gen 3.1 Test - Day 0
-4 flask GREX100 MCS
500mL of CM1 + IL-2 (6000 IU/mL)
+ OKT-3 (15ug)
+250E6 Feeder cells
Day 7
Direct to REP all 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 19

Process Day	Conditions	Gen 3.1 Test
Day 0- pre REP initiation	Media CM1	500 mL
	IL-2 (6000 IU/mL)	+
	OKT-3 (15ug)	+
	Feeders (250 E+06)	+

Process Day	Conditions	Gen 3.1 Test
Day 7- REP initiation	Media CM2	500 mL
	IL-2 (6000 IU/mL)	+
	OKT-3 (30ug) added on Day 7	+
	Feeders Added on Day 7	500 E06
	Total Feeders at Day	750 E+06

Process Day	Conditions	Gen 3.1 Test
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 20

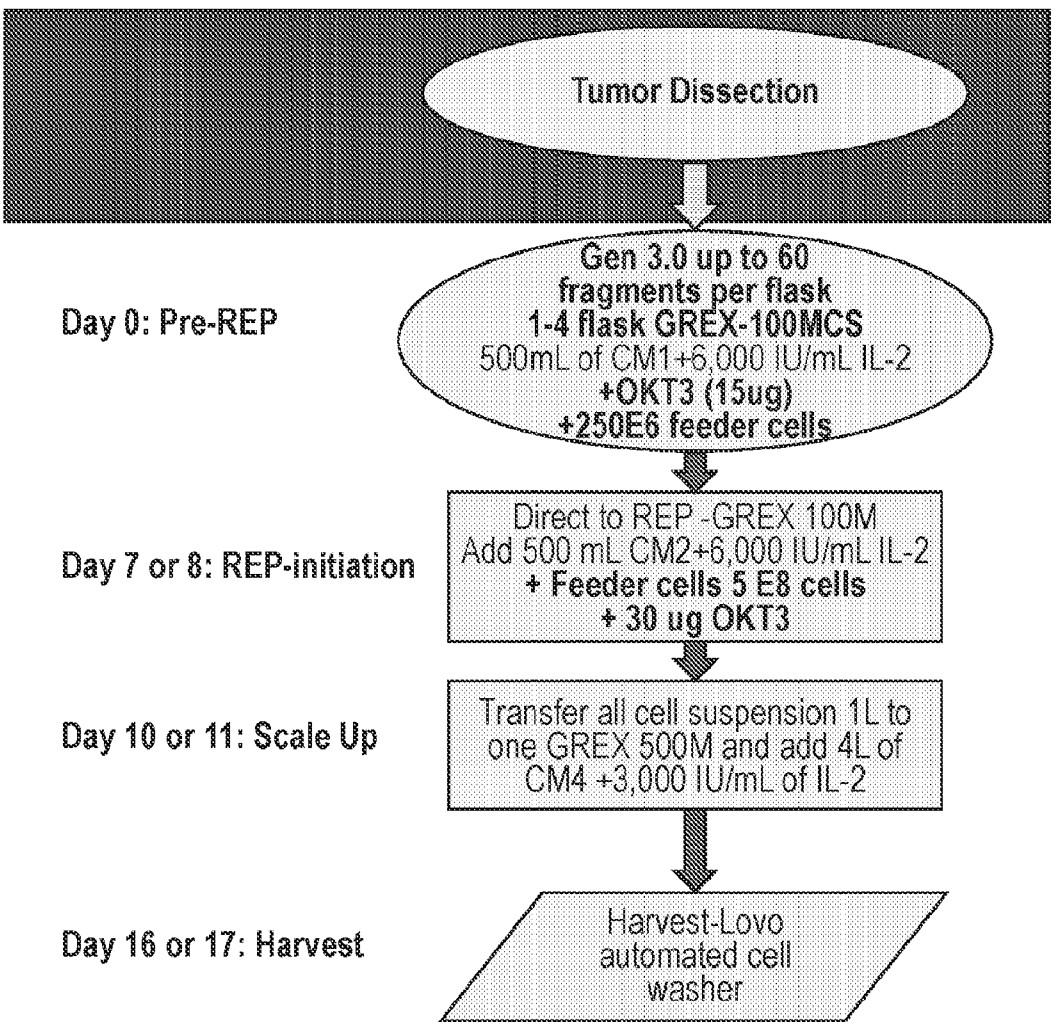

Tumor Dissection
Day 0: Pre-REP
Gen 3.0 up to 60 fragments per flask
1-4 flask GREX-100MCS
500mL of CM1+6,000 IU/mL IL-2
+OKT3 (15ug)
+250E6 feeder cells
Day 7 or 8: REP-initiation
Direct to REP -GREX 100M
Add 500 mL CM2+6,000 IU/mL IL-2
+ Feeder cells 5 E8 cells
+ 30 ug OKT3
Day 10 or 11: Scale Up
Transfer all cell suspension 1L to one GREX 500M and add 4L of CM4 +3,000 IU/mL of IL-2
Day 16 or 17: Harvest
Harvest-Lovo automated cell washer

Figure 21

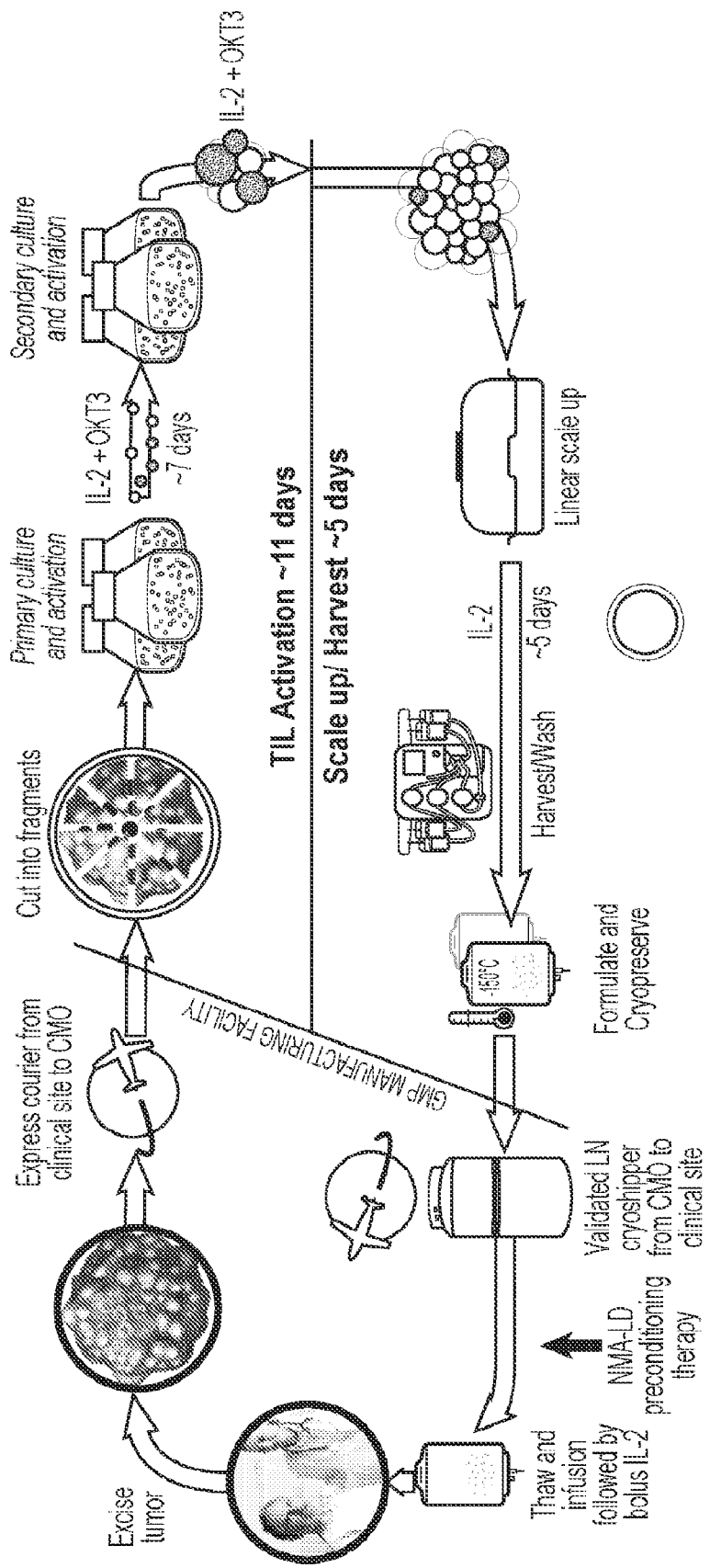

Excise tumor
Thaw and infusion followed by bolus IL-2
NMA-LD preconditioning therapy
Validated LN cryoshipper from CMO to clinical site
Express courier from clinical site to CMO
GMP MANUFACTURING FACILITY
Cut into fragments
Primary culture and activation
IL-2 + OKT3
~7 days
Secondary culture and activation
IL-2 + OKT3
TIL Activation ~11 days
Scale up/ Harvest ~5 days
Linear scale up
IL-2
~5 days
Harvest/Wash
Formulate and Cryopreserve

Figure 22

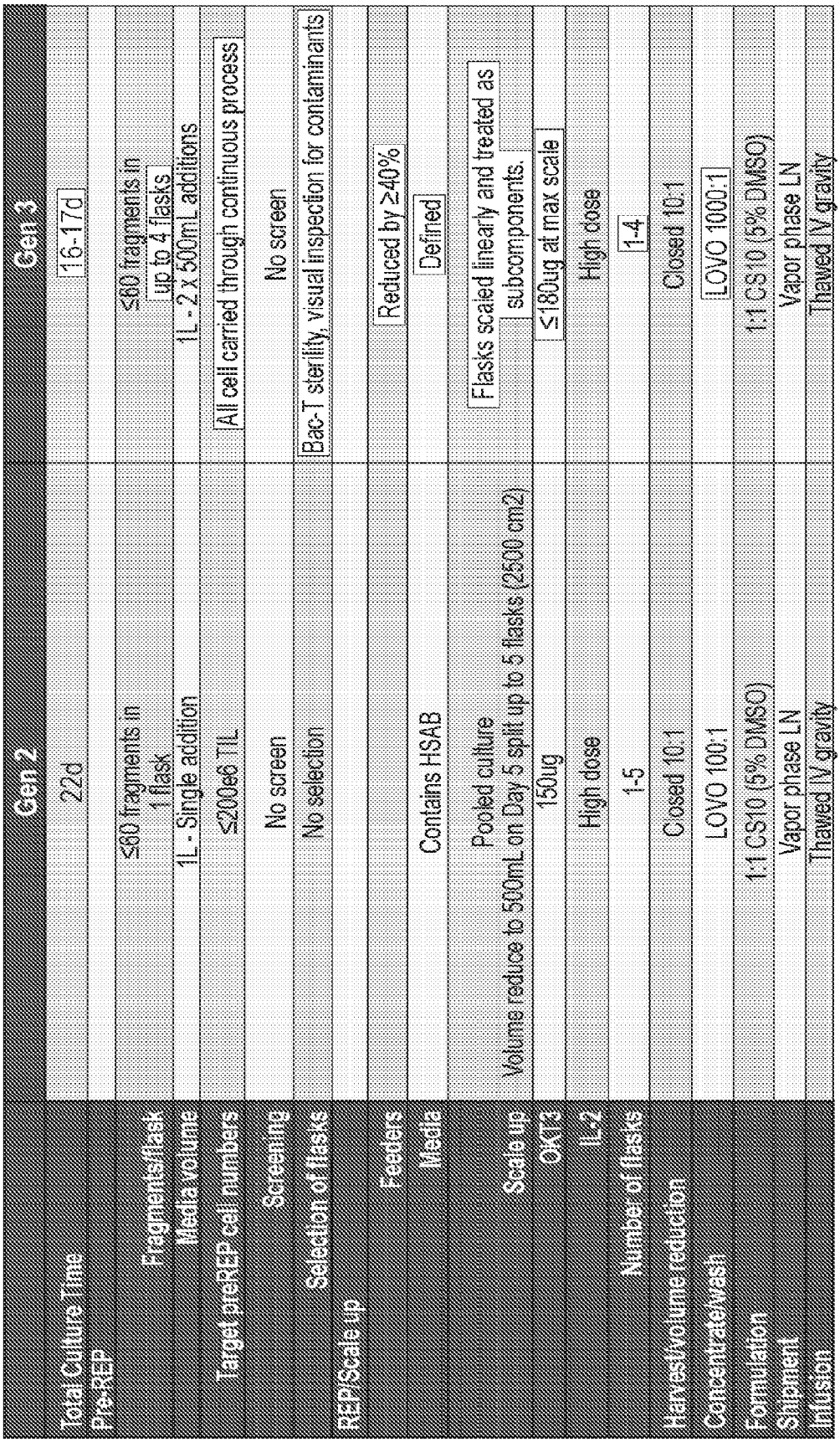

Gen 2
Gen 3
Total Culture Time
22d
16-17d
Pre-REP
Fragments/flask
≤60 fragments in 1 flask
≤60 fragments in up to 4 flasks
Media volume
1L - Single addition
1L - 2 x 500mL additions
Target pre-REP cell numbers
≤200e6 TIL
All cell carried through continuous process
Screening
No screen
No screen
Selection of flasks
No selection
Bac-T sterility, visual inspection for contaminants
REP/Scale up
Feeders
Reduced by ≥40%
Media
Contains HSAB
Defined
Scale up
Pooled culture Volume reduce to 500mL on Day 5 split up to 5 flasks (2500 cm2)
Flasks scaled linearly and treated as subcomponents.
GVT3
150ug
≤180ug at max scale
IL-2
High dose
High dose
Number of flasks
1-5
1-4
Harvest/volume reduction
Closed 10:1
Closed 10:1
Concentrate/wash
LOVO 100:1
LOVO 1000:1
Formulation
1:1 CS10 (5% DMSO)
1:1 CS10 (5% DMSO)
Shipment
Vapor phase LN
Vapor phase LN
Infusion
Thawed IV gravity
Thawed IV gravity

Figure 23A

	Gen 2	Gen 3
Total Culture Time	22d	16-17d
Pre-REP		
Fragments/flask	≤60 fragments in 1 flask	≤60 fragments in up to 4 flasks
Media volume	1L - Single addition	1L - 2 x 500mL additions
Target preREP cell numbers	≤200e6 TIL	All cell carried through continuous process
Screening	No screen	No screen
Selection of flasks	No selection	Bac-T sterility, visual inspection for contaminants
REP/Scale Up		
Feeders		Reduced by ≥40%
Media	Contains HSAB	Defined
Scale Up	Pooled culture Volume reduce to 500mL on Day 5 split up to 5 flasks (2500 cm2)	Flasks scaled linearly and treated as subcomponents.
OKT3	150ug	≤180ug at max scale
IL-2	High dose	High dose
Number of flasks	1-5	1-4
Harvest/volume reduction	Closed 10:1	Closed 10:1
Concentrate/wash	LOVO 100:1	LOVO 1000:1
Formulation	1:1 CS10 (5% DMSO)	1:1 CS10 (5% DMSO)
Shipment	Vapor phase LN	Vapor phase LN
Infusion	Thawed IV gravity	Thawed IV gravity

Figure 23B

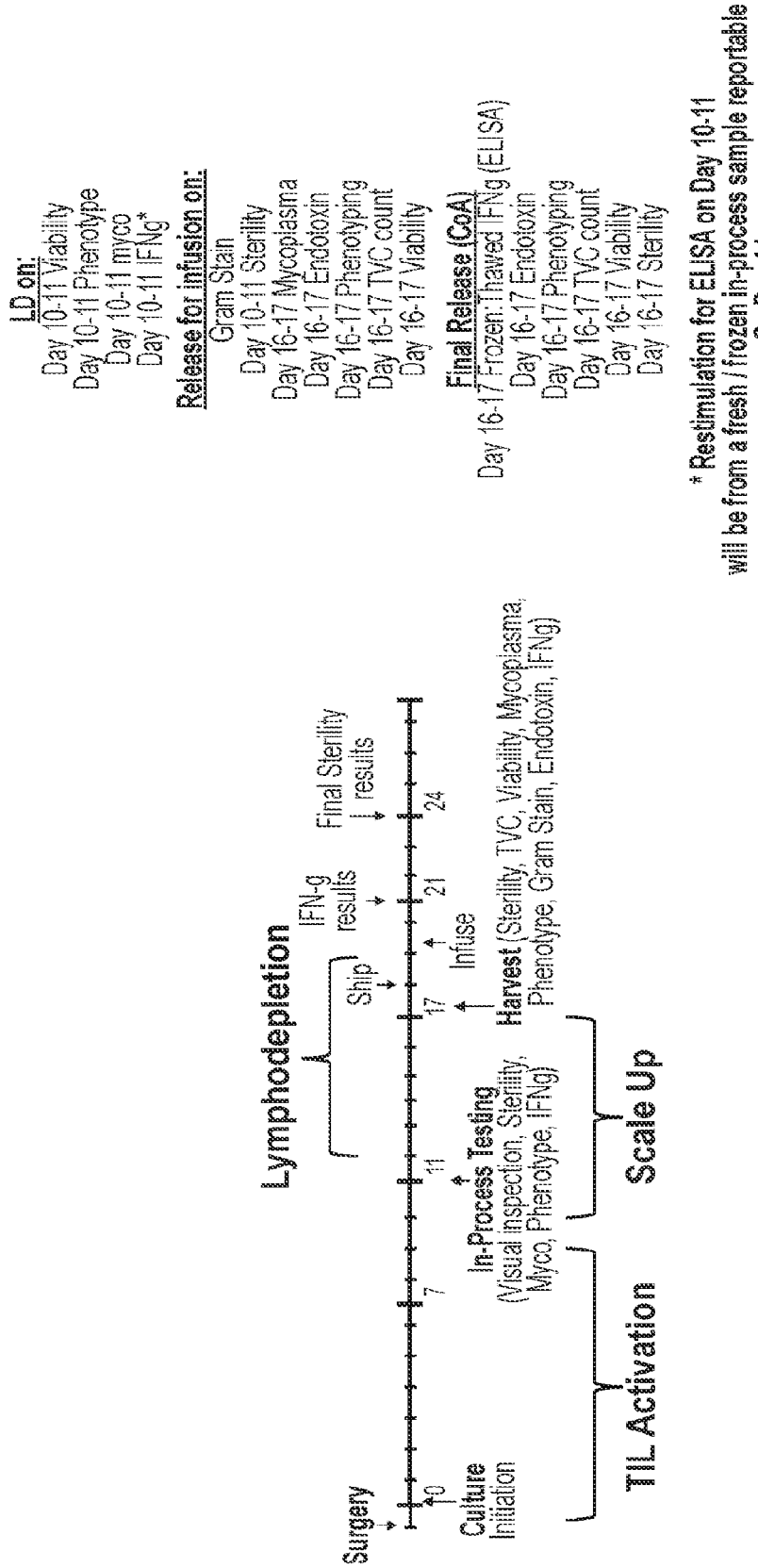

LD on:
Day 10-11 Viability
Day 10-11 Phenotype
Day 10-11 myco
Day 10-11 IFNg*
Release for infusion on:
Gram Stain
Day 10-11 Sterility
Day 16-17 Mycoplasma
Day 16-17 Endotoxin
Day 16-17 Phenotyping
Day 16-17 TVC count*
Day 16-17 Viability
Final Release (CoA)
Day 16-17 Frozen / thawed IFNg (ELISA)
Day 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
Lymphodepletion
IFN-g results
Final Sterility results
Ship
Infuse
Surgery
Culture Initiation
In-Process Testing
(Visual inspection, Sterility, Myco, Phenotype, IFNg)
Harvest (Sterility, TVC, Viability, Mycoplasma, Phenotype, Gram Stain, Endotoxin, IFNg)
0
7
11
17
21
24
TIL Activation
Scale Up

Figure 24

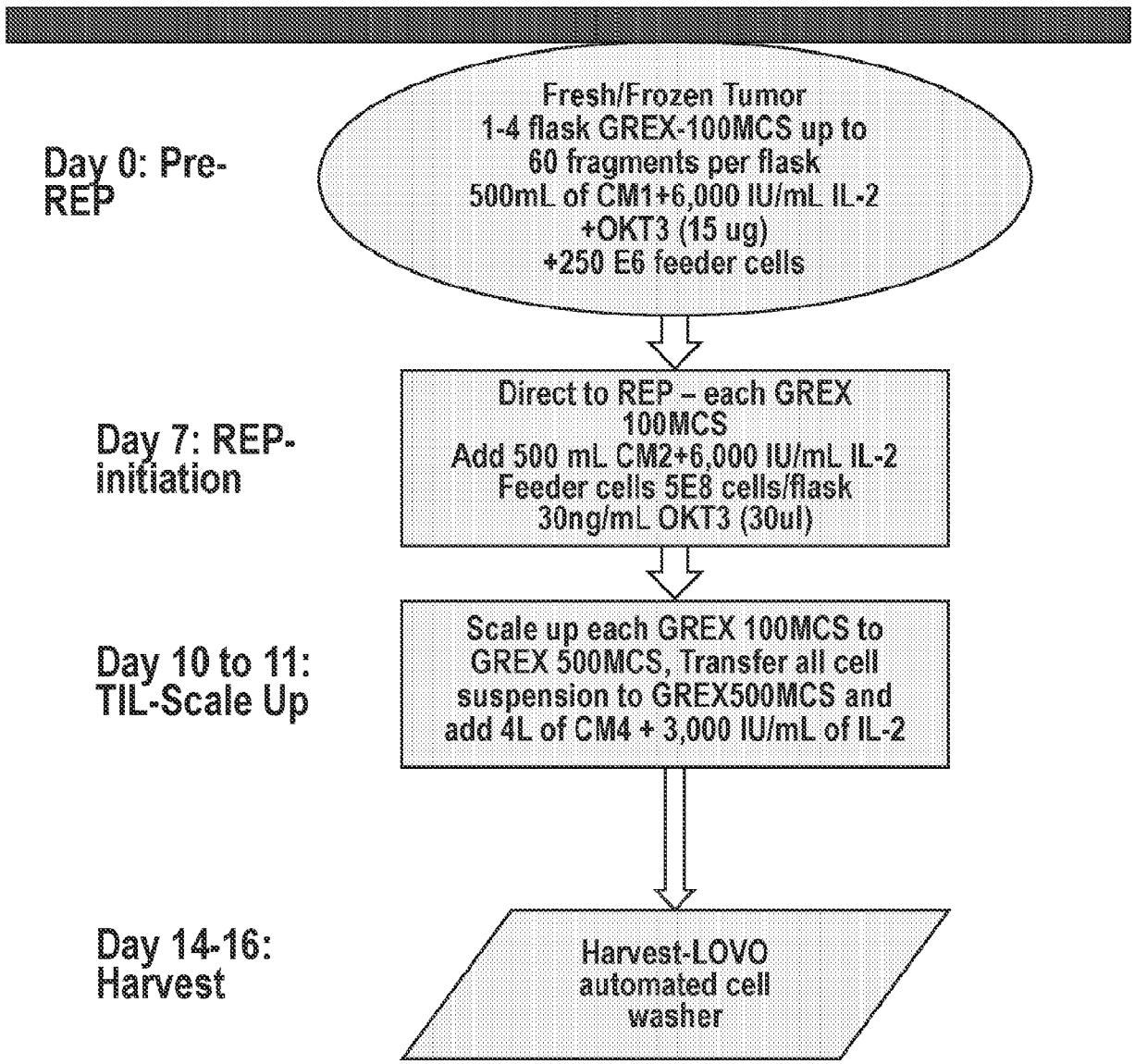

Day 0: Pre-REP
Fresh/Frozen Tumor
1-4 flask GREX-100MCS up to
60 fragments per flask
500mL of CM1+6,000 IU/mL IL-2
+OKT3 (15 ug)
+250 E6 feeder cells
Day 7: REP-initiation
Direct to REP – each GREX 100MCS
Add 500 mL CM2+6,000 IU/mL IL-2
Feeder cells 5E8 cells/flask
30ng/mL OKT3 (30ul)
Day 10 to 11: TIL-Scale 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 25

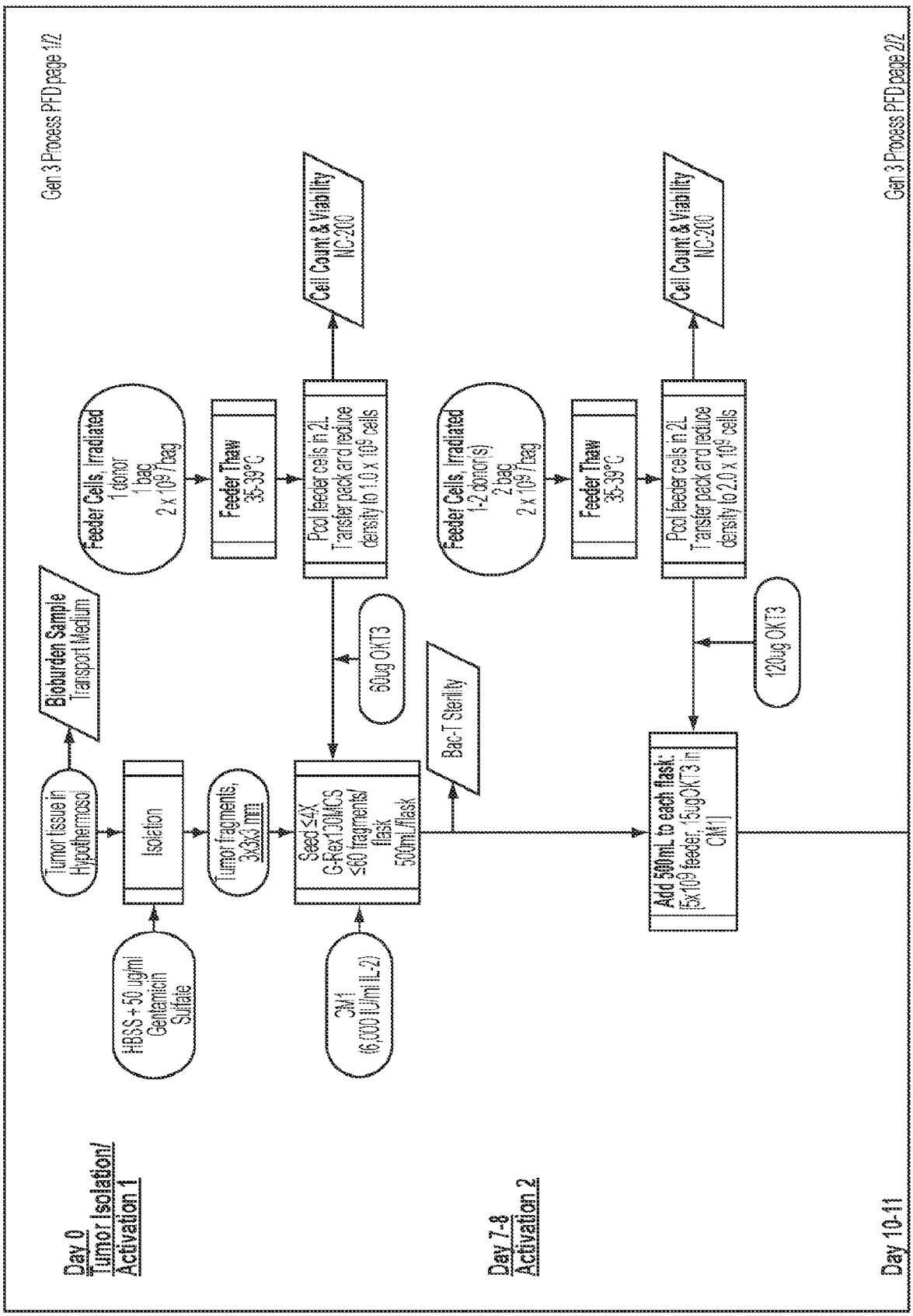

Gen 3 Process PFD page 1/2
Day 0
Tumor Isolation/
Activation 1
Tumor Tissue in Hypothermosol
Bioburden Sample
Transport Medium
Isolation
HBSS + 50 µg/ml Gentamicin Sulfate
Tumor fragments, 3x3x3 mm
Seed <4X
G-Rex100MCS
<60 fragments/
flask
500ml/flask
CM1
(6,000 IU/ml IL-2)
60ug OKT3
Bac-T Sterility
Feeder Cells, Irradiated
1 donor
1 bag
2 x 10^9 /bag
Feeder Thaw
35-39°C
Pool feeder cells in 2L
Transfer pack and reduce
density to 1.0 x 10^6 cells
Cell Count & Viability
NC-200
Day 7-8
Activation 2
Add 500ml to each flask:
5x10^6 feeder, 15ug OKT3 in
CM1
Feeder Cells, Irradiated
1-2 donor(s)
2 bag
2 x 10^9 /bag
Feeder Thaw
35-39°C
Pool feeder cells in 2L
Transfer pack and reduce
density to 2.0 x 10^6 cells
Cell Count & Viability
NC-200
120ug OKT3
Day 10-11
Gen 3 Process PFD page 2/2

Figure 26A

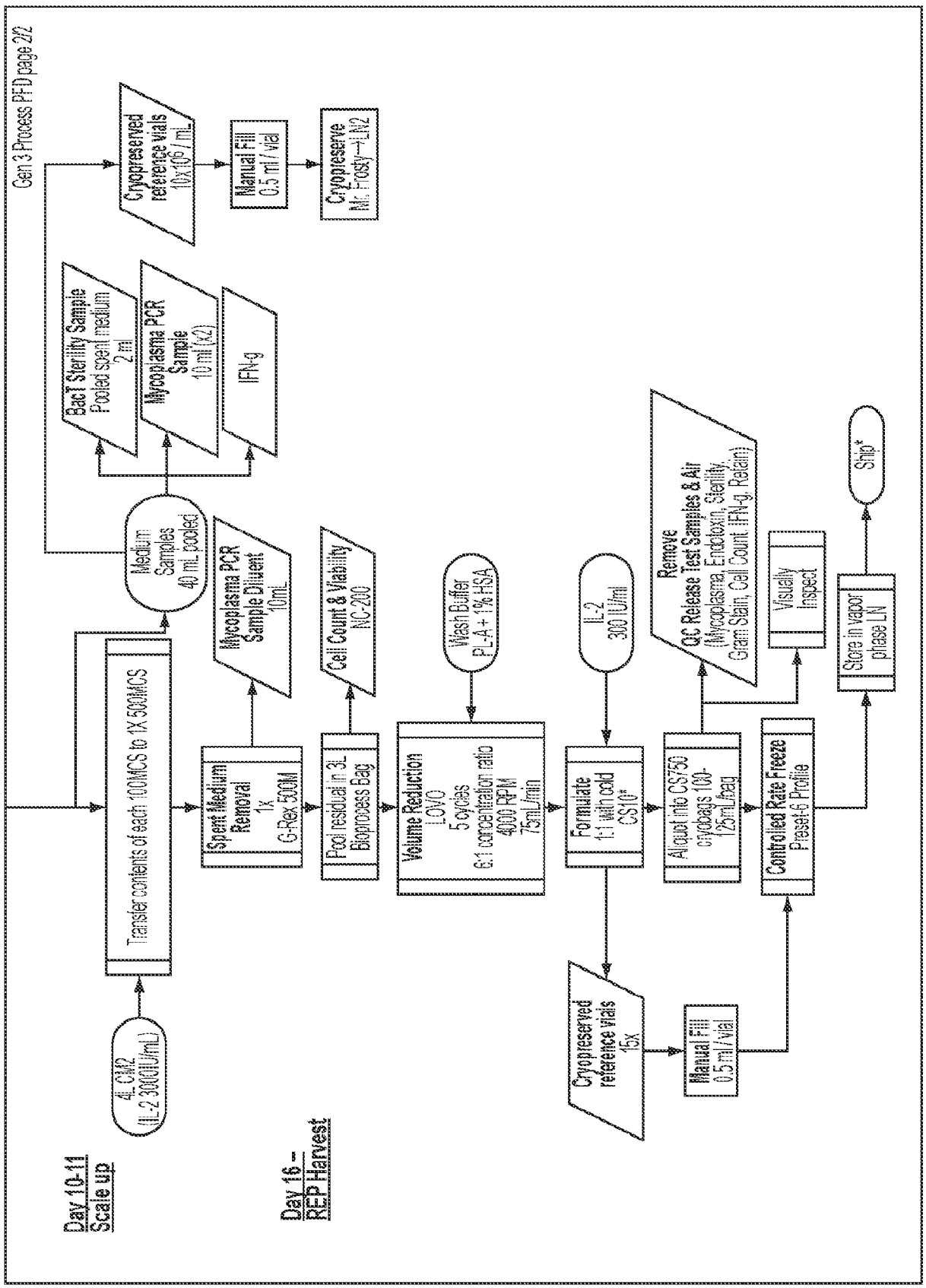

Gen 3 Process PFD page 2/2
Day 10-11
Scale up
Day 16 –
REP Harvest
4L CM2
(IL-2 3000IU/mL)
Transfer contents of each 100MCS to 1X 500MCS
Medium
Samples
40 mL pooled
Bact Sterility Sample
Pooled spent medium
2 ml
Mycoplasma PCR
Sample
10 ml (x2)
IFN-g
Cryopreserved
reference vials
10x1E6 / mL
Manual Fill
0.5 ml / vial
Cryopreserve
Mr. Frosty→LN2
Spent Medium
Removal
1x
G-Rex 500M
Mycoplasma PCR
Sample Diluent
10mL
Pool residual in 3L
Bioprocess Bag
Cell Count & Viability
NC-200
Volume Reduction
LOVO
5 cycles
6:1 concentration ratio
4000 RPM
75mL/min
Wash Buffer
PL-A + 1% HSA
Formulate
1:1 with cold
CS10*
IL-2
300 IU/ml
Cryopreserved
reference vials
15x
Manual Fill
0.5 ml / vial
Aliquot into CS750
cryobags 100-
125mL/bag
Remove
QC Release Test Samples & Air
(Mycoplasma, Endotoxin, Sterility,
Gram Stain, Cell Count, IFN-g, Retain)
Visually
Inspect
Controlled Rate Freeze
Preset-6 Profile
Store in vapor
phase LN
Ship*

Figure 26B

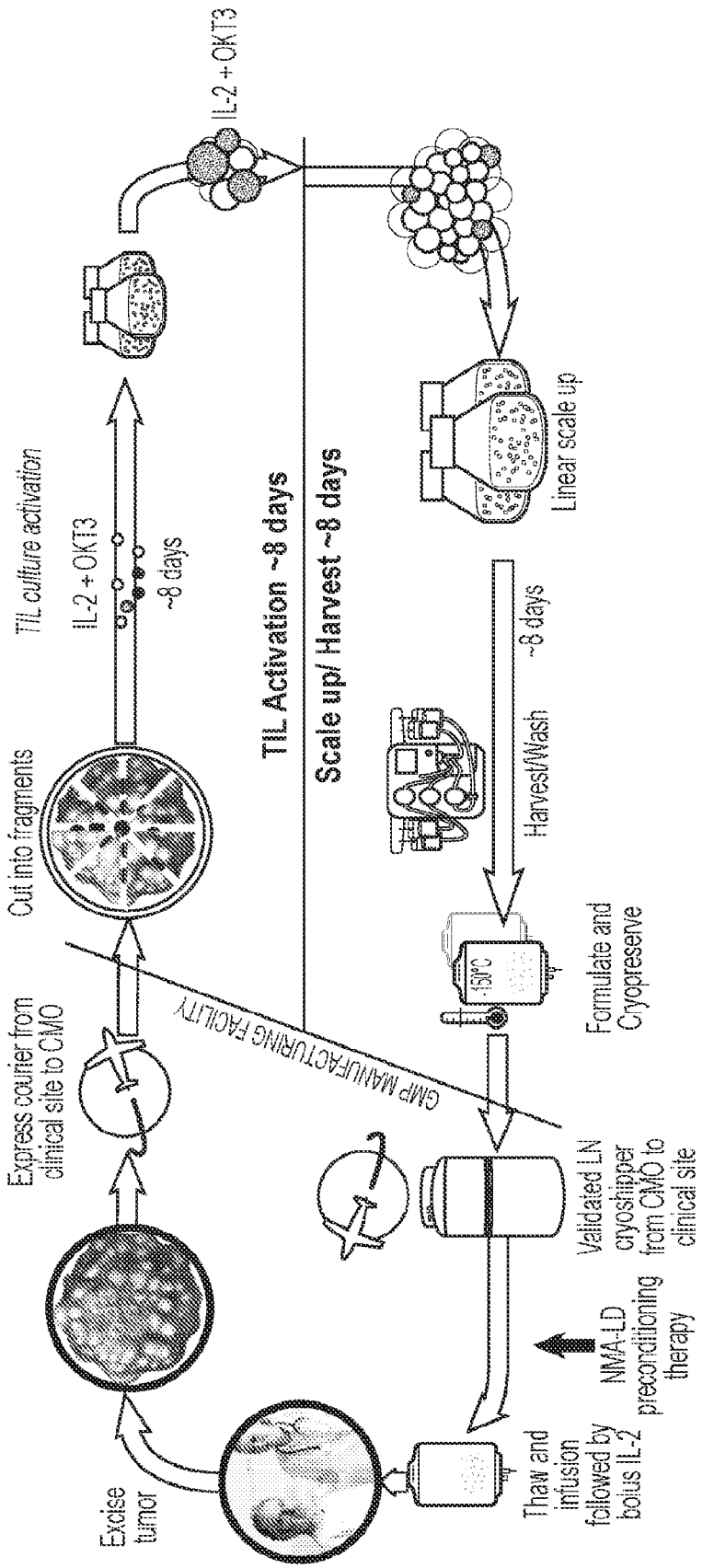

IL-2 + OKT3
TIL culture activation
IL-2 + OKT3
~8 days
Cut into fragments
TIL Activation ~8 days
Scale up/ Harvest ~8 days
Linear scale up
Harvest/Wash
~8 days
Express courier from clinical site to CMO
GMP MANUFACTURING FACILITY
Formulate and Cryopreserve
Validated LN cryoshipper from CMO to clinical site
Excise tumor
Thaw and infusion followed by bolus IL-2
NMA-LD preconditioning therapy

Figure 27

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 250e^6 cells + OKT-3 (15ug)**
REP Initiation	Direct to REP- Day 11- **<200e^6 TIL** 1 G-Rex 500MCS	Direct to REP- Day 11- **<200e^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 500e^6 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 28

Process Comparison	Process Changes	Differences
Gen 2 : Gen 2.1	• Initiate process with two flasks instead of one flask • Divide REP initiation feeder layer between 2 G-Rex500MCS Flasks • Pre-formulate media and warm prior to use	• Potential doubling of final cell count (dose with increased TIL repertoire. • Process redundancy throughout process
Gen 2.1 : Gen 3.1	• Fresh or Frozen tumor • 14-16 day process (from 22 day) • Reduce total feeder layer on process • Feeder layer and OKT3 present at Day 0 • REP initiated with fragments • 100MCS scales to 500MCS • Scales to multiple pre-REP flasks • Standard Media and Defined Media (Serum Free)	• Increased potency • Improved phenotype • Decreased process time • Reduced reagent testing • Decreased process variability • Defined reagents • Increased repertoire • Reduce impurities (feeder) • Comparable or Higher Dose.

Figure 29

Table 4. Description of Gen 3 Optimization Variants.

Process Day	Conditions	Gen 3.0	Gen 3.1 control	Gen 3.1 Test
Day 0 : Tumor Fragment Isolation and Activation	Media (*)	500 mL	500 mL	500 mL
	IL-2	6000 IU/mL	6000 IU/mL	6000 IU/mL
	OKT-3	-	15 ug	15 ug
	Feeders	-	-	2.5E+06
Process Day	**Conditions**	**Gen 3.0**	**Gen 3.1 control**	**Gen 3.1 Test**
Day 7 - 8 : TIL Culture Reactivation	Media (*)	500 mL	500 mL	500 mL
	IL-2	6000 IU/mL	6000 IU/mL	6000 IU/mL
	OKT-3	30 ug	30 ug	30 ug
	Feeders	1 E+09	500 E+06	500E+06
	Total Feeders added through Day 7	1 E+09	500 E+06	750E+06
Process Day	**Conditions**	**Gen 3.0**	**Gen 3.1 control**	**Gen 3.1 Test**
Day 10-11 : Culture Scale Up	From GREX 100 transfer whole TIL suspension to 1 GREX 500 containing 4L media with IL-2 (3000 IU/mL)			
Process Day	**Conditions**	**Gen 3.0**	**Gen 3.1 control**	**Gen 3.1 Test**
Day 16-17: Harvest/Wash/Formulate	LOVO automated cell washer and cryopreservation with CS10.			

(*) Media can be standard media or CTS serum free media.

Figure 30

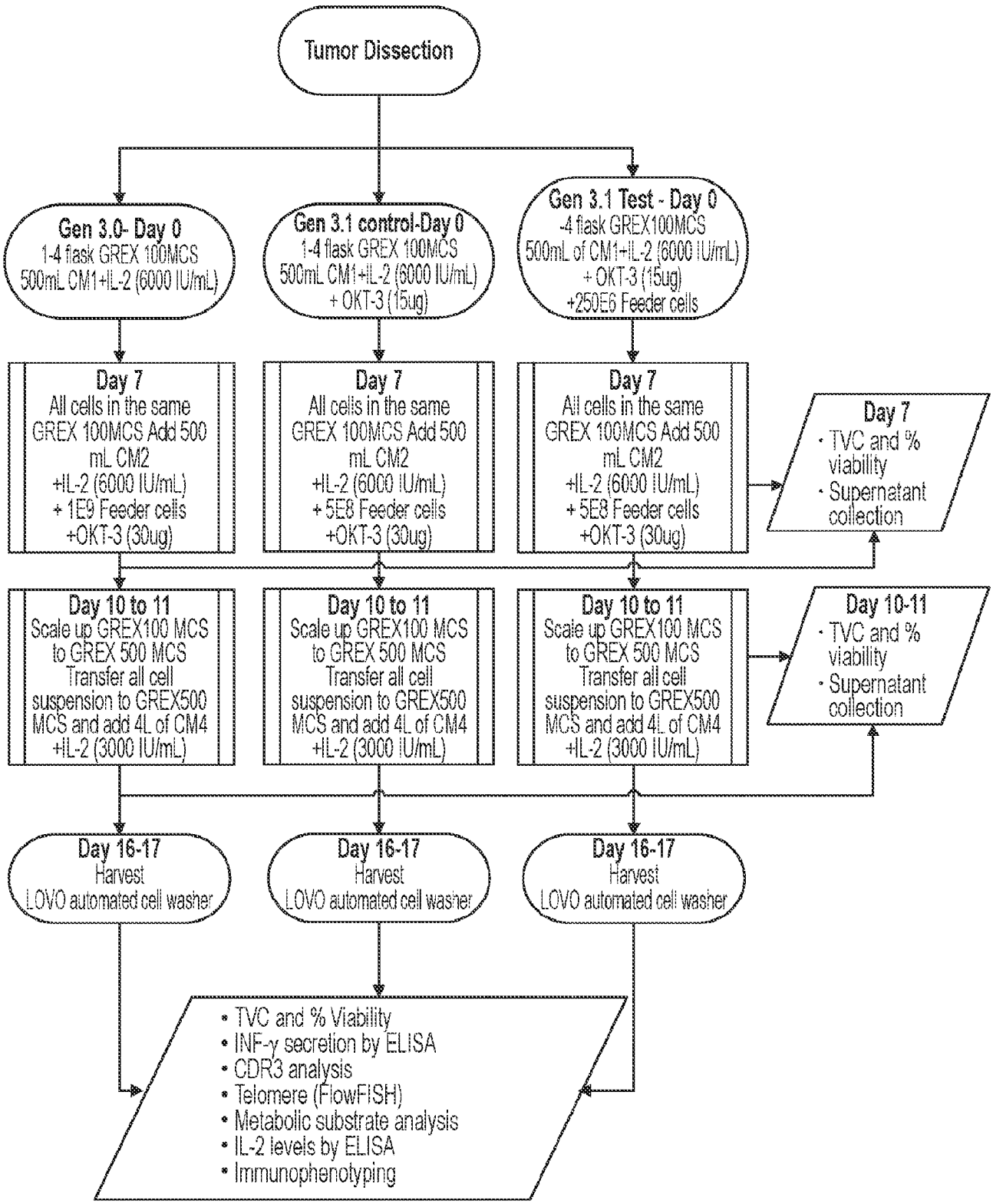

Tumor Dissection
Gen 3.0- Day 0
1-4 flask GREX 100MCS
500mL CM1+IL-2 (6000 IU/mL)
Gen 3.1 control-Day 0
1-4 flask GREX 100MCS
500mL CM1+IL-2 (6000 IU/mL)
+ OKT-3 (15ug)
Gen 3.1 Test - Day 0
-4 flask GREX100MCS
500mL of CM1+IL-2 (6000 IU/mL)
+ OKT-3 (15ug)
+250E6 Feeder cells
Day 7
All cells in the same GREX 100MCS Add 500 mL CM2
+IL-2 (6000 IU/mL)
+ 1E9 Feeder cells
+OKT-3 (30ug)
Day 7
All cells in the same GREX 100MCS Add 500 mL CM2
+IL-2 (6000 IU/mL)
+ 5E8 Feeder cells
+OKT-3 (30ug)
Day 7
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 16-17
Harvest
LOVO automated cell washer
Day 16-17
Harvest
LOVO automated cell washer
Day 16-17
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 31

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 32

Test	Acceptance Criteria	Gen 3.1 Test vs Gen 3.0 Process
Cell Count (TVC)	Gen 3.1 > 30% to Process Gen 3.0	Met
% Viability	≥70% Viability	Met
Immunophenotyping (%CD3+/ %CD45+)	≤5% difference between Gen 3.1 and Gen 3.0 process	Met
IFNγ secretion	Gen 3.1 ≥ to Process Gen 3.0	Met

Figure 33

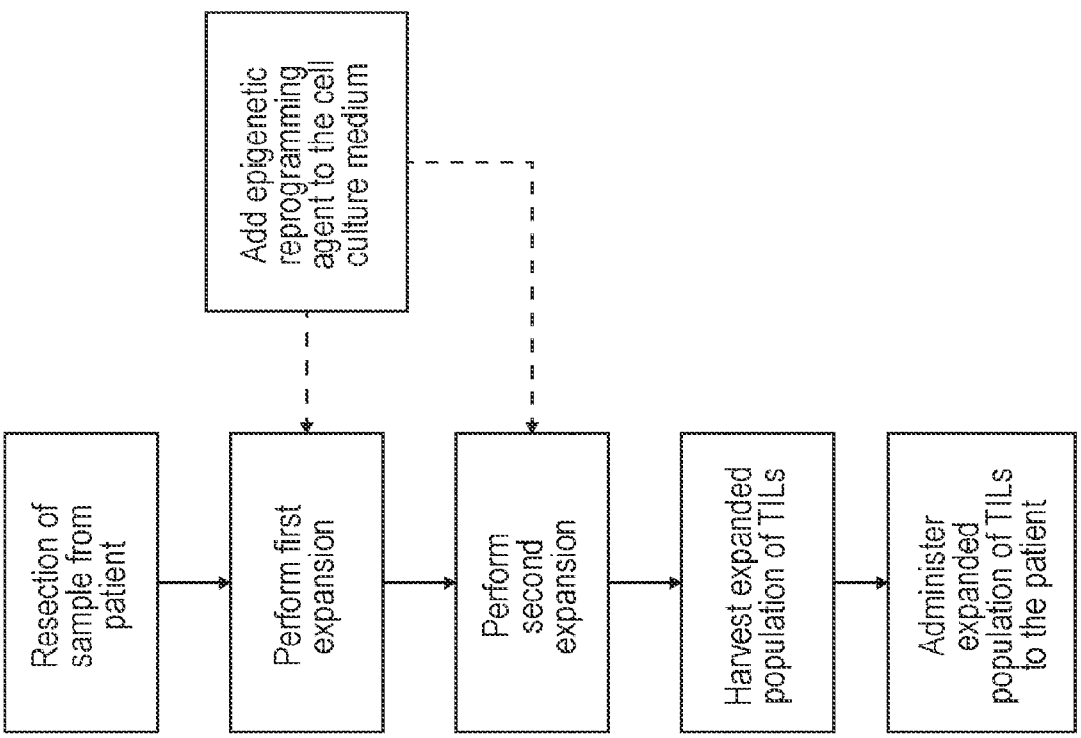

Resection of sample from patient
Perform first expansion
Perform second expansion
Harvest expanded population of TILs
Administer expanded population of TILs to the patient
Add epigenetic reprogramming agent to the cell culture medium

Figure 34

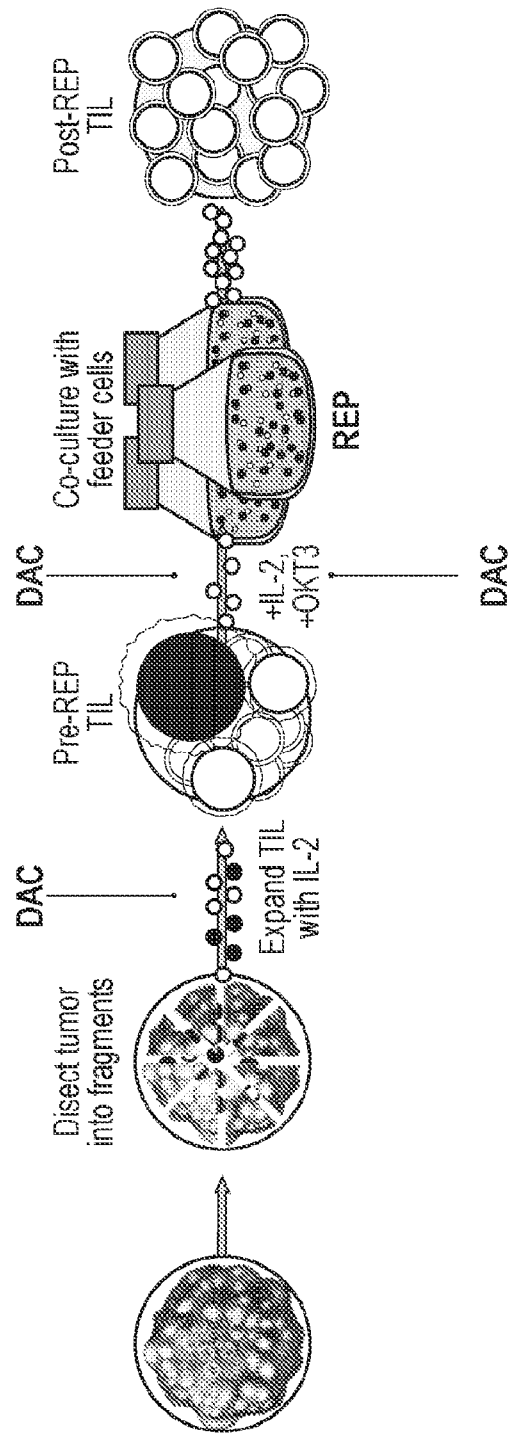

Disect tumor into fragments
DAC
Expand TIL with IL-2
Pre-REP TIL
DAC
+IL-2, +OKT3
DAC
REP
Co-culture with feeder cells
Post-REP TIL

Figure 35

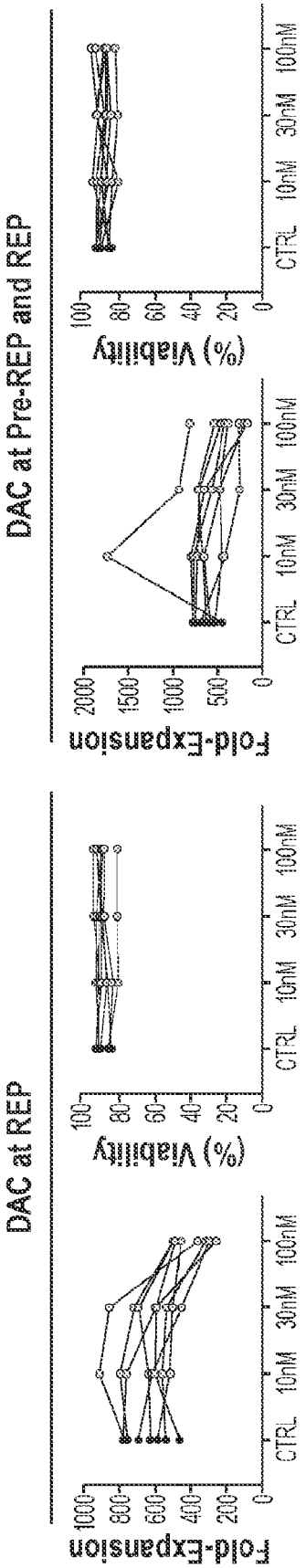

DAC at REP
DAC at Pre-REP and REP
Fold-Expansion
Viability (%)
CTRL
10nM
30nM
100nM

Figure 36A

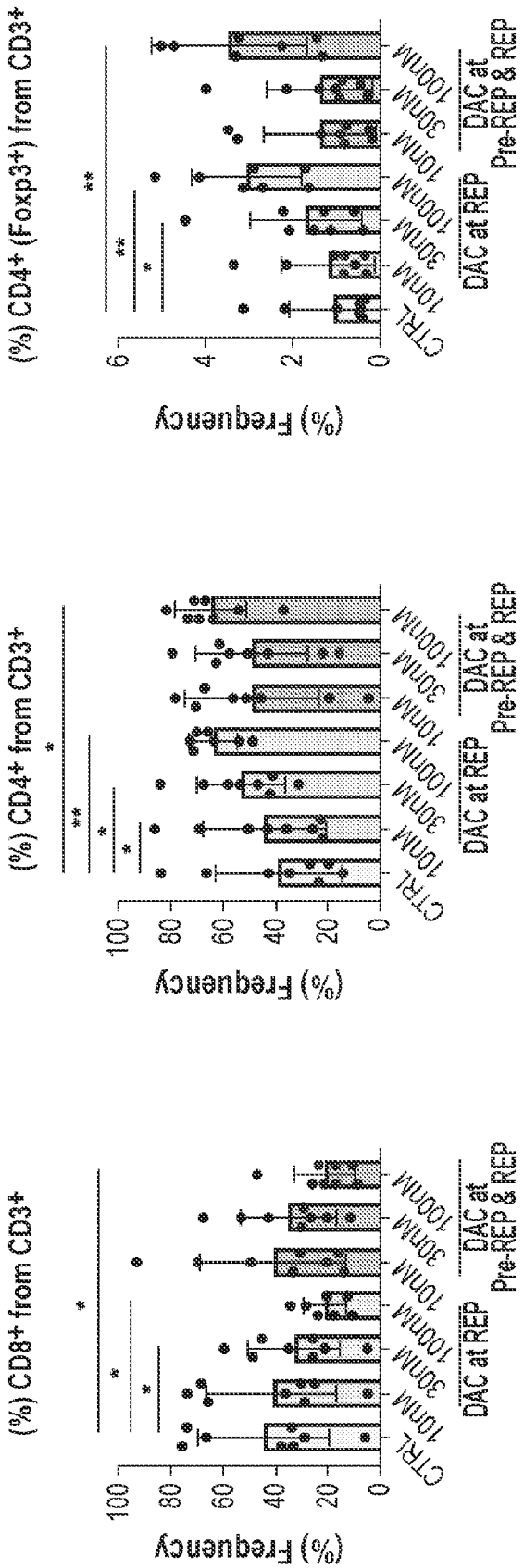

(%) CD8+ from CD3+
(%) CD4+ from CD3+
(%) CD4+ (Foxp3+) from CD3+
Frequency (%)
CTRL
10nM
30nM
100nM
DAC at REP
DAC at Pre-REP & REP

Figure 36B

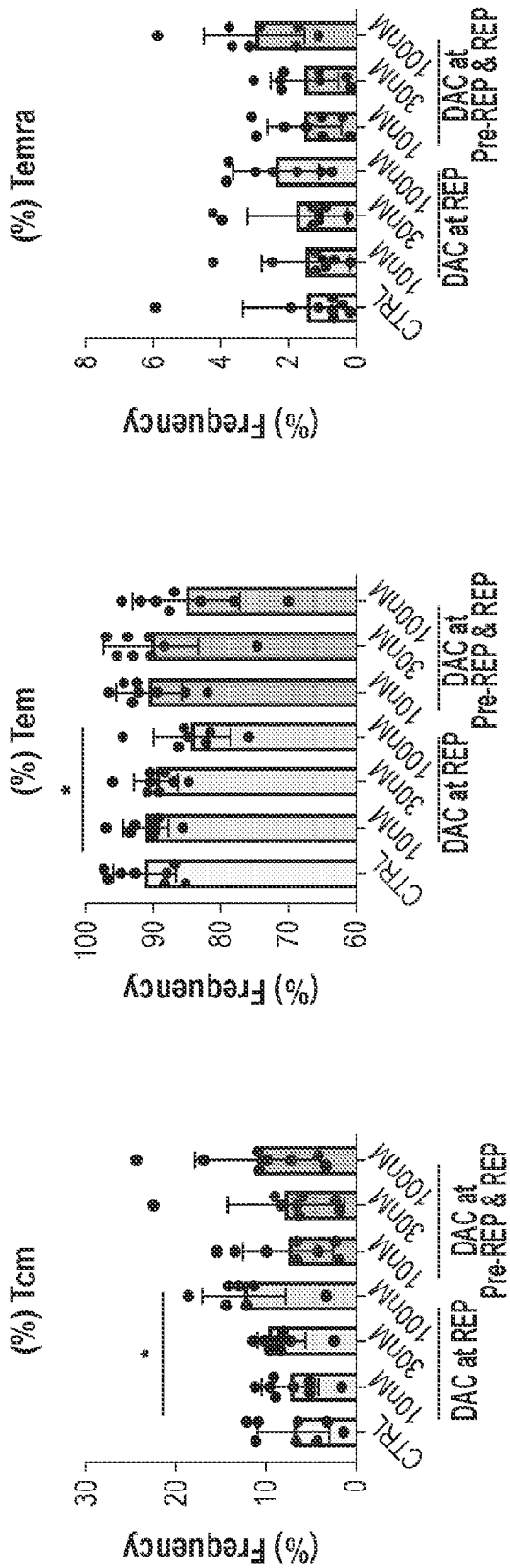

CD8+ TIL
(%) Tcm
(%) Frequency
30
20
10
0
*
CTRL
10nM
30nM
100nM
10nM
30nM
100nM
DAC at REP
DAC at Pre-REP & REP
(%) Tem
(%) Frequency
100
90
80
70
60
*
CTRL
10nM
30nM
100nM
10nM
30nM
100nM
DAC at REP
DAC at Pre-REP & REP
(%) Temra
(%) Frequency
8
6
4
2
0
CTRL
10nM
30nM
100nM
10nM
30nM
100nM
DAC at REP
DAC at Pre-REP & REP

Figure 37A

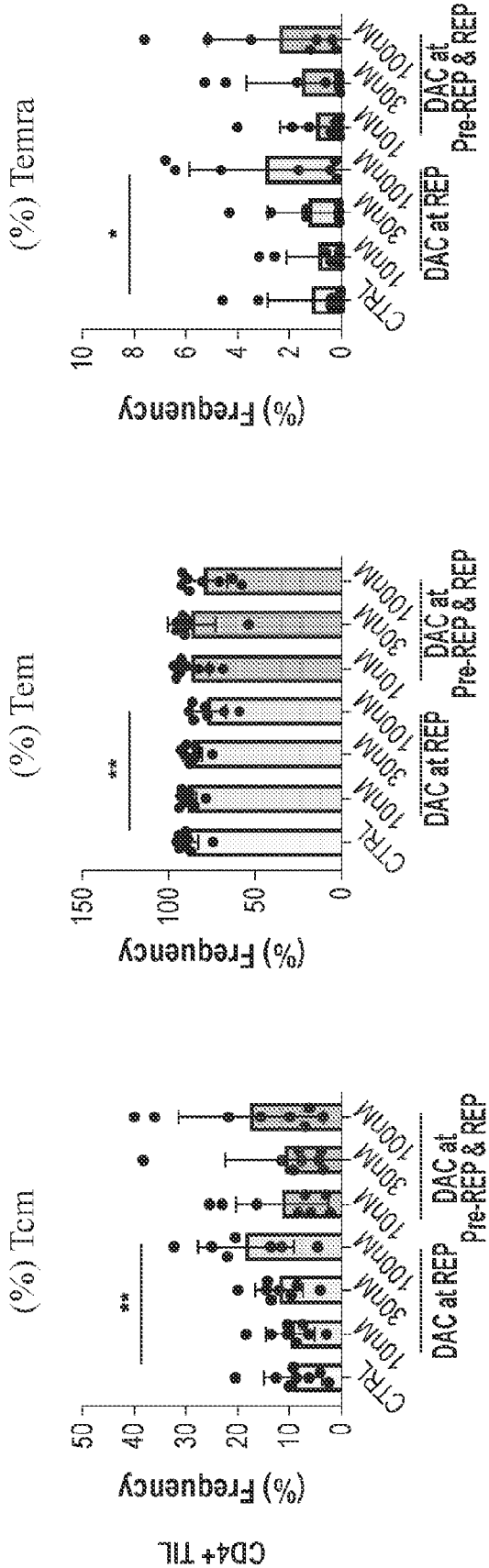

CD4+ TIL
(%) Tcm
(%) Frequency
50
40
30
20
10
0
**
CTRL
10nM
30nM
100nM
10nM
30nM
100nM
DAC at REP
DAC at Pre-REP & REP
(%) Tem
(%) Frequency
150
100
50
0
**
CTRL
10nM
30nM
100nM
10nM
30nM
100nM
DAC at REP
DAC at Pre-REP & REP
(%) Temra
(%) Frequency
10
8
6
4
2
0
*
CTRL
10nM
30nM
100nM
10nM
30nM
100nM
DAC at REP
DAC at Pre-REP & REP

Figure 37B

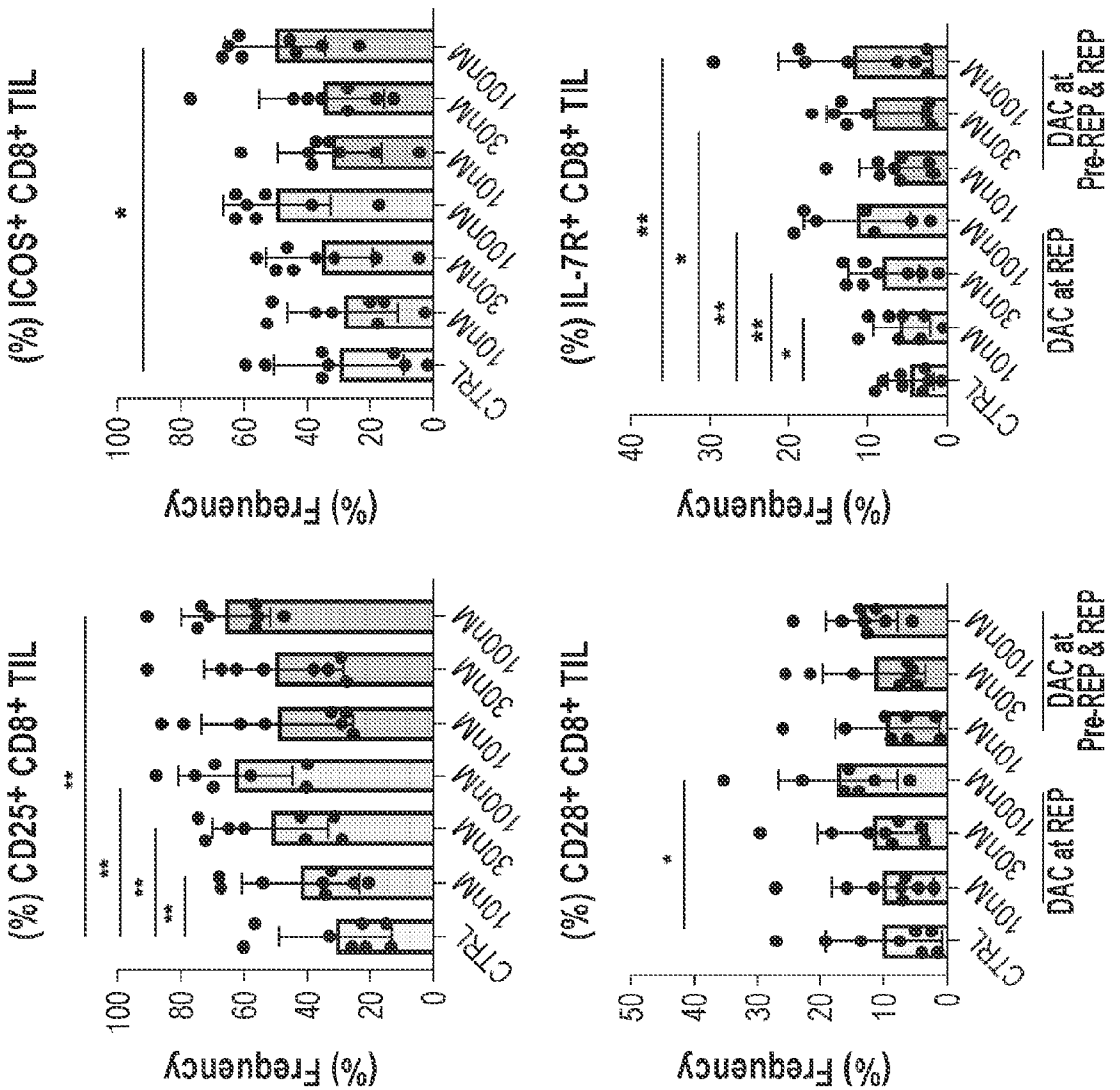

(%) ICOS+ CD8+ TIL
(%) IL-7R+ CD8+ TIL
(%) CD25+ CD8+ TIL
(%) CD28+ CD8+ TIL
Frequency (%)
CTRL
10nM
30nM
100nM
DAC at REP
DAC at Pre-REP & REP

Figure 38A

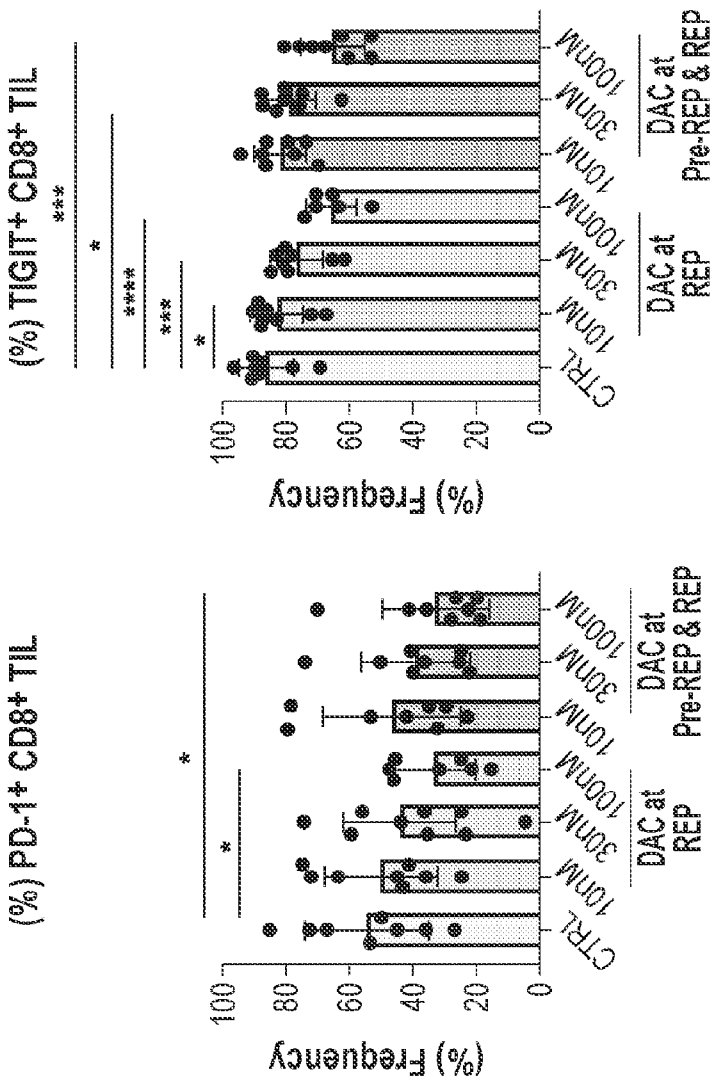

(%) PD-1+ CD8+ TIL
(%) TIGIT+ CD8+ TIL
Frequency (%)
0
20
40
60
80
100
CTRL
10nM
30nM
100nM
10nM
30nM
100nM
DAC at REP
DAC at Pre-REP & REP
*
*
*
***
*
****
***
*

Figure 38B

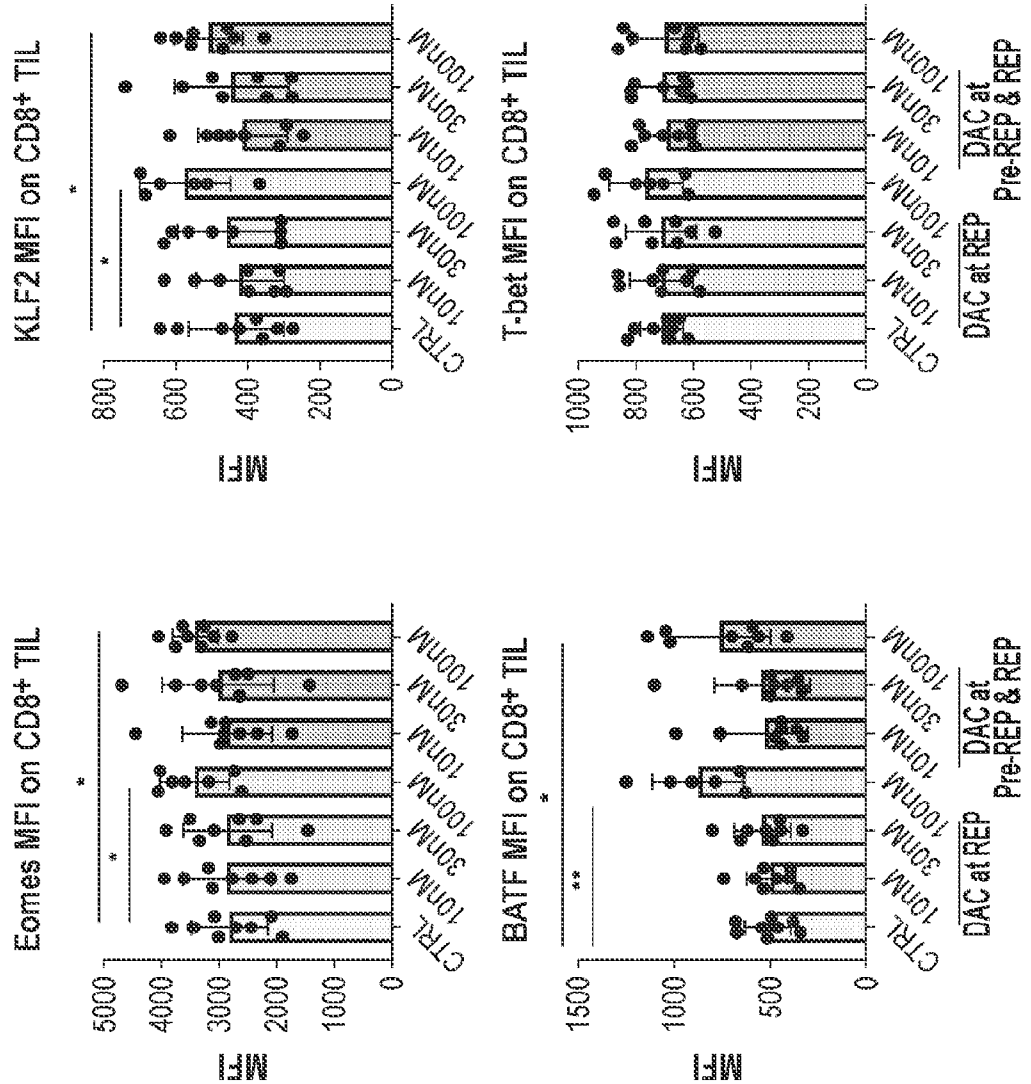

KLF2 MFI on CD8+ TIL
T-bet MFI on CD8+ TIL
Eomes MFI on CD8+ TIL
BATF MFI on CD8+ TIL
MFI
CTRL
10nM
30nM
100nM
DAC at REP
DAC at Pre-REP & REP

Figure 39

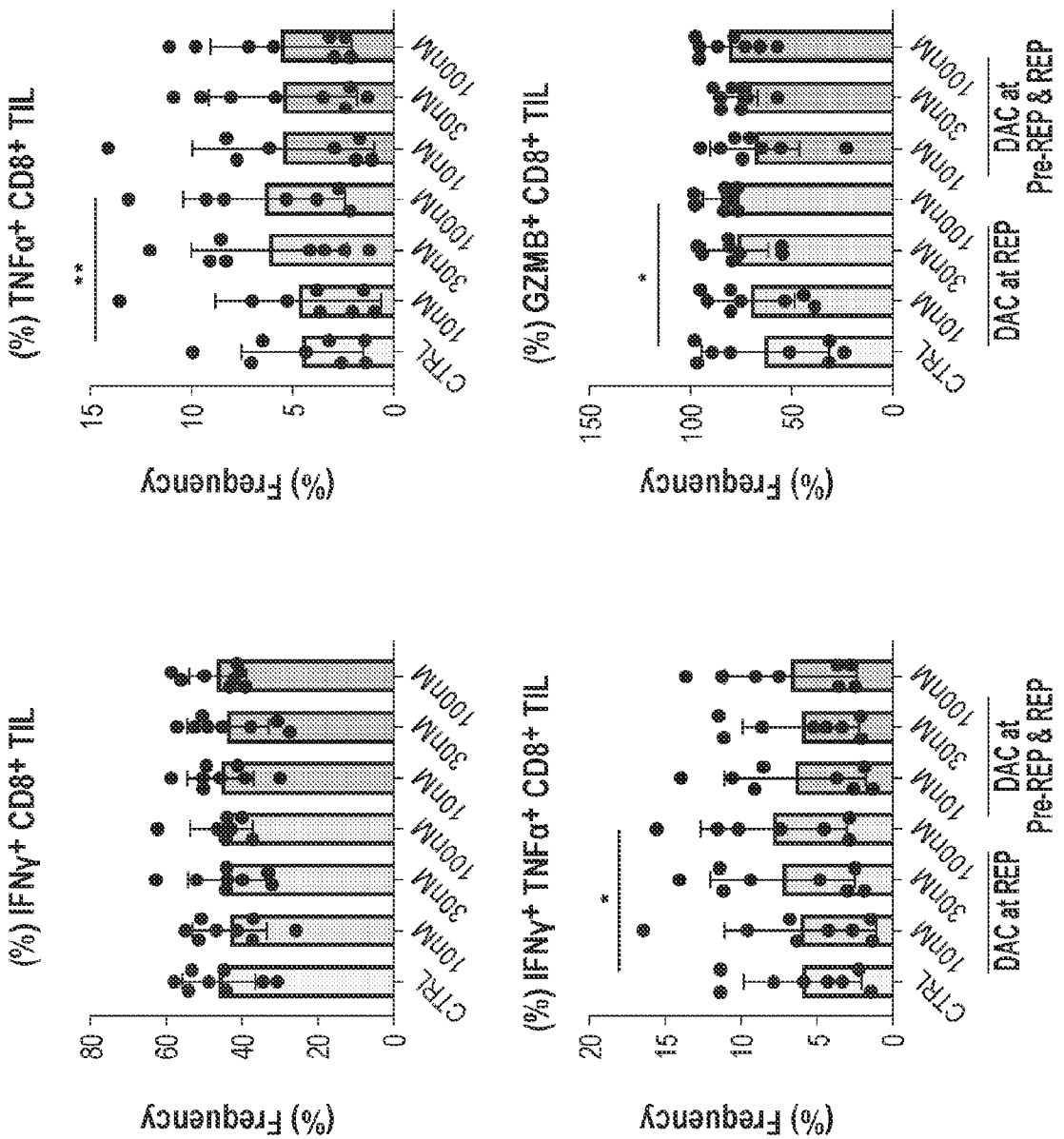

(%) TNFα+ CD8+ TIL
(%) GZMB+ CD8+ TIL
(%) IFNγ+ CD8+ TIL
(%) IFNγ+ TNFα+ CD8+ TIL
(%) Frequency
CTRL
10nM
30nM
100nM
DAC at REP
DAC at Pre-REP & REP

Figure 40

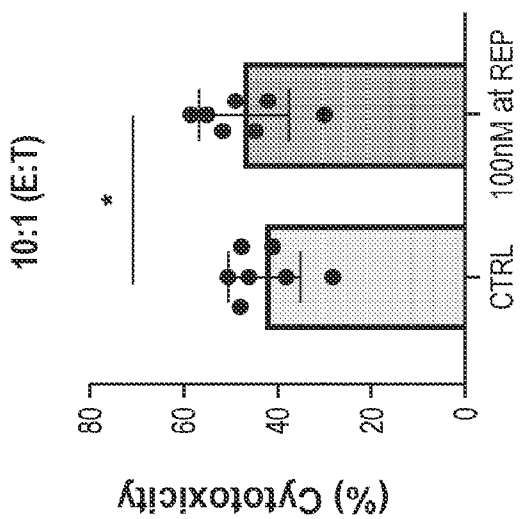


Figure 41A

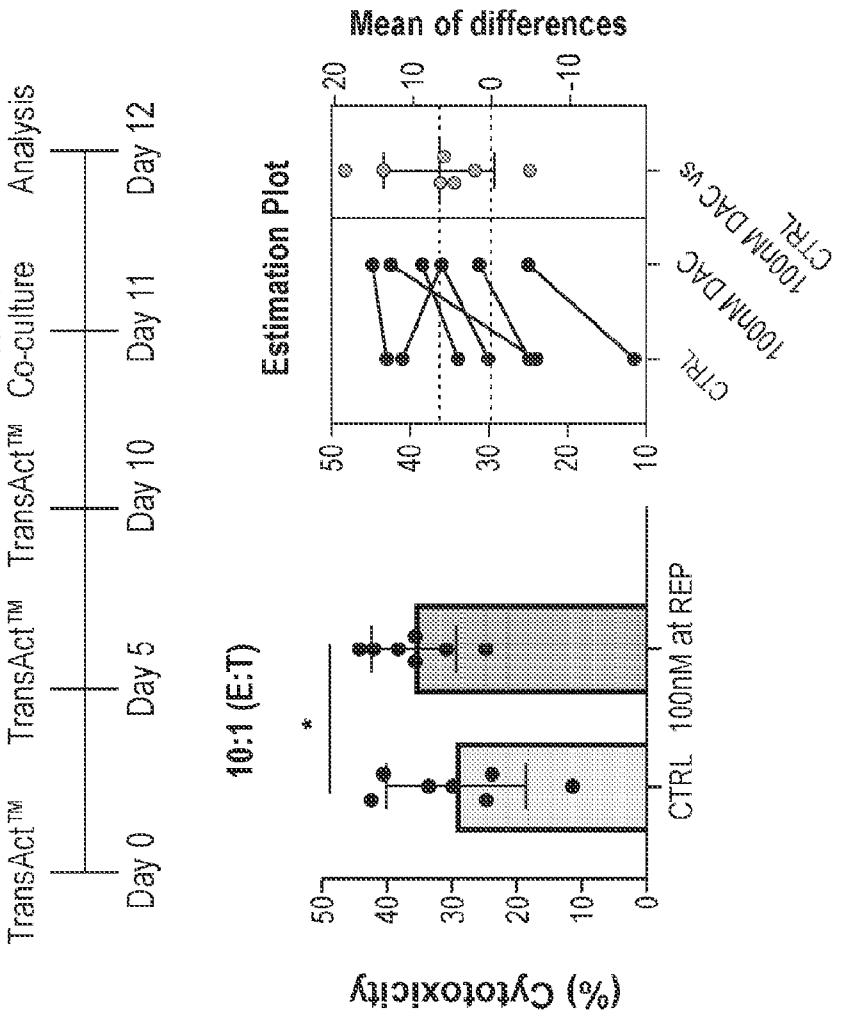

TransAct™
Day 0
TransAct™
Day 5
TransAct™
Day 10
THP-1
Co-culture
Day 11
Analysis
Day 12
10:1 (E:T)
*
CTRL
100nM at REP
(%) Cytotoxicity
Estimation Plot
Mean of differences
CTRL
100nM DAC
100nM DAC vs CTRL

Figure 41B

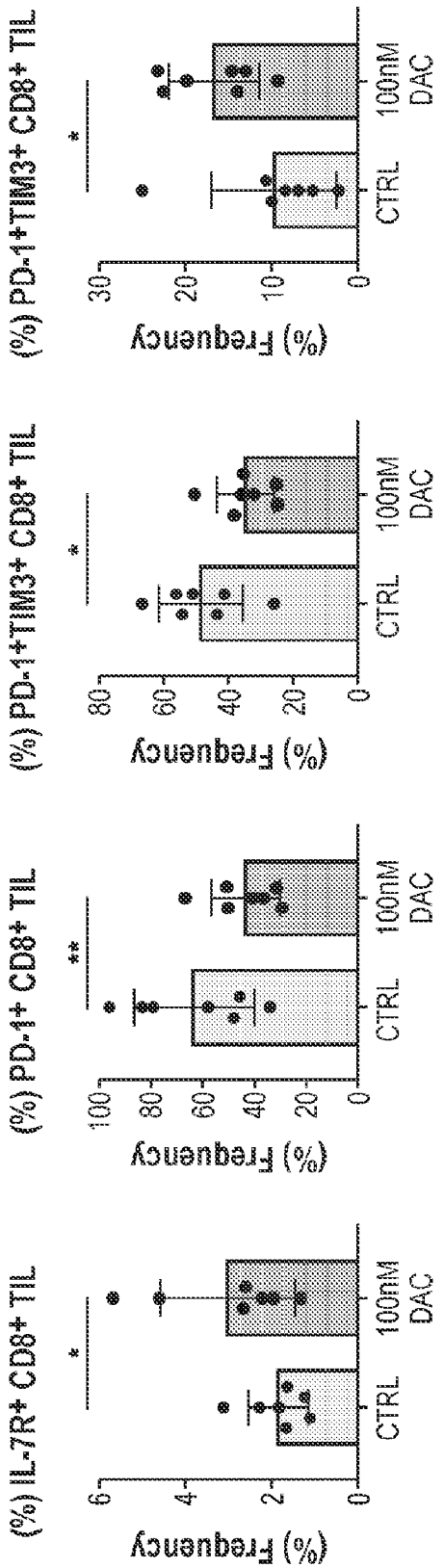

(%) IL-7R+ CD8+ TIL
(%) PD-1+ CD8+ TIL
(%) PD-1+TIM3+ CD8+ TIL
(%) PD-1+TIM3+ CD8+ TIL
Frequency (%)
CTRL
100nM DAC
*
**

Figure 42A

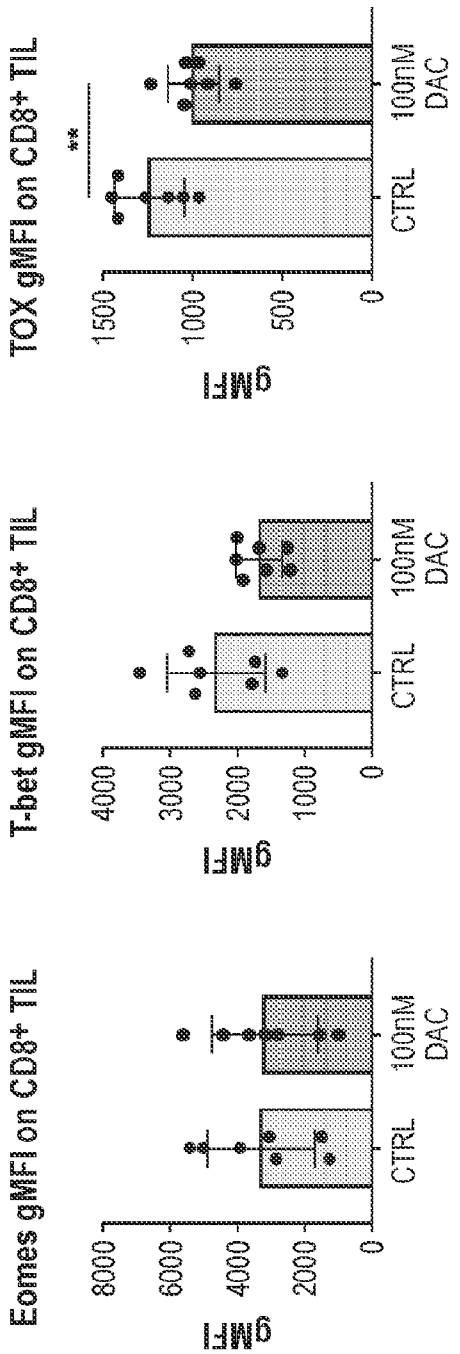

Eomes gMFI on CD8+ TIL
gMFI
8000
6000
4000
2000
0
CTRL
100nM DAC
T-bet gMFI on CD8+ TIL
gMFI
4000
3000
2000
1000
0
CTRL
100nM DAC
TOX gMFI on CD8+ TIL
**
gMFI
1500
1000
500
0
CTRL
100nM DAC

Figure 42B