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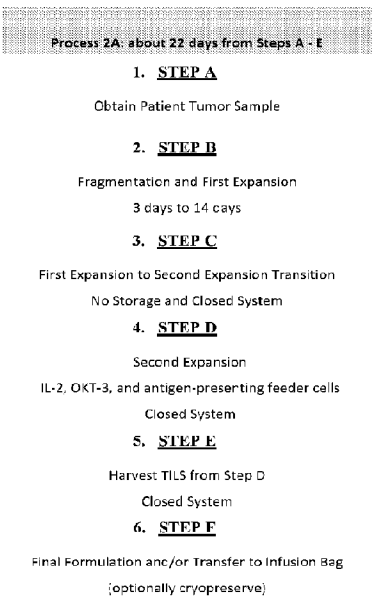
(54) **Titre : PROCEDES DE FABRICATION DE LYMPHOCYTES INFILTRANT LES TUMEURS MODIFIES ET LEUR UTILISATION DANS LA THERAPIE CELLULAIRE ADOPTIVE**
(54) **Title: METHODS OF MAKING MODIFIED TUMOR INFILTRATING LYMPHOCYTES AND THEIR USE IN ADOPTIVE CELL THERAPY**

Figure 1



(57) **Abrégé/Abstract:**
Provided herein are compositions and methods for the treatment of cancers using modified TILs, wherein the modified TILs include one or more immunomodulatory agents (e.g, cytokines) associated with their cell surface. The immunomodulatory agents associated with the TILs provide a localized immunostimulatory effect that can advantageously enhance TIL survival, proliferation and/or anti-tumor activity in a patient recipient. As such, the compositions and methods disclosed herein provide effective cancer therapies.

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Abstract:

Provided herein are compositions and methods for the treatment of cancers using modified TILs, wherein the modified TILs include one or more immunomodulatory agents (e.g, cytokines) associated with their cell surface. The immunomodulatory agents associated with the TILs provide a localized immunostimulatory effect that can advantageously enhance TIL survival, proliferation and/or anti-tumor activity in a patient recipient. As such, the compositions and methods disclosed herein provide effective cancer therapies.

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METHODS OF MAKING MODIFIED TUMOR INFILTRATING LYMPHOCYTES AND THEIR USE IN ADOPTIVE CELL THERAPY

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application Nos. 63/143,736, filed January 29, 2021; 63/146,486, filed February 5, 2021; 63/224,360, filed July 21, 2021; 63/277,571, filed November 9, 2021; and 63/285,956, filed December 3, 2021, all of which are herein incorporated by reference in their entireties.

BACKGROUND

[0002] 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. In some instances, however, the survival and anti-tumor activity of the transferred TILs can decrease following transfer to the patient.

[0003] Administration of supporting immunostimulatory agents (*e.g.*, cytokines) have been explored to enhance T cell therapies. Such immunostimulatory agents, however, require high systemic doses that can lead to undesirable toxicity.

[0004] Thus, there remains a need for improved TIL therapies for the treatment of cancers.

BRIEF SUMMARY

[0005] Provided herein are compositions and methods for the treatment of cancers using modified TILs, wherein the modified TILs include one or more immunomodulatory agents (*e.g.*, cytokines) associated with their cell surface. The immunomodulatory agents associated with the TILs provide a localized immunostimulatory effect that can advantageously enhance TIL survival, proliferation and/or anti-tumor activity in a patient recipient. As such, the compositions and methods disclosed herein provide effective cancer therapies.

[0006] In one aspect, provided herein is a method of treating a cancer in a patient or subject in need thereof comprising administering a population of tumor infiltrating lymphocytes (TILs), optionally wherein the patient or subject has received at least one prior therapy, wherein a portion of the TILs are modified TILs such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0007] In one aspect, provided herein is 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) 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;

(b) adding the first population of TILs into a closed system;

(c) 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 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

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

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

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

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

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

(i) modifying a portion of the TILs at any time prior to the administering (h) such that each of the modified TILs comprises an immunomodulatory composition associated

with its surface membrane.

[0008] In one aspect, provided herein is 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;

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

(c) 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 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

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

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

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

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

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

(i) modifying a portion of the TILs at any time prior to the administering (h) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0009] In one aspect, provided herein is 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) adding the first population of TILs into a closed system;

(c) 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 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

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

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

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

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

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

(i) modifying a portion of the TILs at any time prior to the administering (h) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0010] In another aspect, provided herein is 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 and adding the tumor fragments into a closed system;

(c) 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 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

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

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

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

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

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

(i) modifying a portion of the TILs at any time prior to the administering (h) such that

each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0011] In other aspect, provided herein is 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;

(c) contacting the first population of TILs 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 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) modifying a portion of the TILs at any time prior to the administering (g) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0012] In another aspect, provided herein is 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;

(c) contacting the tumor fragments 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 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

(i) modifying a portion of the TILs at any time prior to the administering (g) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0013] In one aspect, provided herein is 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;

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

(c) performing a priming first expansion by culturing the PD-l enriched TIL population 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;

(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 therapeutic 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;

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

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

(g) modifying a portion of the TILs at any time during the method such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0014] Provided herein is 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;

(b) adding the first population of TILs into a closed system;

(c) 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 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

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

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

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

(g) modifying a portion of the TILs at any time prior to the transfer to the infusion bag in step (f) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0015] Provided herein is 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;

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

(c) 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 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

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

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

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

(g) modifying a portion of the TILs at any time prior to the transfer to the infusion bag in step (f) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0016] In another aspect, provided herein is 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) adding the first population of TILs into a closed system;

(c) 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 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

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

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

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

(g) modifying a portion of the TILs at any time prior to the transfer in step (f) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0017] In one aspect, provided herein is 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 a 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) adding the tumor fragments into a closed system;

(c) 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 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

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

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

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

(g) modifying a portion of the TILs at any time prior to the transfer in step (f) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0018] In some embodiments, of the methods provided herein, the first expansion is divided into a first step and a second step, wherein the method further comprises performing the first step of the first expansion by culturing the first population of TILs in a cell culture medium containing IL-2 to produce TILs that egress from the tumor fragments or sample, separating TILs that remain in the tumor fragments or sample from TILs that egressed from the tumor fragments or sample, optionally digesting the tumor fragments or sample to produce a tumor digest, and performing the second step of the first expansion by culturing in the cell culture medium the TILs remaining in the tumor fragments or sample or tumor digest to produce the second population of TILs.

[0019] In one aspect, provided herein is 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) contacting the first population of TILs with a first cell culture medium;

(c) 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;

(d) 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;

(e) harvesting the third population of TILs; and

(f) modifying a portion of the TILs at any time prior to the harvesting in step (f) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0020] In one aspect, provided herein is 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 a cancer in a 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 of the tumor that contains a mixture of tumor and TIL cells;

(b) fragmenting the tumor into tumor fragments;

(c) contacting the tumor fragments 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 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) modifying a portion of the TILs at any time prior to the harvesting in step (f) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0021] In one aspect, provided herein is 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;

(b) performing a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, optionally OKT-3, and optionally comprising

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

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

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

(e) modifying a portion of the TILs at any time prior to or after the harvesting in step (d) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0022] In some embodiments of this method, the cell culture medium in step (b) 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).

[0023] In one aspect, provided herein is a method of expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

(a) performing a priming first expansion by culturing a first population of TILs, said first population of TILs obtainable by processing a tumor sample from a tumor resected from a cancer in a subject into multiple tumor fragments, in a cell culture medium comprising IL-2, optionally OKT-3, and optionally antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for first period of about 1 to 7/8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

(b) performing a rapid second expansion by contacting the second population of TILs to a cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the number of APCs in the rapid second expansion is at least twice the number of APCs in step (a), wherein the rapid second expansion is performed for a second period of about

1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the rapid second expansion is performed in a container comprising a second gas-permeable surface area;

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

(d) modifying a portion of the TILs at any time prior to or after the harvesting in step (c) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0024] In one aspect, provided herein is a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

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

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

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

(d) modifying a portion of the TILs at any time prior to or after the harvesting in step (c) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0025] In some embodiments of this method, the cell culture medium in step (a) 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).

[0026] In some embodiments of the methods provided herein, the priming first expansion is divided into a first step and a second step, wherein the method further comprises performing the first step of the priming first expansion by culturing the first population of TILs in a cell culture medium containing IL-2 to produce TILs that egress from the tumor fragments or sample, separating TILs that remain in the tumor fragments or sample from TILs that

egressed from the tumor fragments or sample, optionally digesting the tumor fragments or sample to produce a tumor digest, and performing the second step of the priming first expansion in the cell culture medium the TILs remaining in the tumor fragments or sample or tumor digest to produce the second population of TILs.

[0027] In one aspect, provided herein is 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 sample obtained from one or more small biopsies, core biopsies, or needle biopsies of a tumor from a cancer in a subject by culturing the tumor sample in a first cell culture medium comprising IL-2 for about 3 days;

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

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

(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) modifying a portion of the TILs at any time prior to transfer to the infusion bag in step (e) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0028] In another aspect, provided herein is 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 sample obtained from one or more small biopsies, core biopsies, or needle biopsies of a tumor from a cancer in a subject by culturing the tumor sample in a first cell culture medium comprising IL-2 for about 3 days;

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

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

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

(e) modifying a portion of the TILs at any time prior to or after the harvesting in step (f) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

[0029] In some embodiments of the methods provided herein, 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)), renal cancer, and renal cell carcinoma.

[0030] In one aspect, provided herein is a method of expanding T cells comprising:

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

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

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

(d) modifying a portion of the T cells at any time prior to or after the harvesting in step (c) such that each of the modified T cells comprises an immunomodulatory composition associated with its surface membrane.

[0031] In another aspect, provided herein is 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;

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

(d) modifying a portion of the T cells at any time prior to or after the harvesting in step (e) such that each of the modified T cells comprises an immunomodulatory composition associated with its surface membrane.

[0032] In one aspect, provided herein is a method for expanding peripheral blood lymphocytes (PBLs) from peripheral blood, the method comprising the steps of:

(a) obtaining a sample of peripheral blood mononuclear cells (PBMCs) from peripheral blood of a patient;

(b) culturing said PBMCs in a culture comprising a first cell culture medium with IL-2, anti-CD3/anti-CD28 antibodies and a first combination of antibiotics, for a period of time selected from the group consisting of: about 9 days, about 10 days, about 11 days, about 12 days, about 13 days and about 14 days, thereby effecting expansion of peripheral blood lymphocytes (PBLs) from said PBMCs;

(c) harvesting the PBLs from the culture in step (b); and

(d) modifying a portion of the PBLs at any time prior to or after the harvesting in step (c) such that each of the modified PBLs comprises an immunomodulatory composition associated with its surface membrane.

[0033] In some embodiments, the patient is pre-treated with ibrutinib or another interleukin-2 inducible T cell kinase (ITK) inhibitor. In certain embodiments, the patient is refractory to treatment with ibrutinib or such other ITK inhibitor.

[0034] In some embodiments, the immunomodulatory composition comprises one or more membrane anchored immunomodulatory fusion proteins each comprising one or more immunomodulatory agents and a cell membrane anchor moiety.

[0035] In exemplary embodiments, the one or more immunomodulatory agents comprise one or more cytokines. In some embodiments, the one or more cytokines comprise IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, or GCSF or a variant thereof.

[0036] In some embodiments, the one or more cytokines comprise IL-2. In some embodiments, the IL-2 is human IL-2. In exemplary embodiments, the human IL-2 has the amino acid sequence of SEQ ID NO:272.

[0037] In some embodiments, the one or more cytokines comprise IL-12. In certain embodiments, the IL-12 comprises a human IL-12 p35 subunit attached to a human IL-12 p40 subunit. In certain embodiments, the human IL-12 p35 subunit has the amino acid sequence of SEQ ID NO:267 and the human IL-12 p40 subunit has the amino acid sequence of SEQ ID NO:268.

[0038] In some embodiments, the one or more cytokines comprise IL-15. In some embodiments, the IL-15 is human IL-15. In exemplary embodiments, the human IL-15 has the amino acid sequence of SEQ ID NO:258.

[0039] In some embodiments, the one or more cytokines comprise IL-18. In certain embodiments, the IL-18 is human IL-18. In certain embodiments, the human IL-18 has the amino acid sequence of SEQ ID NO:269 or SEQ ID NO:270.

[0040] In some embodiments, the one or more cytokines comprise IL-21. In certain embodiments, the IL-21 is human IL-21. In some embodiments, the human IL-21 has the amino acid sequence of SEQ ID NO:251.

[0041] In some embodiments, the one or more cytokines comprise IL-15 and IL-21. In some embodiments, the IL-15 is human IL-15 and the IL-21 is human IL-21. In certain

embodiments, the human IL-15 has the amino acid sequence of SEQ ID NO: 258 and the human IL-21 has the amino acid sequence of SEQ ID NO:271.

[0042] In some embodiments, the one or more immunomodulatory agents comprise a CD40 agonist. In certain embodiments, the CD40 agonist is an anti-CD40 binding domain or CD40L. In exemplary embodiments, the CD40 agonist is a CD40 binding domain comprising a variable heavy domain (VH) and a variable light domain (VL). In some embodiments, the VH and VL of the CD40 binding domain are selected from the following: a) a VH having the amino acid sequence of SEQ ID NO: 274, and a VL having the amino acid sequence of SEQ ID NO:275; b) a VH having the amino acid sequence of SEQ ID NO: 277, and a VL having the amino acid sequence of SEQ ID NO:278; c) a VH having the amino acid sequence of SEQ ID NO: 280, and a VL having the amino acid sequence of SEQ ID NO:281; and d) a VH having the amino acid sequence of SEQ ID NO: 283, and a VL having the amino acid sequence of SEQ ID NO:284. In exemplary embodiments, the CD40 binding domain is an scFv.

[0043] In some embodiments, the CD40 agonist is a human CD40L having the amino acid sequence of SEQ ID NO: 273.

[0044] In some embodiments, the one or more membrane anchored immunomodulatory fusion proteins are independently according to the formula, from N- to C-terminus: S-IA-L-C, wherein S is a signal peptide, IA is an immunomodulatory agent, L is a linker and C is a cell membrane anchor moiety.

[0045] In some embodiments, the cell membrane anchor moiety comprises a CD8a transmembrane-intracellular domain, a B7-1 transmembrane domain, a B7-2 transmembrane domain, or a CD8a transmembrane domain. In exemplary embodiments, the cell membrane anchor moiety comprises a B7-1 transmembrane domain. In some embodiments, the cell membrane anchor moiety has the amino acid sequence of SEQ ID NO:239.

[0046] In some embodiments, the immunomodulatory composition comprises two or more different membrane anchored immunomodulatory fusion proteins, wherein each of the different membrane anchored immunomodulatory fusion proteins each comprise a different immunomodulatory agent. In some embodiments, the different immunomodulatory agents are selected from: IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, GCSF or a variant thereof, and a CD40 agonist. In some embodiments, the different immunomodulatory agents are selected from: IL-12 and IL-

15, IL-15 and IL-18, IL-15 and IL-21, CD40L and IL-15, IL-15 and IL-21, and IL-2 and IL-12.

[0047] In some embodiments, the modified TILs comprise a first membrane anchored immunomodulatory fusion protein and a second membrane anchored immunomodulatory fusion protein.

[0048] In some embodiments, the first membrane anchored immunomodulatory fusion protein comprises IL-15 and the second membrane anchored immunomodulatory fusion protein comprises IL-21.

[0049] In exemplary embodiments, the first membrane anchored immunomodulatory fusion protein and the second membrane anchored immunomodulatory fusion protein are expressed under the control of an NFAT promoter in the modified TILs.

[0050] In exemplary embodiments, the one or more membrane anchored immunomodulatory fusion proteins are independently according to the formula, from N- to C-terminus: S-IA-L-C, wherein S is a signal peptide, IA is an immunomodulatory agent, L is a linker and C is a cell membrane anchor moiety. In some embodiments, IA is a cytokine. In exemplary embodiments, IA is selected from the group consisting of: IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, or GCSF or a variant thereof. In some embodiments, IA is IL-2. In certain embodiments, IA is IL-12. In some embodiments, IA is IL-15. In certain embodiments, IA is IL-21.

[0051] In exemplary the one or more membrane anchored immunomodulatory fusion proteins are independently according to the formula, from N- to C-terminus: S1-IA1-L1-C1-L2-S2-IA2-L3-C2, wherein S1 and S2 are each independently a signal peptide, IA1 and IA2 are each independently an immunomodulatory agent, L1-L3 are each independently a linker, and C1 and C2 are each independently a cell membrane anchor moiety. In some embodiments, S1 and S2 are the same. In certain embodiments, C1 and C2 are the same. In some embodiments, L2 is a cleavable linker. In exemplary embodiments, L2 is a furin cleavable linker. In some embodiments, IA1 and IA2 are each independently a cytokine.

[0052] In some embodiments, IA1 and IA2 are each independently selected from the group consisting of: IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, or GCSF or a variant thereof. In some embodiments, IA1 and IA2 are each independently selected from the group consisting of IL-2 and IL-12, with the proviso that one of IA1 and IA2 is IL-2 and the other is IL-12. In some

embodiments, IA1 and IA2 are each independently selected from the group consisting of IL-15 and IL-21, with the proviso that one of IA1 and IA2 is IL-15 and the other is IL-21.

[0053] In certain embodiments, the modifying comprises introducing a heterologous nucleic acid encoding the fusion protein into the portion of TILs and expressing the fusion protein on the surface of the modified TILs.

[0054] In certain embodiments, the modifying comprises introducing a heterologous nucleic acid encoding the fusion protein into the portion of TILs and expressing the fusion protein on the surface of the modified TILs. In some embodiments, the heterologous nucleic acid is introduced into the genome of the modified TIL using one or more methods selected from a CRISPR method, a TALE method, a zinc finger method, and a combination thereof.

[0055] In some embodiments, the immunomodulatory composition comprises a fusion protein comprising one or more immunomodulatory agents linked to a TIL surface antigen binding domain. In some embodiments, the one or more immunomodulatory agents comprise one or more cytokines. In some embodiments, the one or more cytokines comprise IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, or GCSF or a variant thereof. In some embodiments, the one or more cytokines comprise IL-12. In certain embodiments, the one or more cytokines comprise IL-15. In some embodiments, the one or more cytokines comprise IL-21. In certain embodiments, the TIL surface antigen binding domain comprises an antibody variable heavy domain and variable light domain. In some embodiments, the TIL surface antigen binding domain comprises an antibody or fragment thereof. In some embodiments, the TIL surface antigen binding domain exhibits an affinity for one or more of following TIL surface antigens: CD45, CD4, CD8, CD3, CDlla, CDllb, CDllc, CD18, CD25, CD127, CD19, CD20, CD22, HLA-DR, CD197, CD38, CD27, CD196, CXCR3, CXCR4, CXCR5, CD84, CD229, CCR1, CCR5, CCR4, CCR6, CCR8, CCR10, CD 16, CD56, CD 137, OX40, or GITR. In certain embodiments, the modifying comprises incubating the fusion protein with the portion of TILs under conditions to permit the binding of the fusion protein to the portion of TILs.

[0056] In some embodiments, the immunomodulatory composition comprises a nanoparticle comprising a plurality of immunomodulatory agents. In some embodiments, the plurality of immunomodulatory agents are covalently linked together by degradable linkers. IN certain embodiments, the nanoparticle comprises at least one polymer, cationic polymer, or cationic block co-polymer on the nanoparticle surface. In some embodiments, the one or more

cytokines comprise IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, or GCSF or a variant thereof. In certain embodiments, the one or more cytokines comprises IL-12. In some embodiments, the one or more cytokines comprises IL-15. In some embodiments, the one or more cytokines comprise IL-21. In some embodiments, the nanoparticle is a liposome, a protein nanogel, a nucleotide nanogel, a polymer nanoparticle, or a solid nanoparticle. In some embodiments, the nanoparticle is a nanogel. In certain embodiments, the nanoparticle further comprises an antigen binding domain that binds to one or more of the following antigens: CD45, CDlla (integrin alpha- L), CD 18 (integrin beta-2), CD1lb, CD11c, CD25, CD8, or CD4. In some embodiments, the modifying comprises attaching the immunomodulatory composition to the surface of the portion of TILs.

[0057] In certain embodiments of the methods provided herein, the modifying is carried out on TILs from the first expansion, or TILs from the second expansion, or both. In certain embodiments, the modifying is carried out on TILs from the priming first expansion, or TILs from the rapid second expansion, or both.

[0058] In some embodiments of the methods provided herein, the modifying is carried out after the first expansion and before the second expansion. In some embodiments, the modifying is carried out after the priming first expansion and before the rapid second expansion, or both. In certain embodiments, the modifying is carried out after the second expansion. In some embodiments, the modifying is carried out after the rapid second expansion. In some embodiments, the modifying is carried out after the harvesting.

[0059] In certain embodiments, the first expansion is performed over a period of about 11 days. In some embodiments, the priming first expansion is performed over a period of about 11 days.

[0060] In some embodiments of the methods provided herein, the IL-2 is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL in the cell culture medium in the first expansion. In certain embodiments, the IL-2 is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL in the cell culture medium in the priming first expansion.

[0061] In some embodiments, the IL-2 in the second expansion step is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL and the OKT-3 antibody is present at an initial concentration of about 30 ng/mL. In some embodiments, the IL-2 in the rapid

second expansion step is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL and the OKT-3 antibody is present at an initial concentration of about 30 ng/mL.

[0062] In some embodiments, the first expansion is performed using a gas permeable container. In certain embodiments, the priming first expansion is performed using a gas permeable container. In some embodiments, the second expansion is performed using a gas permeable container. In certain embodiments, the rapid second expansion is performed using a gas permeable container.

[0063] In some embodiments of the methods provided herein, the cell culture medium of the first expansion further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof. In certain embodiments, the cell culture medium of the priming first expansion further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof. In some embodiments, the cell culture medium of the second expansion further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof. In certain embodiments, the cell culture medium of the rapid second expansion further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

[0064] In some embodiments of the methods of treatment provided herein, the method further includes the step of treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the TILs to the patient. In some embodiments, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for three days. In some embodiments, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day and fludarabine at a dose of 25 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for three days. In certain embodiments, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day and fludarabine at a dose of 25 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for one day. In certain embodiments, the cyclophosphamide is administered with mesna.

[0065] In some embodiments of the methods of treatment provided herein, the method further includes the step of treating the patient with an IL-2 regimen starting on the day after

the administration of TILs to the patient. In some embodiments of the methods of treatment provided herein, the method further includes the step of treating the patient with an IL-2 regimen starting on the same day as administration of TILs to the patient. In some embodiments, the IL-2 regimen is a high-dose IL-2 regimen comprising 600,000 or 720,000 IU/kg of aldesleukin, or a biosimilar or variant thereof, administered as a 15-minute bolus intravenous infusion every eight hours until tolerance.

[0066] In some embodiments of the methods provided herein, the therapeutically effective population of TILs is administered and comprises from about 2.3×10^{10} to about 13.7×10^{10} TILs.

[0067] In some embodiments of the methods provided herein, the priming first expansion and rapid second expansion are performed over a period of 21 days or less. In some embodiments, the priming first expansion and rapid second expansion are performed over a period of 16 or 17 days or less. In certain embodiments, the priming first expansion is performed over a period of 7 or 8 days or less. In some embodiments, the rapid second expansion is performed over a period of 11 days or less.

[0068] In some embodiments, of the methods provided herein the first expansion in step (c) and the second expansion in step (d) are each individually performed within a period of 11 days. In some embodiments of the methods provided herein, steps (a) through (f) are performed in about 10 days to about 22 days.

[0069] In some embodiments of the methods provided herein, the modified TILs further comprise a genetic modification that causes expression of one or more immune checkpoint genes to be silenced or reduced in at least a portion of the therapeutic population of TILs. IN some embodiments, the one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, PKA, CBL-B, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, BTLA, CD160, TIGIT, TET2, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3, TOX, SOCS1, ANKRD11, and BCOR.. In certain embodiments, the one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, and PKA.

[0070] In some embodiments, the modified TILs further comprises a genetic modification that causes expression of one or more immune checkpoint genes to be enhanced in at least a portion of the therapeutic population of TILs, the immune checkpoint gene(s) being selected from the group comprising CCR2, CCR4, CCR5, CXCR2, CXCR3, CX3CR1, IL-2, IL-4, IL-7, IL-10, IL-15, IL-21, the NOTCH 1/2 intracellular domain (ICD), and/or the NOTCH ligand mDLL1. In certain embodiments, the genetic modification is produced using a programmable nuclease that mediates the generation of a double-strand or single-strand break at said one or more immune checkpoint genes. In some embodiments, the genetic modification is produced using one or more methods selected from a CRISPR method, a TALE method, a zinc finger method, and a combination thereof. In certain embodiments, the genetic modification is produced using a CRISPR method. In some embodiments, the CRISPR method is a CRISPR/Cas9 method. In certain embodiments, the genetic modification is produced using a TALE method. In some embodiments, the genetic modification is produced using a zinc finger method.

[0071] In some embodiments, the modified TILs are modified to transiently express the immunomodulatory composition on the cell surface. In some embodiments, the immunomodulatory composition comprises one or more membrane anchored immunomodulatory fusion proteins, wherein each fusion protein comprises one or more immunomodulatory agents and a cell membrane anchor moiety.

[0072] In exemplary embodiments, the one or more immunomodulatory agents comprise one or more cytokines. In some embodiments, the one or more cytokines comprise IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, or GCSF or a variant thereof.

[0073] In some embodiments, the one or more cytokines comprise IL-2. In some embodiments, the IL-2 is human IL-2. In exemplary embodiments, the human IL-2 has the amino acid sequence of SEQ ID NO:272.

[0074] In some embodiments, the one or more cytokines comprise IL-12. In certain embodiments, the IL-12 comprises a human IL-12 p35 subunit attached to a human IL-12 p40 subunit. In certain embodiments, the human IL-12 p35 subunit has the amino acid sequence of SEQ ID NO:267 and the human IL-12 p40 subunit has the amino acid sequence of SEQ ID NO:268.

[0075] In some embodiments, the one or more cytokines comprise IL-15. In some embodiments, the IL-15 is human IL-15. In exemplary embodiments, the human IL-15 has the amino acid sequence of SEQ ID NO:258.

[0076] In some embodiments, the one or more cytokines comprise IL-18. In certain embodiments, the IL-18 is human IL-18. In certain embodiments, the human IL-18 has the amino acid sequence of SEQ ID NO:269 or SEQ ID NO:270.

[0077] In some embodiments, the one or more cytokines comprise IL-21. In certain embodiments, the IL-21 is human IL-21. In some embodiments, the human IL-21 has the amino acid sequence of SEQ ID NO:271.

[0078] In some embodiments, the one or more cytokines comprise IL-15 and IL-21. In some embodiments, the IL-15 is human IL-15 and the IL-21 is human IL-21. In certain embodiments, the human IL-15 has the amino acid sequence of SEQ ID NO: 258 and the human IL-21 has the amino acid sequence of SEQ ID NO:271.

[0079] In some embodiments, the one or more immunomodulatory agents comprise a CD40 agonist. In certain embodiments, the CD40 agonist is an anti-CD40 binding domain or CD40L. In exemplary embodiments, the CD40 agonist is a CD40 binding domain comprising a variable heavy domain (VH) and a variable light domain (VL). In some embodiments, the VH and VL of the CD40 binding domain are selected from the following: a) a VH having the amino acid sequence of SEQ ID NO: 274, and a VL having the amino acid sequence of SEQ ID NO:275; b) a VH having the amino acid sequence of SEQ ID NO: 277, and a VL having the amino acid sequence of SEQ ID NO:278; c) a VH having the amino acid sequence of SEQ ID NO: 280, and a VL having the amino acid sequence of SEQ ID NO:281; and d) a VH having the amino acid sequence of SEQ ID NO: 283, and a VL having the amino acid sequence of SEQ ID NO:284. In exemplary embodiments, the CD40 binding domain is an scFv.

[0080] In some embodiments, the CD40 agonist is a human CD40L having the amino acid sequence of SEQ ID NO: 273. In some embodiments, the membrane anchored immunomodulatory fusion protein is according to the formula, from N- to C-terminus: S-IA-L-C, wherein S is a signal peptide, IA is an immunomodulatory agent, L is a linker and C is a cell membrane anchor moiety.

[0081] In some embodiments, the cell membrane anchor moiety comprises a CD8a transmembrane-intracellular domain, a B7-1 transmembrane domain, a B7-2 transmembrane

domain, or a CD8a transmembrane domain. In exemplary embodiments, the cell membrane anchor moiety comprises a B7-1 transmembrane domain. In some embodiments, the cell membrane anchor moiety has the amino acid sequence of SEQ ID NO:239.

[0082] In some embodiments, the immunomodulatory composition comprises two or more different membrane anchored immunomodulatory fusion proteins, wherein each of the different membrane anchored immunomodulatory fusion proteins each comprise a different immunomodulatory agent. In some embodiments, the different immunomodulatory agents are selected from: IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, GCSF or a variant thereof, and a CD40 agonist. In some embodiments, the different immunomodulatory agents are selected from: IL-12 and IL-15, IL-15 and IL-18, CD40L, IL-15 and IL-21, and IL-15, and IL-2 and IL-12.

[0083] In some embodiments, the modified TILs are modified by transfecting the TILs with a nucleic acid encoding a fusion protein comprising one or more immunomodulatory agents and a cell membrane anchor moiety in order to transiently express the fusion protein on the cell surface. In some embodiments, the nucleic acid is an RNA. In some embodiments, the RNA is a mRNA. In some embodiments, the TILs are transfected with the mRNA by electroporation. In some embodiments, the TILs are transfected with the mRNA by electroporation after the first expansion and before the second expansion. In some embodiments, the TILs are transfected with the mRNA by electroporation before the first expansion. In some embodiments, the method further comprises activating the TILs by incubation with an anti-CD3 agonist before transfecting the TILs with the mRNA. In some embodiments, the anti-CD3 agonist is OKT-3. In some embodiments, the TILs are activated by incubating the TILs with the anti-CD3 agonist for about 1 to 3 days before transfecting the TILs with the mRNA.

[0084] In some embodiments, the modified TILs are transfected with the nucleic acid encoding the fusion protein using a microfluidic device to temporarily disrupt the cell membranes of the TILs, thereby allowing transfection of the nucleic acid.

[0085] In some embodiments, artificial antigen-presenting cells (aAPCs) are used in place of APCs. In some embodiments, the aAPCs comprise a cell that expresses HLA-A/B/C, CD64, CD80, ICOS-L, and CD58. In some embodiments, the aAPCs comprise a MOLM-14 cell. In some embodiments, the aAPCs comprise a MOLM-13 cell. In some embodiments, the aAPCs comprise a MOLM-14 cell that endogenously expresses HLA-A/B/C, CD64, CD80, ICOS-L,

and CD58. In some embodiments, the aAPCs comprise a MOLM-14 cell that endogenously expresses HLA-A/B/C, CD64, CD80, ICOS-L, and CD58, wherein the MOLM-14 cell is permanently gene-edited to express CD86. In some embodiments, the MOLM-14 cell transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid sequence encoding CD86 and a nucleic acid sequence encoding 4-1BBL, and wherein the MOLM-14 cell expresses CD86 and 4-1BBL. In some embodiments, the aAPCs are transiently gene-edited to transiently express on the cell surface an immunomodulatory composition comprising an immunomodulatory fusion protein. In some embodiments, the aAPCs transiently express on the cell surface an immunomodulatory fusion protein comprising a membrane anchor fused to a cytokine. In some embodiments, the aAPCs transiently express on the cell surface a membrane anchor fused to a cytokine selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, and IL-21. In some embodiments, the aAPCs transiently express on the cell surface a membrane anchor fused to a cytokine selected from the group consisting of IL-2, IL-12, IL-15, and IL-21. In some embodiments, the aAPCs transiently express on the cell surface a membrane anchor fused to a cytokine selected from the group consisting of IL-12, IL-15, and IL-21.

[0086] In some embodiments, the modified TILs are genetically modified to express the immunomodulatory composition on the cell surface. In some embodiments, the immunomodulatory composition comprises one or more membrane anchored immunomodulatory fusion proteins each comprising one or more immunomodulatory agents and a cell membrane anchor moiety. In some embodiments, the one or more membrane anchored immunomodulatory fusion proteins comprise IL-2. In certain embodiments, the one or more membrane anchored immunomodulatory fusion proteins comprise IL-15. In exemplary embodiments, the one or more membrane anchored immunomodulatory fusion proteins comprise IL-18. In some embodiments, the one or more membrane anchored immunomodulatory fusion proteins comprise IL-21.

[0087] In certain embodiments, the modified TILs comprise a first membrane anchored immunomodulatory fusion protein and a second membrane anchored immunomodulatory fusion protein. In some embodiments, the first membrane anchored immunomodulatory fusion protein comprises IL-15 and the second membrane anchored immunomodulatory fusion protein comprises IL-21. In some embodiments, the first membrane anchored immunomodulatory fusion protein and the second immunomodulatory fusion protein are expressed under the control of an NFAT promoter in the modified TILs.

[0088] In some embodiments, the one or more membrane anchored immunomodulatory fusion proteins are independently according to the formula, from N- to C-terminus: S-IA-L-C, wherein S is a signal peptide, IA is an immunomodulatory agent, L is a linker and C is a cell membrane anchor moiety. In some embodiments, IA is a cytokine. In exemplary embodiments, IA is selected from the group consisting of: IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, or GCSF or a variant thereof. In some embodiments, IA is IL-2. In certain embodiments, IA is IL-12. In some embodiments, IA is IL-15. In certain embodiments, IA is IL-21. In some embodiments, L is a CD8a transmembrane-intracellular domain, a B7-1 transmembrane domain, a B7-2 transmembrane domain, or a CD8a transmembrane domain. In certain embodiments, L is a B7-1 transmembrane domain. In some embodiments, L has the amino acid sequence of SEQ ID NO:239.

[0089] In exemplary embodiments, the one or more membrane anchored immunomodulatory fusion proteins are independently according to the formula, from N- to C-terminus: S1-IA1-L1-C1-L2-S2-IA2-L3-C2, wherein S1 and S2 are each independently a signal peptide, IA1 and IA2 are each independently an immunomodulatory agent, L1-L3 are each independently a linker, and C1 and C2 are each independently a cell membrane anchor moiety. In some embodiments, S1 and S2 are the same. In exemplary embodiments, C1 and C2 are the same. In some embodiments, L2 is a cleavable linker. In certain embodiments, L2 is a furin cleavable linker.

[0090] In some embodiments, IA1 and IA2 are each independently a cytokine. In some embodiments, IA1 and IA2 are each independently selected from the group consisting of: IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, or GCSF or a variant thereof. In some embodiments, IA1 and IA2 are each independently selected from the group consisting of IL-2 and IL-12, with the proviso that one of IA1 and IA2 is IL-2 and the other is IL-12. In some embodiments, IA1 and IA2 are each independently selected from the group consisting of IL-15 and IL-21, with the proviso that one of IA1 and IA2 is IL-15 and the other is IL-21.

[0091] In exemplary embodiments, C1 and C2 are each independently a CD8a transmembrane-intracellular domain, a B7-1 transmembrane domain, a B7-2 transmembrane domain, or a CD8a transmembrane domain. In some embodiments, C1 and C2 are each a B7-1 transmembrane domain. In some embodiments, C1 and C2 each have the amino acid sequence of SEQ ID NO:239.

[0092] In certain embodiments, the modified TILs express the one or more membrane anchored immunomodulatory fusion proteins under the control of an NFAT promoter. In some embodiments, the modified TILs are transduced with a retroviral vector to express the one or more membrane anchored immunomodulatory fusion proteins. In some embodiments, the modified TILs are transduced with a lentiviral vector to express the one or more membrane anchored immunomodulatory fusion proteins.

BRIEF DESCRIPTION OF THE DRAWINGS

[0093] Figure 1: Exemplary Gen 2 (process 2A) chart providing an overview of Steps A through F.

[0094] Figure 2A-2C: Process flow chart of an embodiment of Gen 2 (process 2A) for TIL manufacturing.

[0095] Figure 3: Shows a diagram of an embodiment of a cryopreserved TIL exemplary manufacturing process (~22 days).

[0096] Figure 4: Shows a diagram of an embodiment of Gen 2 (process 2A), a 22-day process for TIL manufacturing.

[0097] Figure 5: Comparison table of Steps A through F from exemplary embodiments of process 1C and Gen 2 (process 2A) for TIL manufacturing.

[0098] Figure 6: Detailed comparison of an embodiment of process 1C and an embodiment of Gen 2 (process 2A) for TIL manufacturing.

[0099] Figure 7: Exemplary Gen 3 type TIL manufacturing process.

[00100] Figure 8A-8D: A) Shows a comparison between the 2A process (approximately 22-day process) and an embodiment of the Gen 3 process for TIL manufacturing (approximately 14-days to 16-days process). **B)** Exemplary Process Gen 3 chart providing an overview of Steps A through F (approximately 14-days to 16-days process). **C)** Chart providing three exemplary Gen 3 processes with an overview of Steps A through F (approximately 14-days to 16-days process) for each of the three process variations. **D)** Exemplary modified Gen 2-like process providing an overview of Steps A through F (approximately 22-days process).

[00101] **Figure 9:** Provides an experimental flow chart for comparability between Gen 2 (process 2A) versus Gen 3 processes.

[00102] **Figure 10:** Shows a comparison between various Gen 2 (process 2A) and the Gen 3.1 process embodiment.

[00103] **Figure 11:** Table describing various features of embodiments of the Gen 2, Gen 2.1 and Gen 3.0 process.

[00104] **Figure 12:** Overview of the media conditions for an embodiment of the Gen 3 process, referred to as Gen 3.1.

[00105] **Figure 13:** Table describing various features of embodiments of the Gen 2, Gen 2.1 and Gen 3.0 process.

[00106] **Figure 14:** Table comparing various features of embodiments of the Gen 2 and Gen 3.0 processes.

[00107] **Figure 15:** Table providing media uses in the various embodiments of the described expansion processes.

[00108] **Figure 16:** Schematic of an exemplary embodiment of the Gen 3 process (a 16-day process).

[00109] **Figure 17:** Schematic of an exemplary embodiment of a method for expanding T cells from hematopoietic malignancies using Gen 3 expansion platform.

[00110] **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 CH3 and CH2 domains) is then used to link two of the trivalent proteins together through disulfide bonds (small elongated ovals), stabilizing the structure and providing an agonists capable of bringing together the intracellular signaling domains of the six receptors and signaling proteins to form a signaling complex. The TNFRSF binding domains denoted as cylinders may be scFv domains comprising, *e.g.*, a 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.

[00111] **Figure 19:** Schematic of an exemplary embodiment of the Gen 3 process (a 16-day process).

[00112] **Figure 20:** Provides a process overview for an exemplary embodiment of the Gen 3.1 process (a 16 day process).

[00113] **Figure 21:** Schematic of an exemplary embodiment of the Gen 3.1 Test process (a 16-17 day process).

[00114] **Figure 22:** Schematic of an exemplary embodiment of the Gen 3 process (a 16-day process).

[00115] **Figure 23:** Comparison table for exemplary Gen 2 and exemplary Gen 3 processes.

[00116] **Figure 24:** Schematic of an exemplary embodiment of the Gen 3 process (a 16-17 day process) preparation timeline.

[00117] **Figure 25:** Schematic of an exemplary embodiment of the Gen 3 process (a 14-16 day process).

[00118] **Figure 26A-26B:** Schematic of an exemplary embodiment of the Gen 3 process (a 16 day process).

[00119] **Figure 27:** Schematic of an exemplary embodiment of the Gen 3 process (a 16 day process).

[00120] **Figure 28:** Comparison of Gen 2, Gen 2.1 and an embodiment of the Gen 3 process (a 16 day process).

[00121] **Figure 29:** Comparison of Gen 2, Gen 2.1 and an embodiment of the Gen 3 process (a 16 day process).

[00122] **Figure 30:** Gen 3 embodiment components.

[00123] **Figure 31:** Gen 3 embodiment flow chart comparison (Gen 3.0, Gen 3.1 control, Gen 3.1 test).

[00124] **Figure 32:** Shown are the components of an exemplary embodiment of the Gen 3 process (a 16-17 day process).

[00125] **Figure 33:** Acceptance criteria table.

[00126] **Figure 34:** Depiction of some embodiments of a TIL manufacturing process including electroporation step for use with gene-editing processes (including TALEN, zinc finger nuclease, and CRISPR methods as described herein).

[00127] Figure 35: Depiction of embodiments of TIL manufacturing processes including electroporation step for use with gene-editing processes (including TALEN, zinc finger nuclease, and CRISPR methods as described herein).

[00128] Figure 36: Exemplary membrane anchored immunomodulatory fusion proteins that can be included in the TILs described herein.

[00129] Figure 37: Exemplary membrane anchored immunomodulatory fusion proteins that can be included in the TILs described herein.

[00130] Figure 38: Summary of study to assess expression and signaling of membrane bound IL-15/IL-21 transduced pre-REP TILs.

[00131] Figure 39: Summary of study to assess expression of mIL-15/IL21 and CD8 and CD4 T cell subset in mIL-15/IL-21 transduced REP TILs.

[00132] Figure 40: Summary of study to assess phenotype of mIL-15/IL-21 transduced CD8+ REP TILs.

[00133] Figure 41: Summary of study to assess phenotype of mIL-15/IL-21 transduced CD4+.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

[00134] SEQ ID NO:1 is the amino acid sequence of the heavy chain of muromonab.

[00135] SEQ ID NO:2 is the amino acid sequence of the light chain of muromonab.

[00136] SEQ ID NO:3 is the amino acid sequence of a recombinant human IL-2 protein.

[00137] SEQ ID NO:4 is the amino acid sequence of aldesleukin.

[00138] SEQ ID NO:5 is an IL-2 form.

[00139] SEQ ID NO:6 is the amino acid sequence of nemvaleukin alfa.

[00140] SEQ ID NO:7 is an IL-2 form.

[00141] SEQ ID NO:8 is a mucin domain polypeptide.

[00142] SEQ ID NO:9 is the amino acid sequence of a recombinant human IL-4 protein.

[00143] SEQ ID NO:10 is the amino acid sequence of a recombinant human IL-7 protein.

[00144] SEQ ID NO:11 is the amino acid sequence of a recombinant human IL-15 protein.

[00145] SEQ ID NO:12 is the amino acid sequence of a recombinant human IL-21 protein.

[00146] SEQ ID NO:13 is an IL-2 sequence.

[00147] SEQ ID NO:14 is an IL-2 mutein sequence.

[00148] SEQ ID NO:15 is an IL-2 mutein sequence.

[00149] SEQ ID NO:16 is the HCDR1_IL-2 for IgG.IL2R67A.H1.

[00150] SEQ ID NO:17 is the HCDR2 for IgG.IL2R67A.H1.

[00151] SEQ ID NO:18 is the HCDR3 for IgG.IL2R67A.H1.

[00152] SEQ ID NO:19 is the HCDR1_IL-2 kabat for IgG.IL2R67A.H1.

[00153] SEQ ID NO:20 is the HCDR2 kabat for IgG.IL2R67A.H1.

[00154] SEQ ID NO:21 is the HCDR3 kabat for IgG.IL2R67A.H1.

[00155] SEQ ID NO:22 is the HCDR1_IL-2 clothia for IgG.IL2R67A.H1.

[00156] SEQ ID NO:23 is the HCDR2 clothia for IgG.IL2R67A.H1.

[00157] SEQ ID NO:24 is the HCDR3 clothia for IgG.IL2R67A.H1.

[00158] SEQ ID NO:25 is the HCDR1_IL-2 IMGT for IgG.IL2R67A.H1.

[00159] SEQ ID NO:26 is the HCDR2 IMGT for IgG.IL2R67A.H1.

[00160] SEQ ID NO:27 is the HCDR3 IMGT for IgG.IL2R67A.H1.

[00161] SEQ ID NO:28 is the V_H chain for IgG.IL2R67A.H1.

[00162] SEQ ID NO:29 is the heavy chain for IgG.IL2R67A.H1.

[00163] SEQ ID NO:30 is the LCDR1 kabat for IgG.IL2R67A.H1.

[00164] SEQ ID NO:31 is the LCDR2 kabat for IgG.IL2R67A.H1.

[00165] SEQ ID NO:32 is the LCDR3 kabat for IgG.IL2R67A.H1.

[00166] SEQ ID NO:33 is the LCDR1 chothia for IgG.IL2R67A.H1.

[00167] SEQ ID NO:34 is the LCDR2 chothia for IgG.IL2R67A.H1.

[00168] SEQ ID NO:35 is the LCDR3 chothia for IgG.IL2R67A.H1.

[00169] SEQ ID NO:36 is a V_L chain.

[00170] SEQ ID NO:37 is a light chain.

[00171] SEQ ID NO:38 is a light chain.

[00172] SEQ ID NO:39 is a light chain.

[00173] SEQ ID NO:40 is the amino acid sequence of human 4-1BB.

[00174] SEQ ID NO:41 is the amino acid sequence of murine 4-1BB.

[00175] SEQ ID NO:42 is the heavy chain for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00176] SEQ ID NO:43 is the light chain for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00177] SEQ ID NO:44 is the heavy chain variable region (V_H) for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00178] SEQ ID NO:45 is the light chain variable region (V_L) for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00179] SEQ ID NO:46 is the heavy chain CDR1 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00180] SEQ ID NO:47 is the heavy chain CDR2 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00181] SEQ ID NO:48 is the heavy chain CDR3 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00182] SEQ ID NO:49 is the light chain CDR1 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00183] SEQ ID NO:50 is the light chain CDR2 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00184] SEQ ID NO:51 is the light chain CDR3 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00185] SEQ ID NO:52 is the heavy chain for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00186] SEQ ID NO:53 is the light chain for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00187] SEQ ID NO:54 is the heavy chain variable region (VH) for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00188] SEQ ID NO:55 is the light chain variable region (VL) for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00189] SEQ ID NO:56 is the heavy chain CDR1 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00190] SEQ ID NO:57 is the heavy chain CDR2 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00191] SEQ ID NO:58 is the heavy chain CDR3 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00192] SEQ ID NO:59 is the light chain CDR1 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00193] SEQ ID NO:60 is the light chain CDR2 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00194] SEQ ID NO:61 is the light chain CDR3 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00195] SEQ ID NO:62 is an Fc domain for a TNFRSF agonist fusion protein.

[00196] SEQ ID NO:63 is a linker for a TNFRSF agonist fusion protein.

[00197] SEQ ID NO:64 is a linker for a TNFRSF agonist fusion protein.

[00198] SEQ ID NO:65 is a linker for a TNFRSF agonist fusion protein.

[00199] SEQ ID NO:66 is a linker for a TNFRSF agonist fusion protein.

[00200] SEQ ID NO:67 is a linker for a TNFRSF agonist fusion protein.

[00201] SEQ ID NO:68 is a linker for a TNFRSF agonist fusion protein.

[00202] SEQ ID NO:69 is a linker for a TNFRSF agonist fusion protein.

[00203] SEQ ID NO:70 is a linker for a TNFRSF agonist fusion protein.

[00204] SEQ ID NO:71 is a linker for a TNFRSF agonist fusion protein.

[00205] SEQ ID NO:72 is a linker for a TNFRSF agonist fusion protein.

[00206] SEQ ID NO:73 is an Fc domain for a TNFRSF agonist fusion protein.

[00207] SEQ ID NO:74 is a linker for a TNFRSF agonist fusion protein.

[00208] SEQ ID NO:75 is a linker for a TNFRSF agonist fusion protein.

[00209] SEQ ID NO:76 is a linker for a TNFRSF agonist fusion protein.

[00210] SEQ ID NO:77 is a 4-1BB ligand (4-1BBL) amino acid sequence.

[00211] SEQ ID NO:78 is a soluble portion of 4-1BBL polypeptide.

[00212] SEQ ID NO:79 is a heavy chain variable region (V_H) for the 4-1BB agonist antibody 4B4-1-1 version 1.

[00213] SEQ ID NO:80 is a light chain variable region (V_L) for the 4-1BB agonist antibody 4B4-1-1 version 1.

[00214] SEQ ID NO:81 is a heavy chain variable region (V_H) for the 4-1BB agonist antibody 4B4-1-1 version 2.

[00215] SEQ ID NO:82 is a light chain variable region (V_L) for the 4-1BB agonist antibody 4B4-1-1 version 2.

[00216] SEQ ID NO:83 is a heavy chain variable region (V_H) for the 4-1BB agonist antibody H39E3-2.

[00217] SEQ ID NO:84 is a light chain variable region (V_L) for the 4-1BB agonist antibody H39E3-2.

[00218] SEQ ID NO:85 is the amino acid sequence of human OX40.

[00219] SEQ ID NO:86 is the amino acid sequence of murine OX40.

[00220] SEQ ID NO:87 is the heavy chain for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00221] SEQ ID NO:88 is the light chain for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00222] SEQ ID NO:89 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00223] SEQ ID NO:90 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00224] SEQ ID NO:91 is the heavy chain CDR1 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00225] SEQ ID NO:92 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00226] SEQ ID NO:93 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00227] SEQ ID NO:94 is the light chain CDR1 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00228] SEQ ID NO:95 is the light chain CDR2 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00229] SEQ ID NO:96 is the light chain CDR3 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00230] SEQ ID NO:97 is the heavy chain for the OX40 agonist monoclonal antibody 11D4.

[00231] SEQ ID NO:98 is the light chain for the OX40 agonist monoclonal antibody 11D4.

[00232] SEQ ID NO:99 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 11D4.

[00233] SEQ ID NO:100 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 11D4.

[00234] SEQ ID NO:101 is the heavy chain CDR1 for the OX40 agonist monoclonal antibody 11D4.

[00235] SEQ ID NO:102 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody 11D4.

[00236] SEQ ID NO:103 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody 11D4.

[00237] SEQ ID NO:104 is the light chain CDR1 for the OX40 agonist monoclonal antibody 11D4.

[00238] SEQ ID NO:105 is the light chain CDR2 for the OX40 agonist monoclonal antibody 11D4.

[00239] SEQ ID NO:106 is the light chain CDR3 for the OX40 agonist monoclonal antibody 11D4.

[00240] SEQ ID NO:107 is the heavy chain for the OX40 agonist monoclonal antibody 18D8.

[00241] SEQ ID NO:108 is the light chain for the OX40 agonist monoclonal antibody 18D8.

[00242] SEQ ID NO:109 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 18D8.

[00243] SEQ ID NO:110 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 18D8.

[00244] SEQ ID NO:111 is the heavy chain CDR1 for the OX40 agonist monoclonal antibody 18D8.

[00245] SEQ ID NO:112 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody 18D8.

[00246] SEQ ID NO:113 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody 18D8.

[00247] SEQ ID NO:114 is the light chain CDR1 for the OX40 agonist monoclonal antibody 18D8.

[00248] SEQ ID NO:115 is the light chain CDR2 for the OX40 agonist monoclonal antibody 18D8.

[00249] SEQ ID NO:116 is the light chain CDR3 for the OX40 agonist monoclonal antibody 18D8.

[00250] SEQ ID NO:117 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody Hu119-122.

[00251] SEQ ID NO:118 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody Hu119-122.

[00252] SEQ ID NO:119 is the heavy chain CDR1 for the OX40 agonist monoclonal antibody Hu119-122.

[00253] SEQ ID NO:120 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody Hu119-122.

[00254] SEQ ID NO:121 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody Hu119-122.

[00255] SEQ ID NO:122 is the light chain CDR1 for the OX40 agonist monoclonal antibody Hu119-122.

[00256] SEQ ID NO:123 is the light chain CDR2 for the OX40 agonist monoclonal antibody Hu119-122.

[00257] SEQ ID NO:124 is the light chain CDR3 for the OX40 agonist monoclonal antibody Hu119-122.

[00258] SEQ ID NO:125 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody Hu106-222.

[00259] SEQ ID NO:126 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody Hu106-222.

[00260] SEQ ID NO:127 is the heavy chain CDR1 for the OX40 agonist monoclonal antibody Hu106-222.

[00261] SEQ ID NO:128 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody Hu106-222.

[00262] SEQ ID NO:129 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody Hu106-222.

[00263] SEQ ID NO:130 is the light chain CDR1 for the OX40 agonist monoclonal antibody Hu106-222.

[00264] SEQ ID NO:131 is the light chain CDR2 for the OX40 agonist monoclonal antibody Hu106-222.

[00265] SEQ ID NO:132 is the light chain CDR3 for the OX40 agonist monoclonal antibody Hu106-222.

[00266] SEQ ID NO:133 is an OX40 ligand (OX40L) amino acid sequence.

[00267] SEQ ID NO:134 is a soluble portion of OX40L polypeptide.

[00268] SEQ ID NO:135 is an alternative soluble portion of OX40L polypeptide.

[00269] SEQ ID NO:136 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 008.

[00270] SEQ ID NO:137 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 008.

[00271] SEQ ID NO:138 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 011.

[00272] SEQ ID NO:139 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 011.

[00273] SEQ ID NO:140 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 021.

[00274] SEQ ID NO:141 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 021.

[00275] SEQ ID NO:142 is the heavy chain variable region (V_H) for the OX40 agonist monoclonal antibody 023.

[00276] SEQ ID NO:143 is the light chain variable region (V_L) for the OX40 agonist monoclonal antibody 023.

[00277] SEQ ID NO:144 is the heavy chain variable region (V_H) for an OX40 agonist monoclonal antibody.

[00278] SEQ ID NO:145 is the light chain variable region (V_L) for an OX40 agonist monoclonal antibody.

[00279] SEQ ID NO:146 is the heavy chain variable region (V_H) for an OX40 agonist monoclonal antibody.

[00280] SEQ ID NO:147 is the light chain variable region (V_L) for an OX40 agonist monoclonal antibody.

[00281] SEQ ID NO:148 is the heavy chain variable region (V_H) for a humanized OX40 agonist monoclonal antibody.

[00282] SEQ ID NO:149 is the heavy chain variable region (V_H) for a humanized OX40 agonist monoclonal antibody.

[00283] SEQ ID NO:150 is the light chain variable region (V_L) for a humanized OX40 agonist monoclonal antibody.

[00284] SEQ ID NO:151 is the light chain variable region (V_L) for a humanized OX40 agonist monoclonal antibody.

[00285] SEQ ID NO:152 is the heavy chain variable region (V_H) for a humanized OX40 agonist monoclonal antibody.

[00286] SEQ ID NO:153 is the heavy chain variable region (V_H) for a humanized OX40 agonist monoclonal antibody.

[00287] SEQ ID NO:154 is the light chain variable region (V_L) for a humanized OX40 agonist monoclonal antibody.

[00288] SEQ ID NO:155 is the light chain variable region (V_L) for a humanized OX40 agonist monoclonal antibody.

[00289] SEQ ID NO:156 is the heavy chain variable region (V_H) for an OX40 agonist monoclonal antibody.

[00290] SEQ ID NO:157 is the light chain variable region (V_L) for an OX40 agonist monoclonal antibody.

[00291] SEQ ID NO:158 is the heavy chain amino acid sequence of the PD-1 inhibitor nivolumab.

[00292] SEQ ID NO:159 is the light chain amino acid sequence of the PD-1 inhibitor nivolumab.

[00293] SEQ ID NO:160 is the heavy chain variable region (V_H) amino acid sequence of the PD-1 inhibitor nivolumab.

[00294] SEQ ID NO:161 is the light chain variable region (V_L) amino acid sequence of the PD-1 inhibitor nivolumab.

[00295] SEQ ID NO:162 is the heavy chain CDR1 amino acid sequence of the PD-1 inhibitor nivolumab.

[00296] SEQ ID NO:163 is the heavy chain CDR2 amino acid sequence of the PD-1 inhibitor nivolumab.

[00297] SEQ ID NO:164 is the heavy chain CDR3 amino acid sequence of the PD-1 inhibitor nivolumab.

[00298] SEQ ID NO:165 is the light chain CDR1 amino acid sequence of the PD-1 inhibitor nivolumab.

[00299] SEQ ID NO:166 is the light chain CDR2 amino acid sequence of the PD-1 inhibitor nivolumab.

[00300] SEQ ID NO:167 is the light chain CDR3 amino acid sequence of the PD-1 inhibitor nivolumab.

[00301] SEQ ID NO:168 is the heavy chain amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00302] SEQ ID NO:169 is the light chain amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00303] SEQ ID NO:170 is the heavy chain variable region (V_H) amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00304] SEQ ID NO:171 is the light chain variable region (V_L) amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00305] SEQ ID NO:172 is the heavy chain CDR1 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00306] SEQ ID NO:173 is the heavy chain CDR2 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00307] SEQ ID NO:174 is the heavy chain CDR3 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00308] SEQ ID NO:175 is the light chain CDR1 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00309] SEQ ID NO:176 is the light chain CDR2 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00310] SEQ ID NO:177 is the light chain CDR3 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00311] SEQ ID NO:178 is the heavy chain amino acid sequence of the PD-L1 inhibitor durvalumab.

[00312] SEQ ID NO:179 is the light chain amino acid sequence of the PD-L1 inhibitor durvalumab.

[00313] SEQ ID NO:180 is the heavy chain variable region (V_H) amino acid sequence of the PD-L1 inhibitor durvalumab.

[00314] SEQ ID NO:181 is the light chain variable region (V_L) amino acid sequence of the PD-L1 inhibitor durvalumab.

[00315] SEQ ID NO:182 is the heavy chain CDR1 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00316] SEQ ID NO:183 is the heavy chain CDR2 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00317] SEQ ID NO:184 is the heavy chain CDR3 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00318] SEQ ID NO:185 is the light chain CDR1 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00319] SEQ ID NO:186 is the light chain CDR2 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00320] SEQ ID NO:187 is the light chain CDR3 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00321] SEQ ID NO:188 is the heavy chain amino acid sequence of the PD-L1 inhibitor avelumab.

[00322] SEQ ID NO:189 is the light chain amino acid sequence of the PD-L1 inhibitor avelumab.

[00323] SEQ ID NO:190 is the heavy chain variable region (V_H) amino acid sequence of the PD-L1 inhibitor avelumab.

[00324] SEQ ID NO:191 is the light chain variable region (V_L) amino acid sequence of the PD-L1 inhibitor avelumab.

[00325] SEQ ID NO:192 is the heavy chain CDR1 amino acid sequence of the PD-L1 inhibitor avelumab.

[00326] SEQ ID NO:193 is the heavy chain CDR2 amino acid sequence of the PD-L1 inhibitor avelumab.

[00327] SEQ ID NO:194 is the heavy chain CDR3 amino acid sequence of the PD-L1 inhibitor avelumab.

[00328] SEQ ID NO:195 is the light chain CDR1 amino acid sequence of the PD-L1 inhibitor avelumab.

[00329] SEQ ID NO:196 is the light chain CDR2 amino acid sequence of the PD-L1 inhibitor avelumab.

[00330] SEQ ID NO:197 is the light chain CDR3 amino acid sequence of the PD-L1 inhibitor avelumab.

[00331] SEQ ID NO:198 is the heavy chain amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00332] SEQ ID NO:199 is the light chain amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00333] SEQ ID NO:200 is the heavy chain variable region (V_H) amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00334] SEQ ID NO:201 is the light chain variable region (V_L) amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00335] SEQ ID NO:202 is the heavy chain CDR1 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00336] SEQ ID NO:203 is the heavy chain CDR2 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00337] SEQ ID NO:204 is the heavy chain CDR3 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00338] SEQ ID NO:205 is the light chain CDR1 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00339] SEQ ID NO:206 is the light chain CDR2 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00340] SEQ ID NO:207 is the light chain CDR3 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00341] SEQ ID NO:208 is the heavy chain amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00342] SEQ ID NO:209 is the light chain amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00343] SEQ ID NO:210 is the heavy chain variable region (V_H) amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00344] SEQ ID NO:211 is the light chain variable region (V_L) amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00345] SEQ ID NO:212 is the heavy chain CDR1 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00346] SEQ ID NO:213 is the heavy chain CDR2 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00347] SEQ ID NO:214 is the heavy chain CDR3 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00348] SEQ ID NO:215 is the light chain CDR1 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00349] SEQ ID NO:216 is the light chain CDR2 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00350] SEQ ID NO:217 is the light chain CDR3 amino acid sequence of the CTLA-4 inhibitor ipilimumab.

[00351] SEQ ID NO:218 is the heavy chain amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00352] SEQ ID NO:219 is the light chain amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00353] SEQ ID NO:220 is the heavy chain variable region (V_H) amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00354] SEQ ID NO:221 is the light chain variable region (V_L) amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00355] SEQ ID NO:222 is the heavy chain CDR1 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00356] SEQ ID NO:223 is the heavy chain CDR2 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00357] SEQ ID NO:224 is the heavy chain CDR3 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00358] SEQ ID NO:225 is the light chain CDR1 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00359] SEQ ID NO:226 is the light chain CDR2 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00360] SEQ ID NO:227 is the light chain CDR3 amino acid sequence of the CTLA-4 inhibitor tremelimumab.

[00361] SEQ ID NO:228 is the heavy chain amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00362] SEQ ID NO:229 is the light chain amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00363] SEQ ID NO:230 is the heavy chain variable region (V_H) amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00364] SEQ ID NO:231 is the light chain variable region (V_L) amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00365] SEQ ID NO:232 is the heavy chain CDR1 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00366] SEQ ID NO:233 is the heavy chain CDR2 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00367] SEQ ID NO:234 is the heavy chain CDR3 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00368] SEQ ID NO:235 is the light chain CDR1 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00369] SEQ ID NO:236 is the light chain CDR2 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00370] SEQ ID NO:237 is the light chain CDR3 amino acid sequence of the CTLA-4 inhibitor zalifrelimab.

[00371] SEQ ID NO:238 is a CD8a transmembrane domain.

[00372] SEQ ID NO:239 is a B7-1 transmembrane-intracellular domain

[00373] SEQ ID NOs:240-245 are exemplary glycine-serine linkers that are useful in the immunomodulatory fusion proteins described herein.

[00374] SEQ ID NO:246 is an exemplary linker that is useful in the immunomodulatory fusion proteins described herein.

[00375] SEQ ID NO:247 is a 2A peptide C-terminus sequence.

[00376] SEQ ID NO:248 is a porcine teschovirus-1 2A peptide.

[00377] SEQ ID NO:249 is an equine rhinitis A virus 2A peptide.

[00378] SEQ ID NO:250 is a foot-and-mouth disease virus 2A peptide.

[00379] SEQ ID NO:251 is an exemplary furin-cleavable 2A peptide.

[00380] SEQ ID NOs:252 and 253 are human IgE signal peptide sequences.SEQ ID NO:254 is a human IL-2 signal peptide sequence.

[00381] SEQ ID NO:255 is a 6X NFAT IL-2 minimal promoter.

[00382] SEQ ID NO:256 is an NFAT responsive element.

[00383] SEQ ID NO:557 is a human IL-2 promoter sequence.

[00384] SEQ ID NO:258 is human IL-15 (N72D mutant).

[00385] SEQ ID NO:259 is human IL-15R-alpha-Su/Fc domain.

[00386] SEQ ID NO:260 is human IL-15R-alpha-Su (65aa truncated extracellular domain).

[00387] SEQ ID NO:261 is human IL-15 isoform 2.

[00388] SEQ ID NO:262 is human IL-15 isoform 1.

[00389] SEQ ID NO:263 is human IL-15 (without signal peptide).

[00390] SEQ ID NO:264 is human IL-15R-alpha (85 aa truncated extracellular domain).

[00391] SEQ ID NO:265 is human IL-15R-alpha (182aa truncated extracellular domain).

[00392] SEQ ID NO:266 is human IL-15R-alpha.

[00393] SEQ ID NO:267 is human IL-12 p35 subunit.

[00394] SEQ ID NO:268 is human IL-12 p40 subunit.

[00395] SEQ ID NO:269 is human IL-18

[00396] SEQ ID NO:270is a human IL-18 variant

[00397] SEQ ID NO:271 is human IL-21.

[00398] SEQ ID NO: 272 is human IL-2

[00399] SEQ ID NO:273 is human CD40L

[00400] SEQ ID NO:274 is agonistic anti-human CD40 VH (Sotigalimab)

[00401] SEQ ID NO:275 is agonistic anti-human CD40 VL (Sotigalimab)

[00402] SEQ ID NO:276 is agonistic anti-human CD40 scFv (Sotigalimab)

[00403] SEQ ID NO:277 is agonistic anti-human CD40 VH (Dacetuzumab)

[00404] SEQ ID NO:278 is agonistic anti-human CD40 VL (Dacetuzumab)

[00405] SEQ ID NO:279 is agonistic anti-human CD40 scFv (Dacetuzumab)

[00406] SEQ ID NO:280 is agonistic anti-human CD40 VH (Lucatutuzumab)

[00407] SEQ ID NO:281 is agonistic anti-human CD40 VL (Lucatutuzumab)

[00408] SEQ ID NO:282 is agonistic anti-human CD40 scFv (Lucatutuzumab)

[00409] SEQ ID NO:283 is agonistic anti-human CD40 VH (Selicrelumab)

[00410] SEQ ID NO:284 is agonistic anti-human CD40 VL (Selicrelumab)

[00411] SEQ ID NO:285 is agonistic anti-human CD40 scFv (Selicrelumab)

[00412] SEQ ID NO:286 is a target PD-1 sequence.

[00413] SEQ ID NO:287 is a target PD-1 sequence.

[00414] SEQ ID NO:288 is a repeat PD-1 left repeat sequence.

[00415] SEQ ID NO:289 is a repeat PD-1 right repeat sequence.

[00416] SEQ ID NO:290 is a repeat PD-1 left repeat sequence.

[00417] SEQ ID NO:291 is a repeat PD-1 right repeat sequence.

[00418] SEQ ID NO:292 is a PD-1 left TALEN nuclease sequence.

[00419] SEQ ID NO:293 is a PD-1 right TALEN nuclease sequence.

[00420] SEQ ID NO:294 is a PD-1 left TALEN nuclease sequence.

[00421] SEQ ID NO:295 is a PD-1 right TALEN nuclease sequence.

[00422] SEQ ID NO:296 is a nucleic acid sequence that encodes for the tethered IL-15 of SEQ ID NO:328

[00423] SEQ ID NO:297 is a nucleic acid sequence that encodes for the tethered IL-21 fusion protein of SEQ ID NO:.

[00424] SEQ ID NO:298 is a nucleic acid sequence that encodes for the tethered IL-15 fusion protein of SEQ ID NO:328 and tether IL-21 fusion protein of SEQ ID NO:331.

[00425] SEQ ID NO:299 is a nucleic acid sequence that encodes for the tethered IL-12 fusion protein of SEQ ID NO:303. The nucleic acid sequence includes an NFAT promoter.

[00426] SEQ ID NO:300 is a nucleic acid sequence that encodes for the tethered IL-15 fusion protein of SEQ ID NO:328. The nucleic acid sequence includes an NFAT promoter.

[00427] SEQ ID NO:301 is a nucleic acid sequence that encodes for the tethered IL-21 fusion protein of SEQ ID NO:XX. The nucleic acid sequence includes an NFAT promoter.

[00428] SEQ ID NO:302 is a nucleic acid sequence that encodes for the tethered IL-15 fusion protein of SEQ ID NO:328 and tether IL-21 fusion protein of SEQ ID NO:331. The nucleic acid sequence includes an NFAT promoter.

[00429] SEQ ID NO:303 is the amino acid sequence of an exemplary tethered IL-12 (tethered IL-12-Lr1-Ar2).

[00430] SEQ ID NO:304 is a nucleic acid sequence that encodes for the tethered IL-12 of SEQ ID NO:303.

[00431] SEQ ID NO:305 is the amino acid sequence of an exemplary tethered IL-18 (tethered IL-18-Lr1-Ar2).

[00432] SEQ ID NO:306 is a nucleic acid sequence that encodes for the tethered IL-18 of SEQ ID NO:305.

[00433] SEQ ID NO:307 is the amino acid sequence of an exemplary tethered variant IL-18 (tethered DR-IL-18 (6-27 variant)-Lr1-Ar2).

[00434] SEQ ID NO:308 is a nucleic acid sequence that encodes for the tethered variant IL-18 of SEQ ID NO:307.

[00435] SEQ ID NO:309 is the amino acid sequence of an exemplary tethered IL-12/IL-15.

[00436] SEQ ID NO:310 is a nucleic acid sequence that encodes for the tethered IL-12/IL-15 of SEQ ID NO:309.

[00437] SEQ ID NO:311 is the amino acid sequence of an exemplary tethered IL-18/IL-15.

[00438] SEQ ID NO:312 is a nucleic acid sequence that encodes for the tethered IL-18/IL-15 of SEQ ID NO:311.

[00439] SEQ ID NO:313 is the amino acid sequence of an exemplary tethered anti-CD40scFV (APX005M).

[00440] SEQ ID NO:314 is a nucleic acid sequence that encodes for the tethered anti-CD40scFV (APX005M) of SEQ ID NO:313.

[00441] SEQ ID NO:315 is the amino acid sequence of an exemplary tethered anti-CD40scFV (Dacetuzumab).

[00442] SEQ ID NO:316 is a nucleic acid sequence that encodes for the tethered anti-CD40scFV (Dacetuzumab) of SEQ ID NO:315.

[00443] SEQ ID NO:317 is the amino acid sequence of an exemplary tethered anti-CD40scFV (Lucatutuzumab).

[00444] SEQ ID NO:318 is a nucleic acid sequence that encodes for the tethered anti-CD40scFV (Lucatutuzumab) of SEQ ID NO:317.

[00445] SEQ ID NO:319 is the amino acid sequence of an exemplary tethered anti-CD40scFV (Selicrelumab).

[00446] SEQ ID NO:320 is a nucleic acid sequence that encodes for the tethered anti-CD40scFV (Selicrelumab) of SEQ ID NO:319.

[00447] SEQ ID NO:321 is a nucleic acid sequence that encodes for the CD40L of SEQ ID NO:273.

[00448] SEQ ID NO:322 is the amino acid sequence an exemplary tethered CD40L/IL-15.

[00449] SEQ ID NO:323 is a nucleic acid sequence that encodes for the tethered CD40L/IL-15 of SEQ ID NO:311.

[00450] SEQ ID NO:324 is the amino acid sequence of an exemplary tethered IL-2.

[00451] SEQ ID NO:325 is a nucleic acid sequence that encodes for the tethered IL-2 of SEQ ID NO:313.

[00452] SEQ ID NO:326 is the amino acid sequence of an exemplary tethered IL-12.

[00453] SEQ ID NO:327 is a nucleic acid sequence that encodes for the tethered IL-12 of SEQ ID NO:315.

[00454] SEQ ID NO:328 is the amino acid sequence of an exemplary tethered IL-15.

[00455] SEQ ID NO:329 is a nucleic acid sequence that encodes for the tethered IL-15 of SEQ ID NO:317.

[00456] SEQ ID NO:330 is a nucleic acid sequence that encodes for GFP.

DETAILED DESCRIPTION

I. Introduction

[00457] Adoptive cell therapy utilizing TILs is an effective approach for inducing tumor regression in various cancers, including leukemias and melanoma. The use of adjuvants that include immunostimulatory agents has been explored to enhance adoptive cell therapies and to extend such therapies to other solid tumors. Co-administration of immunomodulators such as cytokines (*e.g.*, interleukins), however, can lead to undesirably toxicity due to the high dosages required. Thus, supplying such adjuvants at the right time and site appears crucial to avoid such undesirable effects.

[00458] Provided herein are compositions and methods for the treatment of cancers using modified TILs, wherein the modified TILs include one or more immunomodulatory agents (*e.g.*, cytokines) associated with their cell surface. The immunomodulatory agents associated with the TILs provide a localized immunostimulatory effect that can advantageously enhance TIL survival and/or anti-tumor activity in a patient recipient. As such, the compositions and methods disclosed herein provide effective cancer therapies.

II. Definitions

[00459] 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.

[00460] The terms "co-administration," "co-administering," "administered in combination with," "administering in combination with," "simultaneous," and "concurrent," as used herein, encompass administration of two or more active pharmaceutical ingredients (in a preferred embodiment of the present invention, for example, 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.

[00461] The term "*in vivo*" refers to an event that takes place in a subject's body.

[00462] 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.

[00463] 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.

[00464] 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.

[00465] 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.

[00466] By "population of cells" (including TILs) herein is meant a number of cells that share common traits. In general, populations generally range from 1×10^6 to 1×10^{10} in number, with different TIL populations comprising different numbers. For example, initial

growth of primary TILs in the presence of IL-2 results in a population of bulk TILs of roughly 1×10^8 cells. REP expansion is generally done to provide populations of 1.5×10^9 to 1.5×10^{10} cells for infusion.

[00467] 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.

[00468] 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.

[00469] 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.

[00470] 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.

[00471] 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.

[00472] 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.

[00473] 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.

[00474] 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.

[00475] 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.

[00476] 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+.

[00477] 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.

[00478] 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

[00479] 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.

[00480] 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.

[00481] 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	60 120 180 240

	GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK	300 360 420 452
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 recombinant human IL-7 (rhIL-7)	MDCDIEGKDG KQYESVLMVS IDQLLDSMKE IGSNCLNNEF NFFKRHICDA NKEGMFLFRA ARKLRQFLKM NSTGDFDLHL LKVSEGTTIL LNCTGQVKGR KPAALGEAQP TKSLEENKSL KEQKKLNDLC FLKRLLQEIK TCWNKILMGT KEH	60 120 153
SEQ ID NO: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

[00482] In some embodiments, an IL-2 form suitable for use in the invention includes a antibody cytokine engrafted protein comprises a heavy chain variable region (V_H), comprising complementarity determining regions HCDR1, HCDR2, HCDR3; a light chain variable region (V_L), comprising LCDR1, LCDR2, LCDR3; and an IL-2 molecule or a fragment thereof engrafted into a CDR of the V_H or the V_L, wherein the antibody cytokine engrafted protein preferentially expands T effector cells over regulatory T cells. In some embodiments, the antibody cytokine engrafted protein comprises a heavy chain variable region (V_H), comprising complementarity determining regions HCDR1, HCDR2, HCDR3; a light chain variable region (V_L), comprising LCDR1, LCDR2, LCDR3; and an IL-2 molecule or a fragment thereof engrafted into a CDR of the V_H or the V_L, wherein the IL-2 molecule is a mutein, and wherein the antibody cytokine engrafted protein preferentially expands T effector cells over regulatory T cells. In some embodiments, the IL-2 regimen comprises administration of an antibody 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.

[00483] 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.

[00484] 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.

[00485] 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.

[00486] 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.

[00487] 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 than 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	MYRMQLLSCI ALSLALVTNS APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML	60
IL-2	TFKFYMPKKA TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE	120
	TTFMCEYADE TATIVEFLNR WITFCQSIIS TLT 153	
SEQ ID NO:14	APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTAML TFKFYMPKKA TELKHLQCLE	60
IL-2 mutein	EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR	120
	WITFCQSIIS TLT 133	
SEQ ID NO:15	APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TAKFYMPKKA TELKHLQCLE	60
IL-2 mutein	EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR	120
	WITFCQSIIS TLT 133	
SEQ ID NO:16	GFSLAPTSSS TKKTQLQLEH LLLDLQMILN GINNYKNPKL TAMLTFKFYM PKKATELKHL	60
HCDR1_IL-2	QCLEEELKPL EEVLNLAQSK NFHLRPRDLI SNINVIVLEL KGSETTFMCE YADETATIVE	120
	FLNRWITFCQ SIISTLTSTS GMSVG 145	
SEQ ID NO:17	DIWWDDKKDY NPSLKS 16	
HCDR2		
SEQ ID NO:18	SMITNWYFDV 10	
HCDR3		
SEQ ID NO:19	APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTAML TFKFYMPKKA TELKHLQCLE	60
HCDR1_IL-2 kabat	EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR	120
	WITFCQSIIS TLTSTSGMSV G 141	
SEQ ID NO:20	DIWWDDKKDY NPSLKS 16	
HCDR2 kabat		
SEQ ID NO:21	SMITNWYFDV 10	
HCDR3 kabat		
SEQ ID NO:22	GFSLAPTSSS TKKTQLQLEH LLLDLQMILN GINNYKNPKL TAMLTFKFYM PKKATELKHL	60
HCDR1_IL-2	QCLEEELKPL EEVLNLAQSK NFHLRPRDLI SNINVIVLEL KGSETTFMCE YADETATIVE	120
clothia	FLNRWITFCQ SIISTLTSTS GM 142	
SEQ ID NO:23	WWDDK	5
HCDR2 clothia		
SEQ ID NO:24	SMITNWYFDV 10	
HCDR3 clothia		
SEQ ID NO:25	GFSLAPTSSS TKKTQLQLEH LLLDLQMILN GINNYKNPKL TAMLTFKFYM PKKATELKHL	60
HCDR1_IL-2 IMGT	QCLEEELKPL EEVLNLAQSK NFHLRPRDLI SNINVIVLEL KGSETTFMCE YADETATIVE	120
	FLNRWITFCQ SIISTLTSTS GMS 143	
SEQ ID NO:26	IWWDDKK	7
HCDR2 IMGT		
SEQ ID NO:27	ARSMITNWYF DV 12	
HCDR3 IMGT		
SEQ ID NO:28	QVTLRESGPA LVKPTQTLTL TCTFSGFSLA PTSSSTKKTQ LQLEHLLLDL QMILNGINNY	60
V_H	KNPKLTAMLT FKFYMPKKAT ELKHLQCLEE ELKPLEEVLN LAQSKNFHLR PRDLISNINV	120
	IVLELKGSET TFMCEYADET ATIVEFLNRW ITFCQSIIST LTSTSGMSVG WIRQPPGKAL	180
	EWLADIWWDD KKDYNPSLKS RLTISKDTSK NQVVLKVTNM DPADTATYYC ARSMITNWYF	240
	DVWGAGTTVT VSS 253	
SEQ ID NO:29	QMILNGINNY KNPKLTAMLT FKFYMPKKAT ELKHLQCLEE ELKPLEEVLN LAQSKNFHLR	60
Heavy chain	PRDLISNINV IVLELKGSET TFMCEYADET ATIVEFLNRW ITFCQSIIST LTSTSGMSVG	120
	WIRQPPGKAL EWLADIWWDD KKDYNPSLKS RLTISKDTSK NQVVLKVTNM DPADTATYYC	180
	ARSMITNWYF DVWGAGTTVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV	240
	TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKR	300
	VEPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV AVSHEDPEVK	360
	FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALAAPIEK	420

	TISKAKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT	480
	PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK	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	60
	FSGSGSGTEF TLTISSLQPD DFATYYCFQG SGYPFTFGGG TKLEIK 106	
SEQ ID NO:37 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	
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	

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).

[00488] 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).

[00489] 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).

[00490] 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:12).

[00491] 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.

[00492] 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.

[00493] 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.

[00494] 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.

[00495] 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.

[00496] 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.

[00497] 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.

[00498] 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.

[00499] 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).

[00500] 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.

[00501] 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.

[00502] 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).

[00503] 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.

[00504] 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.

[00505] 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.

[00506] 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.

[00507] 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.

[00508] 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."

[00509] 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.

[00510] 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.

[00511] 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.

[00512] 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 V_H portion and a V_L portion. A scFv molecule is denoted as either V_L-L-V_H if the V_L domain is the N-terminal part of the scFv molecule, or as V_H-L-V_L if the V_H 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.

[00513] 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.

[00514] 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.

[00515] 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*.

[00516] As used herein, "isotype" refers to the antibody class (*e.g.*, IgM or IgG1) that is encoded by the heavy chain constant region genes.

[00517] 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."

[00518] 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.

[00519] 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.

[00520] 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.

[00521] 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.

[00522] 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.

[00523] "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.

[00524] 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.

III. Immunomodulatory Agent Associated Tumor Infiltrating Lymphocytes

[00525] Provided herein are modified tumor infiltrating lymphocytes (TIL) that include one or more immunomodulatory agents associated with the TIL cell surface. In some embodiments, the subject modified TILs exhibit enhanced in vivo survival, proliferation and/or anti-tumor effects in a patient recipient.

[00526] The immunomodulatory agent can be attached to the TIL disclosed herein (*e.g.* therapeutics TILs provided herein) using any suitable method. In some embodiments the one or more immunomodulatory agents are part of an immunomodulatory fusion protein that is attached to the TIL cell surface. In some embodiments, the one or more immunomodulatory agents are included as part of nanoparticles that are associated with the TIL cell surfaces. The immunomodulatory agents can be any immunomodulatory agent that promotes TIL survival proliferation, and/or anti-tumor effects in a patient recipient. In some embodiments, the immunomodulatory agent is a cytokine (*e.g.*, an interleukin). In exemplary embodiments, the TILs include IL-12, IL-15, and/or IL-21.

[00527] Any suitable TIL population can be modified to produce the subject compositions, including TILs produced using the manufacturing processes described herein. In some

embodiments, the modified TILs are derived from TILs produced during any of the steps of the Process 2A method disclosure herein (see, *e.g.*, FIGs 2-6). In exemplary embodiments, the modified TILs are derived from TILs produced during any of the steps of the GEN 3 method disclosure herein (see, *e.g.*, FIG. 7). In some embodiments, the TILs are PD-1 positive TILs that are derived from the methods disclosed herein.

[00528] Aspects of the subject modified TILs are further detailed herein.

A. Immunomodulatory Fusion Proteins

[00529] In some embodiments, the modified TILs provided herein includes an immunomodulatory fusion protein that includes an immunomodulatory agent (*e.g.*, a cytokine) linked to a moiety that facilitates the tethering of the immunomodulatory agent to surface of the TILs. In some embodiments, the fusion protein includes a cell membrane anchor moiety (a transmembrane domain). In certain embodiments, the fusion protein includes a TIL surface antigen binding moiety that binds to a TIL surface antigen. Aspects of these fusion proteins are further discussed in detail below.

1. Membrane Anchored Immunomodulatory Fusion Proteins

[00530] In some embodiments, the modified TILs provided herein include a membrane anchored immunomodulatory fusion protein. The membrane anchored immunomodulatory fusion protein includes one or more of the immunomodulatory agents (*e.g.*, a cytokine) linked to a cell membrane anchor moiety. In such embodiments, the membrane anchored immunomodulatory agent is tethered to the TIL surface membrane via the cell membrane anchor moiety, thus allowing the immunomodulatory agent to exert its effects in a targeted manner.

[00531] The immunomodulatory agent can be any suitable immunomodulatory agent including, for example, any of the immunomodulatory agents provided herein. In some embodiments, the immunomodulatory agent is an interleukin that promotes an anti-tumor response. In some embodiments, the immunomodulatory agent is a cytokine. In particular embodiments, the immunomodulatory agent is IL-2, IL-12, IL-15, IL-18, IL-21, or a CD40 agonist (e.g., CD40L or agonistic anti-CD40 binding domain (e.g., an anti-CD40 scFv)) or a bioactive variant thereof. In certain embodiments, two or more different a membrane anchored immunomodulatory fusion proteins are expressed on a TIL surface. In exemplary embodiments, a TIL includes 2, 3, 4, 5, 6, 7, 8, 9 or 10 different membrane anchored immunomodulatory fusion proteins.

[00532] The immunomodulatory agent is linked to a cell membrane anchor moiety that allows the tethering of the immunomodulatory agent to the TIL cell surface. Suitable cell membrane anchor moieties include, for example, transmembrane domains of endogenous TIL cell surface proteins and fragments thereof. Exemplary transmembrane domains that can be used in the subject fusion proteins, include for example, B7-1, B7-2, and CD8a transmembrane domains and fragments thereof. In some embodiments, the cell membrane anchor moiety further includes a transmembrane and intracellular domain of an endogenous TIL cell surface protein or fragment thereof. In some embodiments, the cell membrane anchor moiety is a B7-1, B7-2 or CD8a transmembrane-intracellular domain or fragment thereof. In certain embodiments, the cell membrane anchor moiety is a CD8a transmembrane domain having the amino acid sequence of IYIWAPLAGTCGVLLLSLVIT (SEQ ID NO:238). In certain embodiments, the cell membrane anchor moiety is a B7-1 transmembrane-intracellular domain having the amino acid sequence of LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO:239). In certain embodiments, the cell membrane anchor moiety is a non-peptide cell membrane anchor moiety. In exemplary embodiments, the non-peptide cell membrane anchor moiety is a glycophosphatidylinositol (GPI) anchor. GPI anchors have a structure that includes a phosphoethanolamine linker, glycan core, and phospholipid tail. In some embodiments, the glycan core is modified with one or more side chains. In some embodiments, the glycan core is modified with one or more of the following side chains: a phosphoethanolamine group, mannose, galactose, sialic acid, or other sugars.

[00533] The membrane anchored immunomodulatory fusion protein include linkers that allow for the linkage of components of the membrane anchored immunomodulatory fusion protein (e.g. an immunomodulatory agent to a cell membrane anchor moiety). Suitable linkers include linkers that are at least about 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 or 30 amino acid residues in length. In some embodiments, the linker is 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 45-50, 50-60 amino acids in length. Suitable linkers include, but are not limited: a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, or a non-helical linker. In some embodiments, the linker is a peptide linker that optionally comprises Gly and Ser. In certain embodiments, the peptide linker utilize a glycine-serine polymer, including for example (GS)n (SEQ ID NO:240), (GSGGS)n (SEQ ID NO:241), (GGGS)n (SEQ ID NO:242), (GGGGS)n (SEQ ID NO:243), (GGGGGS)n (SEQ ID NO:244), and

(GGGGGGS)n (SEQ ID NO:245), where n is an integer of at least one (and generally from 3 to 10). Additional linkers that can be used with the present compositions and methods are described in U.S. Patent Publication Nos. US 2006/0074008, US 20050238649, and US 2006/0024317, each of which is incorporated by reference herein in its entirety, and particularly in pertinent parts related to linkers. In some embodiments, the peptide linker is SGGGGSGGGGSGGGGSGGGGSGGGSLQ (SEQ ID NO:246).

[00534] In some embodiments, the linker is a cleavable linker. In exemplary embodiments, the cleavable linker allows for the release of the immunomodulatory agent into the tumor microenvironment. Cleavable linkers are also useful in embodiments, wherein two membrane anchored immunomodulatory fusion proteins are co-expressed in the same TIL (see, e.g., Figure 36 and Tables 58 and 59). In exemplary embodiments, the linker is a self-cleaving 2A peptide. See, *e.g.*, Liu et al., Sci. Rep. 7(1):2193 (2017), which is incorporated by reference in relevant parts relating to 2A peptides. 2A peptides are viral oligopeptides that mediate cleavage of polypeptides during translation in eukaryotic cells. In some embodiments, the 2A peptide includes a C-terminus having the amino acid sequence GDVEXiNPGP (SEQ ID NO:247), wherein Xi is any naturally occurring amino acid residue. In certain embodiments, the 2A peptide is a porcine teschovirus-1 2A peptide (GSGATNFSLLKQAGDVEENPGP, SEQ ID NO:248). In some embodiments, the 2A peptide is an equine rhinitis A virus 2A peptide (GSGQCTNYALLKLAGDVESNPGP, SEQ ID NO:249). In certain embodiments, the 2A peptide is a foot-and-mouth disease virus 2A peptide: (GSGEGRGSLLTCGDVEENPGP, SEQ ID NO:250). In some embodiments, the cleavable linker includes a furin-cleavable sequence. Exemplary furin-cleavable sequences are described for example, Duckert et al., Protein Engineering, Design & Selection 17(1):107-112 (2004), and US Patent No. 8,871,906, each of which is incorporated herein by reference, particularly in relevant parts relating to furin-cleavable sequences. In some embodiments, the linker includes a 2A peptide and a furin-cleavable sequence. In exemplary embodiments, the furin-cleavable 2A peptide includes the amino acid sequence RAKRSGSGATNFSLLKQAGDVEENPGP (SEQ ID NO:251).

[00535] In some embodiments, the immunomodulatory agents are attached in the membrane anchored immunomodulatory fusion protein by a degradable linker (*e.g.*, a disulfide linker) such that under physiological conditions, the linker degrades, thereby releasing the immunomodulatory agent. In some embodiments, the immunomodulatory agents are reversibly linked to functional groups through a degradable linker such that under

physiological conditions, the linker degrades and releases the immunomodulatory agent. Suitable degradable linkers include, but are not limited to: a protease sensitive linker that is sensitive to one or more enzymes present in biological media such as proteases in a tumor microenvironment such a matrix metalloproteases present in a tumor microenvironment or in inflamed tissue (*e.g.* matrix metalloproteinase 2 (MMP2) or matrix metalloproteinase 9 (MMP9)).

[00536] In other embodiments, the components of the membrane anchored immunomodulatory fusion protein are linked by an enzyme-sensitive linker. Exemplary cleavable linker include those that are recognized by one of the following enzymes: metalloprotease MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-14, plasmin, PSA, PSMA, CATHEPSIN D, CATHEPSIN K, CATHEPSIN S, ADAM10, ADAM12, ADAMTS, Caspase-1, Caspase-2, Caspase-3, Caspase-4, Caspase-5, Caspase-6, Caspase-7, Caspase-8, Caspase-9, Caspase-10, Caspase-11, Caspase-12, Caspase-13, Caspase-14, and TACE. See, *e.g.*, US Patent Nos. 8,541,203 and 8,580,244, each of which is incorporated by reference in its entirety and in pertinent parts related to cleavable linkers.

[00537] In certain embodiments, the membrane anchored immunomodulatory fusion protein includes a signal peptide that facilitates the translocation of the fusion protein to the TIL cell membrane. Any suitable signal peptide that facilities the localization of the fusion protein to the TIL cell membrane can be used. In some embodiments, the signal peptide does not interfere with the bioactivity of the immunomodulatory agent. Exemplary signal peptide sequences include, but are not limited to: human granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor signal sequence, human prolactin signal sequence, and human IgE signal sequence. In certain embodiments, the fusion protein includes a human IgE signal sequence. In exemplary embodiments, the human IgE signal sequence has the amino acid sequence MDWTWILFLVAAATRVHS (SEQ ID NO:252). In some embodiments, the human IgE signal sequence includes the amino acid sequence NIKGSPWKGSLLLLLVSNLLLCQSVAP (SEQ ID NO:253). In some embodiments, the signal peptide sequence is an IL-2 signal sequence having the amino acid sequence MYRMQLLSCIALSLALVTNS (SEQ ID NO:254).

[00538] In some embodiments, the membrane anchored immunomodulatory fusion protein is according to the formula, from N- to C-terminus:

[00539] S-IA-L-C,

[00540] wherein S is a signal peptide, IA is an immunomodulatory agent, L is a linker and C is a cell membrane anchor moiety.

[00541] In some embodiments, the signal peptide S is any one of SEQ ID NOs:252-254. In some emboidments, the cell membrane anchor moiety is SEQ ID NO:277. In exemplary embodiments, the immunomodulatory agent is IL-2, IL-12, IL-15, IL-18, IL-21, or a CD40 agonist (e.g., CD40L or an anti-CD40 scFv as described herein). In some embodimnets, C is a B7-1 trnasmembrane-intracellular domain (e.g., SEQ ID NO:239). Exemplary membrane anchored immunomodulatory fusion proteins according to the above formula are depicted in Figures 36 and 37.

[00542] In some embodiments, the TIL includes two or more different membrane anchored immunomodulatory fusion proteins according to the formula, from N- to C-terminus: S-IA-L-C, wherein each of the different membrane anchored immunomodulatory fusion proteins includes a different immunomodulatory agent. In some embodiments, the two or more different immunomodulatory agents are selected from the group consisting of: IL-12 and IL-15, IL-15 and IL-18, CD40L and IL-15, IL-15 and IL-21, and IL-2 and IL-12.

[00543] In some embodiments that includes two membrane anchored immunomodulatory fusion proteins, the membrane anchored immunomodulatory fusion proteins are arranged according to the formula, from N- to C-terminus:

[00544] S1-IA1-L1-C1-L2-S2-IA2-L3-C2,

[00545] wherein S1 and S2 are each a signal peptide, IA1 and IA2 are each an immunomodulatory agent, L1-L3 are each a linker, and C1 and C2 are each a cell membrane anchor moiety. In some embodiments, IA1 and IA2 are the same immunomodulatory agent. In certain embodiments, IA1 and IA2 are different immunomodulatory agents. Suitable immunomodulatory agents including any of those described herein. In some embodiments, IA1 and IA2 are independently selected from IL-2, IL-12, IL-15, IL-18, IL-21, a CD40 agonist (e.g., CD40L or an agonistic anti-CD40 binding domain (e.g., an anti-CD40 scFv)) or a bioactive variant thereof. In some embodiments, IA1 and IA2 are selected from the group consisting of: IL-12 and IL-15, IL-15 and IL-18, CD40L and IL-15, IL-15 and IL-21, and IL-2 and IL-12. In some embodiments, one or more of L1-L3 is a cleavable linker. In some embodiments two or more of L1-L3 are different linkers. In exemplary embodiments L2 is a cleavable linker. In some embodiments, L2 is furin cleavable P2A linker (e.g., SEQ ID NO:251). In some embodiments, C1 and C2 are independently transmembrane domains

and/or transmembrane-intracellular domains. In certain embodiments C1 and C2 are the same. In exemplary embodiments, C1 and C2 are each a B7-1 transmembrane-intracellular domain (e.g., SEQ ID NO:239). In exemplary embodiments, C1 and C2 are different. Exemplary constructs that include two membrane anchored immunomodulatory fusion proteins according to the above formula are depicted in Figure 36, and Tables 58 and 59.

[00546] Modified TILs that include cell membrane anchored immunomodulatory fusion proteins associated with their surfaces can be made by genetically modifying a populations of TILs to include a nucleic acid encoding the fusion protein. Any suitable genetic modification method can be used to produce such modified TILs including, for example, CRISPR, TALE, and zinc finger method described herein.

[00547] Any suitable population of TILs can be genetically modified to make the subject modified TIL compositions. In some embodiments, a population TILs produced during any of the steps of the Process 2A method disclosure herein (see, *e.g.*, FIGs 2-6) is genetically modified to produce the subject modified TILs. In exemplary embodiments, a population TILs produced during any of the steps of the GEN 3 method disclosure herein (see, *e.g.*, FIG. 7) is genetically modified to produce the subject modified TILs. In exemplary embodiments, TILs produced from the second step in the Process 2A method and/or the rapid expansion step in the GEN 3 method provided herein are genetically modified to produce the subject modified TILs. In some embodiments, PD-1 positive TILs that have been preselected using the methods described herein are genetically modified to produce the subject modified TILs.

[00548] Any suitable population of TILs can be transiently modified to make the subject transiently modified TIL compositions. In some embodiments, a population of TILs produced during any of the steps of the Process 2A method disclosure herein (see, *e.g.*, FIGS. 2-6) is transfected with nucleic acid encoding a cell membrane anchored immunomodulatory fusion protein to transiently express the cell membrane anchored immunomodulatory fusion protein in the subject transiently modified TILs. In exemplary embodiments, a population of TILs produced during any of the steps of the GEN 3 method disclosure herein (see, *e.g.*, FIG. 7) is transfected with nucleic acid encoding a cell membrane anchored immunomodulatory fusion protein to transiently express the cell membrane anchored immunomodulatory fusion protein in the subject transiently modified TILs. In exemplary embodiments, TILs produced from the first expansion step in the Process 2A method and/or the priming expansion step in the GEN 3 method provided herein are transfected with nucleic acid encoding a cell

membrane anchored immunomodulatory fusion protein to transiently express the cell membrane anchored immunomodulatory fusion protein in the subject transiently modified TILs. In exemplary embodiments, TILs produced from the second expansion step in the Process 2A method and/or the rapid expansion step in the GEN 3 method provided herein are transfected with nucleic acid encoding a cell membrane anchored immunomodulatory fusion protein to transiently express the cell membrane anchored immunomodulatory fusion protein in the subject transiently modified TILs. In some embodiments, PD-1 positive TILs that have been preselected using the methods described herein are transfected with nucleic acid encoding a cell membrane anchored immunomodulatory fusion protein to transiently express the cell membrane anchored immunomodulatory fusion protein in the subject transiently modified TILs.

[00549] Also provided herein are nucleic acids encoding the membrane anchored immunomodulatory fusion proteins, expression vectors that include such nucleic acids, and host cells that include the nucleic acids or expression vectors. Any suitable promoter can be used for the expression of the membrane anchored immunomodulatory fusion protein. In exemplary embodiments, the promoter is an inducible promoter. Exemplary nucleic acids that encode for exemplary membrane anchored immunomodulatory fusion proteins and components of such fusion proteins are depicted in Figures 36 and 37, and Tables 58 and 59.

[00550] In some embodiments, the nucleic acids encoding the membrane anchored immunomodulatory fusion protein is mRNA. In exemplary embodiments, the mRNA includes one or more modifications that improves intracellular stability and/or translation efficiency of the mRNA. In some embodiments, the mRNA includes a 5' cap or cap analog that improves mRNA half-life. Exemplary cap structures, include, but are not limited to ARCA, mCAP, m^7GpppN (cap 0), m^7GpppNm (cap 1), and m^7GpppNmpNm (cap 2) caps. In some embodiments, the 5' cap is according ot the formula: $^{m7}Gppp[N_{2'Ome}]_n[N]_m$ wherein ^{m7}G is N7-methylated guanosine or any guanosine analog, N is any natural, modified or unnatural nucleoside, "n" can be any integer from 0 to 4 and "m" can be an integer from 1 to 9. Exemplary 5' caps are disclosed in US Patent No. 10,703,789 and WO2017053297, which are incorporated by reference in their entirety, and specifically for disclosures relating to 5' caps and cap analogs.

[00551] In some embodiments, the nucleic acids encoding the membrane anchored immunomodulatory fusion protein is mRNA further includes a 3' untranslated region (UTR) or modified UTR. 3′ UTRs are known to have stretches of adenosines and uridines. These

AU rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, the AU rich elements (AREs) can be separated into three classes (Chen et al, 1995): Class I AREs contain several dispersed copies of an AUUUA motif within U-rich regions. C-Myc and MyoD contain class I AREs. Class II AREs possess two or more overlapping UUAUUUA(U/A)(U/A) nonamers. Molecules containing this type of AREs include GM-CSF and TNF-a. Class III ARES are less well defined. These U rich regions do not contain an AUUUA motif. c-Jun and Myogenin are two well-studied examples of this class. Most proteins binding to the AREs are known to destabilize the messenger, whereas members of the ELAV family, most notably HuR, have been documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3′ UTR of nucleic acid molecules will lead to HuR binding and thus, stabilization of the message in vivo.

[00552] Introduction, removal or modification of 3′ UTR AU rich elements (AREs) can be used to modulate the stability of the nucleic acids described herein . When engineering specific nucleic acids, one or more copies of an ARE can be introduced to make polynucleotides of the invention less stable and thereby curtail translation and decrease production of the resultant protein. Likewise, AREs can be identified and removed or mutated to increase the intracellular stability and thus increase translation and production of the resultant protein. Transfection experiments can be conducted in relevant cell lines, using nucleic acids, and protein production can be assayed at various time points post-transfection. For example, cells can be transfected with different ARE-engineering molecules and by using an ELISA kit to the relevant protein and assaying protein produced at 6 hour, 12 hour, 24 hour, 48 hour, and 7 days post-transfection.

[00553] In some embodiments, the nucleic acid encoding the membrane anchored immunomodulatory fusion proteins is operably linked to a nuclear factor of activated T-cells (NFAT) promoter or a functional portion or functional variant thereof. "NFAT promoter" as used herein means one or more NFAT responsive elements linked to a minimal promoter of any gene expressed by T-cells. Preferably, the minimal promoter of a gene expressed by T-cells is a minimal human IL-2 promoter. The NFAT responsive elements may comprise, e.g., NFATl, NFAT2, NFAT3, and/or NFAT4 responsive elements. The NFAT promoter (or functional portion or functional variant thereof) may comprise any number of binding motifs, e.g., at least two, at least three, at least four, at least five, or at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or up to twelve binding motifs.

TABLE 4 – NFAT Promoter Related Sequences.

Description	Amino Acid Sequence
6X NFAT IL-2 minimal promoter	TCGAGGTCGACGGTATCGATAAGCTTGAT ATCGAATTAGGAGGAAAAACTGTTTCATA CAGAAGGCGTCAATTAGGAGGAAAAACTG TTTCATACAGAAGGCGTCAATTAGGAGGA AAAACTGTTTCATACAGAAGGCGTCAATT GGTCCCATCGAATTAGGAGGAAAAACTGT TTCATACAGAAGGCGTCAATTAGGAGGAA AAACTGTTTCATACAGAAGGCGTCAATTA GGAGGAAAAACTGTTTCATACAGAAGGCG TCAATTGGTCCCGGGACATTTTGACACCCC CATAATATTTTTCCAGAATTAACAGTATAA ATTGCATCTCTTGTTCAAGAGTTCCCTATC ACTCTCTTTAATCACTACTCACAGTAACCT CAACTCCTGGCCACC (SEQ ID NO: 255)
NFAT responsive element	GGAGGAAAAACTGTTTCATACAGAAGGCG T (SEQ ID NO: 256)
Human IL-2 Promoter	CATTTTGACACCCCCATAATATTTTTCCAG AATTAACAGTATAAATTGCATCTCTTGTTC AAGAGTTCCCTATCACTCTCTTTAATCACT ACTCACAGTAACCTCAACTCCTG (SEQ ID NO:257)

[00554] In a preferred embodiment, the NFAT promoter comprises six NFAT binding motifs. See, e.g., US Patent No. 8,556,882, which is incorporated by reference in its entirety and particularly for pertinent parts relating to NFAT promoters. In some embodiments, the NFAT promoter system controls expression of an immunomodulatory fusion protein that includes any of the immunomodulatory agents described herein. In certain embodiments, the immunomodulatory agent is selected from: IL-2, IL-12, IL-15, IL-18, IL-21, and a CD40 agonist (e.g., CD40L or agonistic anti-CD40 binding domain (e.g., an anti-CD40 scFv)) or a bioactive variant thereof. Exemplary nucleic acids encoding exemplary subject membrane anchored immunomodulatory fusion proteins operably linked to a NFAT promoter are depicted in Table 59. In some embodiments, the NFAT promoter system controls expression of an immunomodulatory fusion protein that includes IL-15. In some embodiments, the NFAT promoter system controls expression of an immunomodulatory fusion protein that includes IL-21. In some embodiments, the NFAT promoter system controls expression of an immunomodulatory fusion protein that includes IL-15 and IL-21.

[00555] In some embodiments, the invention provides TILs genetically modified to comprise DNA encoding an immunomodulatory fusion protein operably linked to the NFAT

promoter. In some embodiments, the NFAT promoter controls expression of DNA encoding an immunomodulatory fusion protein that includes any of the immunomodulatory agents described herein. In certain embodiments, the immunomodulatory agent is selected from: IL-2, IL-12, IL-15, IL-18, IL-21, and a CD40 agonist (e.g., CD40L or agonistic anti-CD40 binding domain (e.g., an anti-CD40 scFv)) or a bioactive variant thereof. In some embodiments, the NFAT promoter controls expression of DNA encoding an immunomodulatory fusion protein that includes IL-15. In some embodiments, the NFAT promoter controls expression of DNA encoding an immunomodulatory fusion protein that includes IL-21. In some embodiments, the NFAT promoter controls expression of DNA encoding an immunomodulatory fusion protein that includes IL-15 and IL-21.

[00556] In some embodiments, the invention provides TILs genetically modified to comprise DNA encoding an immunomodulatory fusion protein operably linked to the NFAT promoter, wherein the immunomodulatory fusion protein is arranged according to the formula, from N- to C-terminus:

[00557] S1-IA1-L1-C1-L2-S2-IA2-L3-C2,

[00558] wherein S1 and S2 are each a signal peptide, IA1 and IA2 are each an immunomodulatory agent, L1-L3 are each a linker, and C1 and C2 are each a cell membrane anchor moiety. In some embodiments, IA1 and IA2 are the same immunomodulatory agent. In certain embodiments, IA1 and IA2 are different immunomodulatory agents. Suitable immunomodulatory agents including any of those described herein. In some embodiments, IA1 and IA2 are independently selected from IL-2, IL-12, IL-15, IL-18, IL-21, a CD40 agonist (e.g., CD40L or an agonistic anti-CD40 binding domain (e.g., an anti-CD40 scFv)) or a bioactive variant thereof. In some embodiments, IA1 and IA2 are selected from the group consisting of: IL-12 and IL-15, IL-15 and IL-18, CD40L and IL-15, IL-15 and IL-21, and IL-2 and IL-12. In some embodiments, IA1 and IA2 are independently selected from IL-15 and IL-21. In some embodiments, IA1 is IL-15 and IA2 is IL-21. In some embodiments, IA1 is IL-21 and IA2 is IL-15. In some embodiments, one or more of L1-L3 is a cleavable linker. In some embodiments two or more of L1-L3 are different linkers. In exemplary embodiments L2 is a cleavable linker. In some embodiments, L2 is furin cleavable P2A linker (e.g., SEQ ID NO:251). In some embodiments, C1 and C2 are independently transmembrane domains and/or transmembrane-intracellular domains. In certain embodiments C1 and C2 are the same. In exemplary embodiments, C1 and C2 are each a B7-1 transmembrane-intracellular domain (e.g., SEQ ID NO:239). In exemplary embodiments, C1 and C2 are different.

Exemplary constructs that include two membrane anchored immunomodulatory fusion proteins according to the above formula are depicted in Figure 36.

[00559] Nucleic acids encoding the subject membrane anchored immunomodulatory fusion proteins may be introduced into a population of TILs to produce transiently modified or genetically modified TILs that express the membrane anchored immunomodulatory fusion proteins using any suitable method. In some embodiments, nucleic acids encoding the membrane anchored immunomodulatory fusion proteins are introduced into a population of TILs using a microfluidic platform. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform. See, e.g., International Patent Application Publication Nos. WO 2013/059343A1, WO 2017/008063A1, or WO 2017/123663A1, or U.S. Patent Application Publication Nos. US 2014/0287509A1, US 2018/0201889A1, or US 2018/0245089A1, all of which are incorporated by reference herein in their entireties, and particularly for disclosures of microfluidic platforms for nucleic acid delivery. In the SQZ platform, the cell membranes of the cells for modification (e.g., TILs) are temporarily disrupted by microfluidic constriction, thereby allowing the delivery of nucleic acids encoding the membrane anchored immunomodulatory fusion proteins into the cells.

[00560] In some embodiments, the nucleic acid encoding the membrane anchored immunomodulatory fusion protein is mRNA and the microfluidic platform (e.g., SQZ vector-free microfluidic platform) is used to deliver the mRNA into TILs to produce transiently modified TILs. In some embodiments, the nucleic acid encoding the membrane anchored immunomodulatory fusion protein is DNA and the microfluidic platform (e.g., SQZ vector-free microfluidic platform) is used to deliver the DNA into TILs to produce stable genetically-modified TILs. The microfluidic platform (e.g., SQZ vector-free microfluidic platform) may be used to deliver the nucleic acid to any population of TILs produced during any steps of the Process 2A method disclosure herein (see, *e.g.*, FIGS. 2-6) or GEN 3 method disclosure herein (see, *e.g.*, FIG. 7) to produce the modified TILs. In some embodiments, the membrane anchored immunomodulatory fusion protein includes an IL-2, an IL-12, an IL-15, an IL-18, an IL-21, a CD40 agonist (e.g., CD40L or agonistic anti-CD40 binding domain (e.g., an anti-CD40 scFv)) or any combination thereof.

[00561] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-15. In some embodiments, the second

immunomodulatory agent is IL-2, IL-12, IL-18, IL-21, CD40L or an anti-CD40 binding domain (e.g., an anti-CD40 scFv).

[00562] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is CD40L. In some embodiments, the second immunomodulatory agent is IL-2, IL-12, IL-15, IL-18, IL-21, a CD40 agonist (e.g., CD40L or an agonistic anti-CD40 binding domain (e.g., an anti-CD40 scFv)) or a bioactive variant thereof.

[00563] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-12. In some embodiments, the second immunomodulatory agent is IL-2, IL-15, IL-18, IL-21, CD40L or an anti-CD40 binding domain (e.g., an anti-CD40 scFv).

[00564] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-18. In some embodiments, the second immunomodulatory agent is IL-2, IL-12, IL-15, IL-21, CD40L or an anti-CD40 binding domain (e.g., an anti-CD40 scFv).

[00565] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-21. In some embodiments, the second immunomodulatory agent is IL-2, IL-12, IL-15, IL-18, CD40L or an anti-CD40 binding domain (e.g., an anti-CD40 scFv).

[00566] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-2. In some embodiments, the second

immunomodulatory agent is IL-2, IL-12, IL-15, IL-18, IL-21, CD40L or an anti-CD40 binding domain (e.g., an anti-CD40 scFv).

[00567] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-2 and the second immunomodulatory agent is IL-12.

[00568] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-2 and the second immunomodulatory agent is IL-15.

[00569] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-2 and the second immunomodulatory agent is IL-18.

[00570] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-2 and the second immunomodulatory agent is IL-21.

[00571] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-2 and the second immunomodulatory agent is CD40L or an anti-CD40 binding domain (e.g., an anti-CD40 scFv).

[00572] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-12 and the second immunomodulatory agent is IL-15.

[00573] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-12 and the second immunomodulatory agent is IL-18.

[00574] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-12 and the second immunomodulatory agent is IL-21.

[00575] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-12 and the second immunomodulatory agent is CD40L or an anti-CD40 binding domain (e.g., an anti-CD40 scFv).

[00576] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-15 and the second immunomodulatory agent is IL-18.

[00577] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-15 and the second immunomodulatory agent is IL-21.

[00578] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-15 and the second immunomodulatory agent is CD40L or an anti-CD40 binding domain (e.g., an anti-CD40 scFv).

[00579] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-18 and the second immunomodulatory agent is IL-21.

[00580] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-18 and the second immunomodulatory agent is CD40L or an anti-CD40 binding domain (e.g., an anti-CD40 scFv).

[00581] In exemplary embodiments, the modified TILs provided herein include two membrane anchored immunomodulatory fusion proteins that each include a different immunomodulatory agent (i.e., a first and second immunomodulatory agent), wherein the first immunomodulatory agents is IL-21 and the second immunomodulatory agent is CD40L or an anti-CD40 binding domain (e.g., an anti-CD40 scFv).

[00582] Additional membrane anchored immunomodulatory fusion proteins that can be included in the modified TILs provided herein are described in WO 2019/157130 A1, which is incorporated by reference in its entirety, particularly in relevant parts related to membrane anchored immunomodulatory fusion proteins.

[00583] Exemplary membrane anchored immunomodulatory fusion proteins to be included in the modified TILs provided herein are depicted in Figures 36 and 37, and Tables 58 and 59.

[00584] In some embodiments, the nucleic acid encoding any of the membrane anchored immunomodulatory fusion proteins described above is operably linked to an NFAT promoter or a functional portion or functional variant thereof.

2. Immunomodulatory Agent-TIL Antigen Binding Domain Fusion Proteins

[00585] In some embodiments, the modified TILs provided herein include immunomodulatory fusion proteins, wherein such fusion proteins include one or more immunomodulatory agents linked to a TIL antigen binding domain (ABD). In some embodiments, the one or more immunomodulatory agents is tethered to the TIL surface membrane upon TIL ABD binding to a TIL surface antigen.

[00586] The TIL antigen binding domain includes an antibody variable heavy domain (VH) and variable light domain (VL). In some embodiments, the TIL antigen binding domain is a full length antibody that includes a heavy chain according to the formula: VH-CH1-hinge-CH2-CH3 and a light chain according to the formula: VL-CL, wherein VH is a variable heavy domain; CH1, CH2, CH3 are heavy chain constant domains, VL is a variable light domain and CL is a light chain constant domain. In some embodiments, the TIL antigen binding domain is antibody fragment. In certain embodiments, TIL antigen binding domain is a Fab, Fab', F(ab')2, F(ab)2, variable fragment (Fv), domain antibody (dAb), or single chain variable fragment (scFv).

[00587] The TIL antigen binding domain can bind to any suitable TIL antigen that allows for the attachment of the immunomodulatory agent-TIL ABD fusion protein to the surface of the TIL. In exemplary embodiments, the TIL antigen binding domain is capable of binding to a TIL surface antigen. TIL surface antigens include, but are not limited to D16, CD45, CD4, CD8, CD3, CD11a, CD11b, CD11c, CD18, LFA-1, CD25, CD127, CD56, CD19, CD20, CD22, HLA-DR, CD197, CD38, CD27, CD137, OX40, GITR, CD56, CD196, CXCR3, CXCR4, CXCR5, CD84, CD229, CCR1, CCR5, CCR4, CCR6, CCR8, and/or CCR10. In some embodiments, the ABD binds to CD45. In particular embodiments, the ABD binds to a CD45 isoform selected from CD45RA, CD45RB, CD45RC or CD45Rβ. In particular embodiments, the ABD binds to a CD45 expressed primary on T cells.

[00588] In certain embodiments, the ABD binds to a checkpoint inhibitor. Exemplary checkpoint inhibitors include, but are not limited to PD-1, PD-L1, LAG-3, TIM-3 and CTLA-4 (see, *e.g.*, Qin et al., Molecular Cancer 18:155 (2019)). In some embodiments, the ABD binds to a checkpoint inhibitor expressed on an immune effector cell (*e.g.*, a T cell or NK cell). Exemplary anti-PD-1 antibodies are disclosed, for example, in US Patent Nos. US 7,695,715, US 7,332,582, US 9,205,148, US 8,686,119, US 8,735,553, US 7,488,802, US 8,927,697, US 8,993,731, and US 9,102,727, which are incorporated by reference in their entireties, particularly in pertinent parts relating to anti-PD-1 antibodies. Exemplary anti PD-L1 antibodies are disclosed in US Patent Nos. US 8,217,149, US 8,779,108, US 8,168,179, US 8,552,154, US 8,460,927, and US 9,175,082, which are incorporated by reference in their entireties, particularly in pertinent parts relating to anti-PD-L1 antibodies. Exemplary anti-LAG-3 antibodies are disclosed in US Patent Nos. US 9,244,059, US 9,244,059, US 9,505,839, which are incorporated by reference in their entireties, particularly in pertinent parts relating to anti-LAG-3 antibodies. Exemplary TIM-3 antibodies are disclosed in WO 2016/161270, US 8,841,418, and US 9,163,087, which are incorporated by reference in their entireties, particularly in pertinent parts relating to anti-TIM-3 antibodies. Exemplary CTLA-4 antibodies are disclosed in US 6,984,720 and US 7,411,057, which are incorporated by reference in their entireties, particularly in pertinent parts relating to anti-CTLA-4 antibodies.

[00589] In some embodiments, the ABD is an anti-CD45 antibody or a fragment thereof. In certain embodiments, the anti-CD45 antibody is a human anti-CD45 antibody, a humanized anti-CD45 antibody, or a chimeric anti-CD45 antibody. In exemplary embodiments, the ABD includes the vhCDR1-3 and vlCDR1-3 of anti-CD45 antibody BC8 (see US20170326259, incorporated by reference herein, particularly in relevant parts relating

to anti-CD45 antibody sequences). In some embodiments, the ABD includes the variable heavy domain and variable domain of anti-CD45 antibody BC8. In some embodiments, the ABD includes the vhCDR1-3 and vlCDR1-3 or VH and VL of one of the following anti-CD45 antibodies: 10G10, UCHL1, 9.4, 4B2, or GAP8.3 (see Spertini et al., Immunology 113(4):441-452 (2004), Buzzi et al., Cancer Research 52:4027-4035 (1992)).

[00590] The immunomodulatory fusion proteins can be any suitable immunomodulatory agent including, for example, any of the immunomodulatory agents provided herein. In some embodiments, the immunomodulatory agent is an interleukin that promotes an anti-tumor response. In some embodiments, the immunomodulatory agent is a cytokine. In particular embodiments, the immunomodulatory agent is IL-2, IL-12, IL-15, IL-21 or a bioactive variant thereof. In certain embodiments, the fusion protein includes more than one immunomodulatory agents. In exemplary embodiments, the fusion protein includes 2, 3, 4, 5, 6, 7, 8, 9 or 10 different immunomodulatory agents.

[00591] The TIL antigen binding domain is attached to the immunomodulatory agent using any suitable linker. Suitable linkers include, but are not limited: a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, or a non-helical linker. In some embodiments, the linker is a peptide linker that optionally comprises Gly and Ser. Suitable linkers include linkers that are at least about 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 or 30 amino acid residues in length. In some embodiments, the linker is 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 45-50, or 50-60 amino acids in length. In certain embodiments, the peptide linker is a $(GGGS)_n$ or $(GGGGS)_n$ linker, wherein n indicates the number of repeats of the motif and is an integer selected from 1-10. In some embodiments, the linker is an antibody hinge domain or a fragment thereof. In certain embodiments, the linker is a human immunoglobulin (Ig) hinge domain (*e.g.*, an IgG1, IgG2, IgG3, IgG4, IgD, IgE, IgM or IgA hinge) or a fragment thereof. In some embodiments, the immunomodulatory agent is directly coupled to the TIL without a linker.

[00592] The immunomodulatory agent can be attached to the TIL antigen binding domain at a suitable position that does not impede binding of the fusion protein to a TIL. In some embodiments wherein the antigen binding domain is a full length antibody, the immunomodulatory agent is attached to the C-terminus or N-terminus of either the heavy chain or light chain. In some embodiments wherein the antigen binding domain is an scFv, the immunomodulatory agent is attached to the C-terminus or N-terminus of the variable

heavy domain or variable light domain. In some embodiments wherein the antigen binding domain is an Fab, the immunomodulatory agent is attached to the C-terminus or N-terminus of the variable heavy domain or variable light domain. In some embodiments wherein the antigen binding domain is an Fab', the immunomodulatory agent is attached to the C-terminus or N-terminus of the variable heavy domain or variable light domain. In some embodiments wherein the antigen binding domain is an Fab'_2, the immunomodulatory agent is attached to the C-terminus or N-terminus of the variable heavy domain or variable light domain.

[00593] In some embodiments wherein the fusion protein includes two or more immunomodulatory agents, the immunomodulatory agents are attached to each other using any of the linkers described herein. In some embodiments, the two or more immunomodulatory agents are attached to different locations of the antigen binding domain. For example, in some embodiments wherein the TIL antigen binding domain is a full length antibody, the two or more immunomodulatory agents are attached at (i) different locations on the heavy chain (ii) different locations on the light chain or (iii) different locations on the heavy chain and/or light chain.

[00594] The subject immunomodulatory agent-TIL antigen binding domain fusion proteins can be made using any suitable method. In one aspect, provided herein are nucleic acids that encode the subject fusion proteins, expression vectors that include such nucleic acids, and host cells that include the expression vectors. Host cells that include the expression vectors encoding the subject fusion proteins are cultured under conditions for the expression of the fusion proteins and the fusion proteins are subsequently isolated and purified. In some embodiments, the purified fusion proteins are then incubated with a population of TILs under conditions that allow for the binding of the fusion protein to the TILs.

[00595] In some embodiments, the subject immunomodulatory agent-TIL antigen binding domain fusion proteins are attached to TILs produced during any of the steps of the Process 2A method disclosure herein (see, *e.g.*, FIGs 2-6). In exemplary embodiments, the fusion proteins are attached to TILs produced during any of the steps of the GEN 3 method disclosure herein (see, *e.g.*, FIG. 7). In exemplary embodiments, the fusion proteins are attached to TILs produced from the first expansion step in the Process 2A method and/or the priming expansion step in the GEN 3 method provided herein. In exemplary embodiments, the fusion proteins are attached to TILs produced from the second expansion step in the

Process 2A method and/or the rapid expansion step in the GEN 3 method provided herein. In some embodiments, the TILs are PD-1 positive TILs that have been preselected using the methods described herein.

[00596] Nucleic acids encoding the subject the subject immunomodulatory agent-TIL antigen binding domain fusion proteins may be introduced into a population of TILs to produce transiently modified or genetically modified TILs that express the subject immunomodulatory agent-TIL antigen binding domain fusion proteins using any suitable method. In some embodiments, nucleic acids encoding the subject immunomodulatory agent-TIL antigen binding domain fusion proteins are introduced into a population of TILs using a microfluidic platform. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform. See, e.g., International Patent Application Publication Nos. WO 2013/059343A1, WO 2017/008063A1, or WO 2017/123663A1, or U.S. Patent Application Publication Nos. US 2014/0287509A1, US 2018/0201889A1, or US 2018/0245089A1, all of which are incorporated by reference herein in their entireties, and particularly for disclosures of microfluidic platforms for nucleic acid delivery. In the SQZ platform, the cell membranes of the cells for modification (e.g., TILs) are temporarily disrupted by microfluidic constriction, thereby allowing the delivery of nucleic acids encoding the immunomodulatory agent-TIL antigen binding domain fusion protein into the cells.

[00597] In some embodiments, the nucleic acid encoding the subject immunomodulatory agent-TIL antigen binding domain fusion protein is mRNA and the microfluidic platform (e.g., SQZ vector-free microfluidic platform) is used to deliver the mRNA into TILs to produce transiently modified TILs. In some embodiments, the nucleic acid encoding the subject immunomodulatory agent-TIL antigen binding domain fusion protein is DNA and the microfluidic platform (e.g., SQZ vector-free microfluidic platform) is used to deliver the nucleic acid into TILs to produce stable genetically-modified TILs. The microfluidic platform (e.g., SQZ vector-free microfluidic platform) may be used to deliver the nucleic acid to any population of TILs produced during any steps of the Process 2A method disclosure herein (see, *e.g.*, FIGS. 2-6) or GEN 3 method disclosure herein (see, *e.g.*, FIG. 7) to produce the modified TILs. In some embodiments, the membrane anchored immunomodulatory fusion protein comprises an IL-2, an IL-12, an IL-15, an IL-21 or combinations thereof (e.g., IL-15 and IL-21).

[00598] Exemplary immunomodulatory agent-TIL antigen binding domain fusion proteins useful for the compositions and methods provided herein are further described, for example, in US Patent Application Publication No. 20200330514, which is incorporated by reference in its entirety and in pertinent parts related to immunomodulatory agent-TIL antigen binding domain fusion proteins.

B. Nanoparticle Compositions

[00599] In some embodiments, the subject modified TILs provided herein include one or more nanoparticles, and those nanoparticles include one or more immunomodulatory agents. In some embodiments, the nanoparticles provided herein include a plurality of two or more proteins that are coupled to each other and/or a second component of the particle (*e.g.*, reversibly linked through a degradable linker). In some embodiments, the proteins of the nanoparticles are present in a polymer or silica. In certain embodiments, the nanoparticle includes a nanoshell. The nanoparticles provided herein include one or more immunomodulatory agent. In some embodiments, the immunomodulatory agent is IL-2, IL-12, IL-15, IL-18, IL-21, a CD40 agonist (e.g., CD40L or agonistic anti-CD40 binding domain (e.g., an anti-CD40 scFv)) or a bioactive variant thereof. Nanoparticles are attached to the surface of the TIL using any suitable technique described herein.

[00600] Exemplary nanoparticles of use in the subject modified TILs provided herein include without limitation a liposome, a protein nanogel, a nucleotide nanogel, a polymer nanoparticle, or a solid nanoparticle. In some embodiments, the nanoparticle includes a liposome. In exemplary embodiments, the nanoparticle includes an immunomodulatory agent nanogel. In particular embodiments, the nanoparticle is an immunomodulatory agent nanogel with a plurality of immunomodulatory agents (*e.g.*, cytokines) covalently linked to each other. In some embodiments, the nanoparticle includes at least one polymer, cationic polymer, or cationic block co-polymer on the nanoparticle surface. Exemplary nanoparticles that can be used in the compositions provided herein are disclosed, for example, in US Patent Nos. 9,283,184 and 9,603,944, each of which is incorporated by reference in its entirety and in pertinent parts related to nanoparticles.

[00601] The immunomodulatory agent can be any suitable immunomodulatory agent including, for example, any of the immunomodulatory agents provided herein. In some embodiments, the immunomodulatory agent is an interleukin that promotes an anti-tumor response. In some embodiments, the immunomodulatory agent is a cytokine. In particular

embodiments, the immunomodulatory agent is IL-2, IL-12, IL-15, IL-21 or a bioactive variant thereof. In certain embodiments, the fusion protein includes more than one immunomodulatory agents. In exemplary embodiments, the fusion protein includes 2, 3, 4, 5, 6, 7, 8, 9 or 10 different immunomodulatory agents.

[00602] In some embodiments, the nanoparticle includes proteins that are covalently cross-linked to each other and/or a second component (*e.g.*, a degradable linker). In some embodiments, the nanoparticle includes immunomodulatory agents that are reversibly linked through a degradable linker to a function group or polymer, or "reversibly modified." In some embodiments, the nanoparticle is a nanogel that includes a plurality of immunomodulatory agents cross-linked to each other through a degradable linker (see US Patent No. 9,603,944). In exemplary embodiments, the protein of the nanogel are cross-linked to a polymer (*e.g.*, polyethylene glycol (PEG)). In some embodiments, the polymers are cross-linked to the nanogel surface.

[00603] In some embodiments, the immunomodulatory agents of the nanoparticles are reversibly linked to each other through a degradable linker (*e.g.*, a disulfide linker) such that under physiological conditions, the linker degrades, thereby releasing the immunomodulatory agent. In some embodiments, the immunomodulatory agents of the nanoparticles are reversibly linked to functional groups through a degradable linker such that under physiological conditions, the linker degrades and releases the immunomodulatory agent. Suitable degradable linkers include, but are not limited to: two N-hydroxysuccinimide (NHS) ester groups joined together by a flexible disulfide-containing linker that is sensitive to a reductive physiological environment; a hydrolysable linker that is sensitive to an acidic physiological environment (pH < 7, for example, a pH of 4-5, 5-6, or 6- to less than 7, *e.g.*, 6.9), or a protease sensitive linker that is sensitive to one or more enzymes present in biological media such as proteases in a tumor microenvironment such a matrix metalloproteases present in a tumor microenvironment or in inflamed tissue (*e.g.* matrix metalloproteinase 2 (MMP2) or matrix metalloproteinase 9 (MMP9)). A crosslinker sensitive to a reductive physiological environment is, for example, a crosslinker with disulfide containing linker that will react with amine groups on proteins by the presence of NHS groups which cross-link the proteins into high density protein nanogels. In some embodiments, the degradable cross-linker includes Bis[2-(N-succinimidyl-oxycarbonyloxy)ethyl] disulfide.

[00604] In some embodiments, the degradable linker includes at least one N-hydroxysuccinimide ester. In some embodiments, the degradable linker is a redox responsive linker. In some embodiments, the redox responsive linker includes a disulfide bond. In some embodiments, the degradable linkers provided herein include at least one N-hydroxysuccinimide ester, which is capable of reacting with proteins at neutral pH (*e.g.*, about 6 to about 8, or about 7) without substantially denaturing the protein. In some embodiments, the degradable linkers are "redox responsive" linkers, meaning that they degrade in the presence of a reducing agent (*e.g.*, glutathione, GSH) under physiological conditions (*e.g.*, 20-40 °C and/or pH 4-8), thereby releasing intact protein from the compound to which it is reversibly linked. In some embodiments, the protein of the nanoparticles are linked to the degradable linker through a terminal or internal-NH_2 functional group (*e.g.*, a side chain of a lysine).

[00605] In other embodiments, the proteins of the nanoparticle are linked by an enzyme-sensitive linker. Exemplary cleavable linker include those that are recognized by one of the following enzymes: metalloprotease MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-14, plasmin, PSA, PSMA, CATHEPSIN D, CATHEPSIN K, CATHEPSIN S, ADAM10, ADAM12, ADAMTS, Caspase-1, Caspase-2, Caspase-3, Caspase-4, Caspase-5, Caspase-6, Caspase-7, Caspase-8, Caspase-9, Caspase-10, Caspase-11, Caspase-12, Caspase-13, Caspase-14, and TACE. See, *e.g.*, US Patent Nos. 8,541,203 and 8,580,244, each of which is incorporated by reference in its entirety and in pertinent parts related to cleavable linkers.

[00606] In some embodiments, the nanoparticles are nanogels that include a monodispersed plurality of immunomodulatory agents (*e.g.*, cytokines). In some embodiments, the immunomodulatory agents of the nanogels are cross-linked to polymer. In certain embodiments, the polymer is cross-linked to the surface of the nanogel. In particular embodiments, the nanogel includes: a) one more immunomodulatory agents reversibly and covalently cross-linked to each other through a degradable linker; and b) polymers cross-linked to surface exposed proteins of the nanogels. Such nanogels can be made by contacting the one or more immunomodulatory agents with a degradable linker under conditions that permit reversible covalent crosslinking of the immunomodulatory agents to each other through the degradable linker to form a plurality of immunomodulatory agent nanogels. Subsequently, the immunomodulatory agent nanogels are contacted with a polymer (*e.g.*, polyethylene glycol) under conditions that permit crosslinking of the polymer to the

immunomodulatory agents of the immunomodulatory agent nanogels, thereby producing a plurality of immunomodulatory agent-polymer nanogels.

[00607] In some embodiments, the nanoparticles include one or more polymers. Exemplary polymers include, but are not limited to: aliphatic polyesters, poly (lactic acid) (PLA), poly (glycolic acid) (PGA), co-polymers of lactic acid and glycolic acid (PLGA), polycarprolactone (PCL), polyanhydrides, poly(ortho)esters, polyurethanes, poly(butyric acid), poly(valeric acid), and poly(lactide-co-caprolactone), and natural polymers such as alginate and other polysaccharides including dextran and cellulose, collagen, chemical derivatives thereof, including substitutions, additions of chemical groups such as for example alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art), albumin and other hydrophilic proteins, zein and other prolamines and hydrophobic proteins, copolymers and mixtures thereof. In some embodiments, the immunomodulatory agents of the nanoparticles are linked to hydrophilic polymers. Exemplary hydrophilic polymers include, but are not limited to: polyethylene glycol (PEG), polyethylene glycol-b-poly lysine (PEG-PLL), and/or polyethylene glycol-b-poly arginine (PEG-PArg).

[00608] In some embodiments, the nanoparticle (*e.g.*, nanogel) includes one or more polycations on its surface. Exemplary polycations for use in the subject nanoparticles include, but are not limited to, polylysine (poly-L-lysine and/or poly-D-lysine), poly(argininate glyceryl succinate) (PAGS, an arginine-based polymer), polyethyleneimine, polyhistidine, polyarginine, protamine sulfate, polyethylene glycol-b-polylysine (PEG-PLL), and polyethylene glycol-g-polylysine.

[00609] In some embodiments, the nanoparticle is associated with the TIL surface by electrostatic attraction to the TIL. In certain embodiments, the nanoparticle includes a ligand that has affinity for a surface molecule of the TIL (*e.g.*, a surface protein, carbohydrate and/or lipid).

[00610] In particular embodiments, the nanoparticle includes an antigen binding domain that binds a TIL surface antigen as described herein. In some embodiments, the antigen binding domain is an antibody or fragment thereof. In exemplary embodiments, the TIL surface antigen is CD45, LFA-1, CD 11a (integrin alpha- L), CD 18 (integrin beta-2), CD11b, CD11c, CD25, CD8, or CD4. In exemplary embodiments, the antigen binding domain (ABD) is an anti-CD45 antibody or a fragment thereof. In certain embodiments, the

anti-CD45 antibody is a human anti-CD45 antibody, a humanized anti-CD45 antibody, or a chimeric anti-CD45 antibody. In exemplary embodiments, the ABD includes the vhCDR1-3 and vlCDR1-3 of anti-CD45 antibody BC8 (see US20170326259, incorporated by reference herein, particularly in relevant parts relating to anti-CD45 antibody sequences). In some embodiments, the ABD includes the variable heavy domain and variable domain of anti-CD45 antibody BC8. In some embodiments, the ABD includes the vhCDR1-3 and vlCDR1-3 or VH and VL of one of the following anti-CD45 antibodies: 10G10, UCHL1, 9.4, 4B2, or GAP8.3 (see Spertini et al., Immunology 113(4):441-452 (2004), Buzzi et al., Cancer Research 52:4027-4035 (1992)). In such embodiments, the nanoparticles are attached to the surface of a population of TILs by incubating the TILs in the presence of the nanoparticles under conditions wherein the nanoparticles bind to the surface of the TILs.

[00611] In some embodiments, the nanoparticle is associated with the TIL cell surface by electrostatic attraction. In some embodiments the nanoparticle is covalently conjugated to the TIL. In other embodiments, the nanoparticle is not covalently conjugated to the TIL.

[00612] In some embodiments, the subject nanoparticles are attached to TILs produced during any of the steps of the Process 2A method disclosure herein (see, *e.g.*, FIGs 2-6). In exemplary embodiments, the subject nanoparticles are attached to TILs produced during any of the steps of the GEN 3 method disclosure herein (see, *e.g.*, FIG. 7). In exemplary embodiments, the subject nanoparticles are attached to TILs produced from the first expansion step in the Process 2A method and/or the priming expansion step in the GEN 3 method provided herein. In exemplary embodiments, the subject nanoparticles are attached to TILs produced from the second expansion step in the Process 2A method and/or the rapid expansion step in the GEN 3 method provided herein. In some embodiments, the TILs are PD-1 positive TILs that have been preselected using the methods described herein.

[00613] Additional suitable nanoparticles for use in the modified TILs provided herein are disclosed in US Patent Application Publication No. US20200131239 and WO2020205808, each of which is incorporated by reference in its entirety and in relevant parts related to nanoparticles.

C. Immunomodulatory Agents

[00614] The modified TILs provided herein include one or more immunomodulatory agents attached to its surface. The immunomodulatory agents can be incorporated into any of the immunomodulatory fusion proteins described herein, including, for example, the

membrane anchored immunomodulatory fusion proteins described herein. Any suitable immunomodulatory agent can be included in the subject modified TIL. In some embodiments, the immunomodulatory agent enhances TIL survival and/or anti-tumor activity once transferred to a patient. Exemplary immunomodulatory agents include, for example, cytokines. In some embodiments, the modified TIL includes one or more of the following cytokines: IL-2, IL-7, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IL-4, IL-1α, IL-1β, IL-5, IFNγ, TNF α (TNFa), IFNα, IFNβ, GM-CSF, or GCSF or a biologically active variant thereof. In some embodiments, the immunomodulatory agent is a costimulatory molecule. In particular embodiments, the costimulatory molecule is one of the following: OX40, CD28, GITR, VISTA, CD40, CD3, or an agonist of CD137. In some embodiments, the immunomodulatory agent is a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). Exemplary immunomodulatory agents are discussed in detailed further below.

1. IL-15

[00615] In some embodiments, the modified TILs provided herein include an IL-15. In exemplary embodiments, the IL-15 is included as part of an immunomodulatory fusion protein as described herein (e.g., a membrane anchored immunomodulatory fusion protein).

[00616] As used herein, "interleukin 15", "IL-15" and "IL15" all refer to an interleukin that binds to and signals through a complex composed of an IL-15 specific receptor alpha chain (IL-15Rα), an IL-2/IL-15 receptor beta chain (CD122) and the common gamma chain (gamma-C, CD132) (e.g., Genbank Accession numbers: NM_00000585, NP_000576 and NP_751915 (human); and NM_001254747 and NP_001241676 (mouse)). IL-15 has been shown to stimulate T cell proliferation inside tumors. IL-15 also is able to extend the survivability of effector memory CD8+ T cells and is critical for the development of NK cells. Therefore, without being bound by any particular theory of operation, it is believed that modified TILs associated with an IL-15s described herein exhibit enhanced survival and/or anti-tumor effects.

[00617] IL-15 has a short half-life of less than 40 minutes in vivo. Modifications to IL-15 monomer can improve its in vivo pharmacokinetics in the treatment of cancers. These modifications have generally centered on improving the trans-presentation of IL-15 with the alpha subunit of IL-15 receptor, IL-15Rα. Such modifications include: 1) pre-association of IL-15 and its soluble receptor a-subunit-Fc fusion to form IL-15: IL-15Rα-Fc complex (see,

e.g., Rubinstein et al., Proc Natl Acad Sci U.S.A. 103:9166–71 (2006)); 2) expression of the superagonist IL-15-sIL-15Rα-sushi protein (see, *e.g.*, Bessard et al., Molecular cancer therapeutics 8: 2736-45 (2009)); and 3) pre-association of human IL-15 mutant IL-15N72D with IL-15Rα-Fc sushi-Fc fusion complex (see, *e.g.*, Zhu et al., Journal of Immunology 183: 3598-6007 (2009)).

[00618] In some embodiments, the IL-15 associated with the modified TIL is a full length IL-15, a fragment or a variant of IL-15. In some embodiments, the IL-15 is a human IL-15 or a variant human IL-15. In exemplary embodiments, the IL-15 is a biological active human IL-15 variant. In some embodiments, the IL-15 includes a 1, 2, 3,4 ,5 ,6 7, 8, 9, or 10 mutations as compared to a wild-type IL-15. In certain embodiments, the IL-15 includes an N72D mutation relative to a wild type human IL-15. In some embodiments, the variant IL-15 exhibits IL-15Rα binding activity.

[00619] In some embodiments, the immunomodulatory agent includes an IL-15 and an extracellular domain of an IL-15Rα. In certain embodiments, the immunomodulatory agent includes an IL-15 and an IL-15Rα fused to an Fc domain (IL-15Rα-Fc)

TABLE 5 – IL-15 Related Sequences.

Description	Amino Acid Sequence
Human IL-15 (N72D mutant)	NWVNVISDLKKIEDLIQSMHIDATLYTESDV HPSCKVTAMKCFLLELQVISLESGDASIHDT VENLIILANDSLSSNGNVTESGCKECEELEEK NIKEFLQSFVHIVQMFINTS (SEQ ID NO: 258)
Human IL-15R-alpha-Su/Fc domain	ITCPPPMSVEHADIWVKSYSLYSRERYICNSG FKRKAGTSSLTECVLNKATNVAHWTTPSLK CIREPKSCDKTHTCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 259)
Human IL-15R-alpha-Su (65aa truncated extracellular domain)	ITCPPPMSVEHADIWVKSYSLYSRERYICNSG FKRKAGTSSLTECVLNKATNVAHWTTPSLK CIR (SEQ ID NO: 260)

Human IL-15 isoform 2	MVLGTIDLCSCFSAGLPKTEANWVNVISDLK KIEDLIQSMHIDATLYTESDVHPSCKVTAMK CFLLELQVISLESGDASIHDTVENLIILANNSL SSNGNVTESGCKECEELEEKNIKEFLQSFVHI VQMFINTS (SEQ ID NO: 261)
Human IL-15 isoform 1	MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHV FILGCFSAGLPKTEANWVNVISDLKKIEDLIQ SMHIDATLYTESDVHPSCKVTAMKCFLLEL QVISLESGDASIHDTVENLIILANNSLSSNGN VTESGCKECEELEEKNIKEFLQSFVHIVQMFI NTS (SEQ ID NO: 262)
Human IL-15 (without signal peptide)	NWVNVISDLKKIEDLIQSMHIDATLYTESDV HPSCKVTAMKCFLLELQVISLESGDASIHDT VENLIILANNSLSSNGNVTESGCKECEELEEK NIKEFLQSFVHIVQMFINTS (SEQ ID NO: 263)
Human IL-15R-alpha (85 aa truncated extracellular domain)	ITCPPPMSVEHADIWVKSYSLYSRERYICNSG FKRKAGTSSLTECVLNKATNVAHWTTPSLK CIRDPALVHQRPAPPSTVTTAGV (SEQ ID NO: 264)
Human IL-15R-alpha (182aa truncated extracellular domain)	ITCPPPMSVEHADIWVKSYSLYSRERYICNSG FKRKAGTSSLTECVLNKATNVAHWTTPSLK CIRDPALVHQRPAPPSTVTTAGVTPQPESLSP SGKEPAASSPSSNNTAATTAAIVPGSQLMPS KSPSTGTTEISSHESSHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTST (SEQ ID NO: 265)
Human IL-15R-alpha	MAPRRARGCRTLGLPALLLLLLLRPPATRGI TCPPPMSVEHADIWVKSYSLYSRERYICNSG FKRKAGTSSLTECVLNKATNVAHWTTPSLK CIRDPALVHQRPAPPSTVTTAGVTPQPESLSP SGKEPAASSPSSNNTAATTAAIVPGSQLMPS KSPSTGTTEISSHESSHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTSTVLLCGL SAVSLLACYLKSRQTPPLASVEMEAMEALP VTWGTSSRDEDLENCSHHL (SEQ ID NO: 266)

[00620] In some embodiments the immunostimulatory protein is a superagonist IL-15 (IL-15SA) that includes a complex of human IL-15 and soluble human IL-15Rα. The combination of human IL-15 with soluble human IL-15Rα forms an IL-15 SA complex that possesses greater biological activity than human IL-15 alone. Soluble human IL-15Rα, as well as truncated versions of the extracellular domain, has been described in the art (Wei et al., 2001 J of Immunol. 167: 277-282). The amino acid sequence of human IL-15Rα is set forth in SEQ ID NO: 266. In some embodiments, the IL-15SA includes a complex of human

IL-15 and soluble human. IL-15Rα comprising all or a portion of the extracellular domain, without the transmembrane or cytoplasmic domain. In some embodiments, the IL-15SA includes a complex of human IL-15 and soluble human IL-15Rα that includes the full extracellular domain or a truncated form of the extracellular domain which retains IL-15 binding activity.

[00621] In some embodiments, the IL-15SA includes a complex of human IL-15 and soluble human IL-15Rα that includes a truncated form of the extracellular domain which retains IL-15 binding activity. In some embodiments, the soluble human IL-15Rα includes amino acids 1-60, 1-61, 1-62, 1-63, 1-64 or 1-65 of human IL-15Rα. In some embodiments, the soluble human IL-15Rα includes amino acids 1-80, 1-81, 1-82, 1-83, 1-84 or 1-85 of human IL-15Rα. In some embodiments, the soluble human IL-15Rα includes amino acids 1-180, 1-181, or 1-182 of human IL-15Rα.

[00622] In some embodiments, the immunomodulatory agent is an IL-15SA comprising a complex of human IL-15 and soluble human IL-15Rα comprising a truncated form of the extracellular domain which retains IL-15 binding activity and comprises a Sushi domain. The Sushi domain of IL-15Rα is described in the art as approximately 60 amino acids in length and comprises 4 cysteines. (Wei et al., 2001). Truncated forms of soluble human IL-15Rα which retain IL-15 activity and comprise a Sushi domain are useful in IL-15SA of the present disclosure.

[00623] In some embodiments, the immunomodulatory agent includes a complex comprising soluble human IL-15Rα expressed as a fusion protein, such as an Fc fusion as described herein (*e.g.*, human IgG1 Fc), with IL-15. In some embodiments, IL-15SA comprises a dimeric human IL-15RαFc fusion protein (*e.g.*, human IgG1 Fc) complexed with two human IL-15 molecules.

[00624] In some embodiments, the immunomodulatory agent is an IL-15SA cytokine complex that includes an IL-15 molecule comprising an amino acid sequence set forth in SEQ ID NO: 258, SEQ ID NO: 261, SEQ ID NO:262, or SEQ ID NO:263. In some embodiments, an IL-15SA cytokine complex comprises a soluble IL-15Rα molecule comprising a sequence of SEQ ID NO:260, SEQ ID NO: 264 or SEQ ID NO:265.

[00625] In some embodiments, the immunomodulatory agent is an IL-15SA cytokine complex that includes a dimeric IL-15RαFc fusion protein complexed with two IL-15 molecules. In some embodiments, IL-15-SA comprises a dimeric IL-15RαSu (Sushi

domain)/Fc (SEQ ID NO:259) and two IL-15N72D (SEQ ID NO:258) molecules (also known as ALT-803), as described in US20140134128, incorporated herein by reference. In some embodiments, the IL-15SA comprises a dimeric IL-15RαSu/Fc molecule (SEQ ID NO: 259) and two IL-15 molecules (SEQ ID NO: 261). In some embodiments, the IL-15SA comprises a dimeric IL-15RαSu/Fc molecule (SEQ ID NO: 259) and two IL-15 molecules (SEQ ID NO:262). In some embodiments, the IL-15SA comprises a dimeric IL-15RαSu/Fc molecule (SEQ ID NO:259) and two IL-15 molecules (SEQ ID NO:263).

[00626] In some embodiments, the IL-15SA includes a dimeric IL-15RαSu/Fc molecule (SEQ ID NO:259) and two IL-15 molecules having amino acid sequences selected from SEQ ID NO: 258, 258, 262, and 263.

[00627] In some embodiments, the IL-15SA includes a soluble IL-15Rα molecule (SEQ ID NO:260) and two IL-15 molecules (SEQ ID NO:258). In some embodiments, the IL-15SA comprises a soluble IL-15Rα molecule (SEQ ID NO:260) and two IL-15 molecules (SEQ ID NO:261). In some embodiments, the IL-15SA comprises a soluble IL-15Rα molecule (SEQ ID NO:260) and two IL-15 molecules (SEQ ID NO:262). In some embodiments, the IL-15SA comprises a soluble IL-15Rα molecule (SEQ ID NO:260) and two IL-15 molecules (SEQ ID NO:263).

[00628] In some embodiments, the IL-15SA comprises a soluble IL-15Rα molecule (SEQ ID NO:264) and two IL-15 molecules (SEQ ID NO:258). In some embodiments, the IL-15SA comprises a soluble IL-15Rα molecule (SEQ ID NO:264) and two IL-15 molecules (SEQ ID NO:261). In some embodiments, the IL-15SA comprises a soluble IL-15Rα molecule (SEQ ID NO:264) and two IL-15 molecules (SEQ ID NO:262). In some embodiments, the IL-15SA comprises a soluble IL-15Rα molecule (SEQ ID NO:264) and two IL-15 molecules (SEQ ID NO:261).

[00629] In some embodiments, the IL-15SA includes a soluble IL-15Rα molecule (SEQ ID NO:265) and two IL-15 molecules (SEQ ID NO:258). In some embodiments, the IL-15SA comprises a soluble IL-15Rα molecule (SEQ ID NO:265) and two IL-15 molecules (SEQ ID NO:261). In some embodiments, the IL-15SA comprises a soluble IL-15Rα molecule (SEQ ID NO:265) and two IL-15 molecules (SEQ ID NO:262). In some embodiments, the IL-15SA comprises a soluble IL-15Rα molecule (SEQ ID NO:265) and two IL-15 molecules (SEQ ID NO:263).

[00630] In some embodiments, the IL-15SA comprises a dimeric IL-15RαSu/Fc (SEQ ID NO:269) molecule and two IL-15 molecules (SEQ ID NO:262). In some embodiments, the IL-15SA includes a dimeric IL-15RαSu/Fc (SEQ ID NO:259) molecule and two IL-15 molecules (SEQ ID NO:263).

[00631] In some embodiments, the IL-15SA includes SEQ ID NO:259 and SEQ ID NO:260. In some embodiments IL-15SA comprises SEQ ID NO:261 or SEQ ID NO:262. In some embodiments the IL-15SA comprises SEQ ID NO:261 and SEQ ID NO:259. In some embodiments the IL-15SA comprises SEQ ID NO:262 and SEQ ID NO:259. In some embodiments the IL-15SA comprises SEQ ID NO:263 and SEQ ID NO:259. In some embodiments, the IL-15SA comprises SEQ ID NO:261 and SEQ ID NO:260. In some embodiments the IL-15SA comprises SEQ ID NO:262 and SEQ ID NO:260.

[00632] In some embodiments, the TIL compositions include an immunomodulatory fusion protein or nanoparticle composition that includes a IL-15 or a bioactive variant thereof. Exemplary fusion proteins that include IL-15 are depicted in Figures 36 and 37, and Tables 58 and 59.

[00633] In exemplary embodiments the TIL compositions provided herein includes a nucleic acid encoding an immunomodulatory fusion protein that includes an IL-15, wherein the nucleic acid is operably linked to a NFAT promoter, as described herein. Exemplary NFAT promoter-driven constructs for expression of immunomodulatory fusion proteins that include IL-15 are depicted in Table 59.

2. IL-12

[00634] In some embodiments, the modified TIL is associated with an IL-12 or a variant thereof. In exemplary embodiments, the IL-12 is included as part of an immunomodulatory fusion protein as described herein (e.g., a membrane anchored immunomodulatory fusion protein).

[00635] As used herein, "interleukin 12", "IL-12" and "IL12" all refer to an interleukin that is a heterodimeric cytokine encoded by the IL-12A and IL-12B genes (Genbank Accession numbers: NM_000882 (IL-12A) and NM_002187 (IL-12B)). IL-12 is composed of a bundle of four alpha helices and is involved in the differentiation of native T cells into TH1 cells. It is encoded by two separate genes, IL-12A (p35) and IL-12B (p40). The active heterodimer (referred to as 'p70'), and a homodimer of p40 are formed following protein

synthesis. IL-12 binds to the IL-12 receptor, which is a heterodimeric receptor formed by IL-12R-β1 and IL-12R-β2. IL-12 is known as a T cell-stimulating factor that can stimulate the growth and function of T cells. In particular, IL-12 can stimulate the production of interferon gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α) from T cells and natural killer (NK) cells and reduce IL-4 mediated suppression of IFN-γ. IL-12 can further mediate enhancement of the cytotoxic activity of NK cells and CD8+ cytotoxic T lymphocytes. Moreover, IL-12 can also have anti-angiogenic activity by increasing production of interferon gamma, which in turn increases the production of the chemokine inducible protein-10 (IP-10 or CXCL10). IP-10 then mediates this anti-angiogenic effect. Thus, without being bound by any particular theory of operation, it is believed that IL-12 can increase the survivability and/or anti-tumor effects of the TIL compositions provided herein.

[00636] In some embodiments, the IL-12 associated with the modified TIL is a full length IL-12, a fragment or a variant of IL-12. In some embodiments, the IL-12 is a human IL-12 or a variant human IL-12. In exemplary embodiments, the IL-12 is a biological active human IL-12 variant. In some embodiments, the IL-12 includes a 1, 2, 3,4 ,5 ,6 7, 8, 9, or 10 mutations as compared to a wild-type IL-12.

[00637] In some embodiments, the IL-12 included in the modified TIL compositions include an IL-12 p35 subunit or a variant thereof. In some embodiments, the IL-12 p35 subunit is a human IL-12 p35 subunit. In some embodiments, the IL-12 p35 subunit has the amino acid sequence In certain embodiments, the IL-12 included in the modified TIL compositions include an IL-12 p40 subunit or a variant thereof. In certain embodiments, the IL-12 is a single chain IL-12 polypeptide comprising an IL-12 p35 subunit attached to an IL-12 p40 subunit. Such IL-12 single chain polypeptides advantageously retain one or more of the biological activities of wildtype IL-12. In some embodiments, the single chain IL-12 polypeptide described herein is according to the formula, from N-terminus to C-terminus, (p40)-(L)-(p35), wherein "p40" is an IL-12 p40 subunit, "p35" is IL-12 p35 subunit and L is a linker. In other embodiments, the single chain IL-12 is according to the formula from N-terminus to C-terminus, (p35)-(L)-(p40). Any suitable linker can be used in the single chain IL-12 polypeptide including those described herein. Suitable linkers can include, for example, linkers having the amino acid sequence $(GGGGS)_x$ wherein x is an integer from 1-10. Other suitable linkers include, for example, the amino acid sequence GGGGGGS. Exemplary single chain IL-12 linkers than can be used with the subject single chain IL-12 polypeptides are also described in Lieschke et al., *Nature Biotechnology* 15: 35-40 (1997),

which is incorporated herein in its entirety by reference and particularly for its teaching of IL-12 polypeptide linkers. In an exemplary embodiment, the single chain IL-12 polypeptide is a single chain human IL-12 polypeptide (*i.e.*, it includes a human p35 and p40 IL-12 subunit).

TABLE 6 – IL-12 Related Sequences.

Description	Amino Acid Sequence
Human IL-12 p35 subunit	RNLPVATPDPGMFPCLHHSQNLLRAVSNML QKARQTLEFYPCTSEEIDHEDITKDKTSTVEA CLPLELTKNESCLNSRETSFITNGSCLASRKT SFMMALCLSSIYEDLKMYQVEFKTMNAKLL MDPKRQIFLDQNMLAVIDELMQALNFNSET VPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTI DRVMSYLNAS (SEQ ID NO:267)
Human IL-12 p40 subunit	IWELKKDVYVVELDWYPDAPGEMVVLTCD TPEEDGITWTLDQSSEVLGSGKTLTIQVKEF GDAGQYTCHKGGEVLSHSLLLLHKKEDGIW STDILKDQKEPKNKTFLRCEAKNYSGRFTC WWLTTISTDLTFSVKSSRGSSDPQGVTCGAA TLSAERVRGDNKEYEYSVECQEDSACPAAE ESLPIEVMVDAVHKLKYENYTSSFFIRDIIKP DPPKNLQLKPLKNSRQVEVSWEYPDTWSTP HSYFSLTFCVQVQGKSKREKKDRVFTDKTS ATVICRKNASISVRAQDRYYSSSWSEWASVP CS (SEQ ID NO:268)

[00638] In some embodiments, the TIL compositions include an immunomodulatory fusion protein or nanoparticle composition that includes a IL-12 or a bioactive variant thereof.

[00639] In exemplary embodiments the TIL compositions provided herein includes a nucleic acid encoding an immunomodulatory fusion protein that includes an IL-12, wherein the nucleic acid is operably linked to a NFAT promoter, as described herein. See, e.g., US Patent No. 8,556,882, which is incorporated by reference in its entirety and particularly for pertinent parts relating to NFAT promoters for IL-12 expression. Exemplary fusion proteins that include IL-12 are depicted in Figures 36 and 37, and Table 58.

3. IL-18

[00640] In some embodiments, the modified TIL is associated with an IL-18 or a variant thereof. In exemplary embodiments, the IL-18 is included as part of an immunomodulatory fusion protein as described herein (e.g., a membrane anchored immunomodulatory fusion protein).

[00641] As used herein, "interleukin 18", "IL-18," "IL18," "IGIF," "IL-1g," "interferon-gamma inducing factor," and "IL1F4," all refer to an interleukin that is a heterodimeric cytokine encoded by the IL-18 gene (e.g., Genbank Accession numbers: NM_001243211, NM_001562 and NM_001386420). IL-18, structurally similar to IL-1β, is a member of IL-1 superfamily of cytokines. This cytokine, which is expressed by many human lymphoid and nonlymphoid cells, has an important role in inflammatory processes. IL-18 in combination with IL-12 can activate cytotoxic T cells (CTLs), as well as natural killer (NK) cells, to produce IFN-γ and, therefore, contributes to tumor immunity. Thus, without being bound by any particular theory of operation, it is believed that IL-18 can enhance the anti-tumor effects of the TIL compositions provided herein.

[00642] In some embodiments, the IL-18 associated with the modified TIL is a full length IL-18, a fragment or a variant of IL-18. In some embodiments, the IL-18 is a human IL-18 or a variant human IL-18. In exemplary embodiments, the IL-18 is a biological active human IL-18 variant. In some embodiments, the IL-18 includes 1, 2, 3,4 ,5 ,6 7, 8, 9, or 10 mutations as compared to a wild-type IL-18. In some embodiments, the variant IL-18 has the amino acid sequence:

TABLE 7 – IL-18 Related Sequences.

Description	Amino Acid Sequence
Human IL-18	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFE DMTDSDCRDNAPRTIFIISMYKDSQPRGMAV TISVKCEKISTLSCENKIISFKEMNPPDNIKDT KSDIIFFQRSVPGHDNKMQFESSSYEGYFLA CEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO: 269)
Human IL-18 variant	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFE DMTDSDCRDNAPRTIFIISKYSDSRARGLAV TISVKCEKISTLSCENKIISFKEMNPPDNIKDT KSDIIFFARVPGHGRKTQFESSSYEGYFLACE KERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO: 270)

[00643] In some embodiments, the TIL compositions include an immunomodulatory fusion protein or nanoparticle composition that includes a IL-18 or a bioactive variant thereof. Exemplary fusion proteins that include IL-18 are depicted in Figure 36.

[00644] In exemplary embodiments the TIL compositions provided herein includes a nucleic acid encoding an immunomodulatory fusion protein that includes an IL-18, wherein the nucleic acid is operably linked to a NFAT promoter, as described herein. Exemplary

NFAT promoter-driven constructs for expression of immunomodulatory fusion proteins that include IL-21 are depicted in Table 59.

4. IL-21

[00645] In some embodiments, the modified TIL is associated with an IL-21 or a variant thereof. In exemplary embodiments, the IL-21 is included as part of an immunomodulatory fusion protein as described herein (e.g., a membrane anchored immunomodulatory fusion protein).

[00646] In certain embodiments, the cytokine-ABD includes an IL-21 molecule or fragment thereof. As used herein, "interleukin 21" "IL-21", and "IL21" (e.g., Genbank Accession numbers: NM_001207006 and NP_001193935 (human); and NM_0001291041 and NP_001277970 (mouse)) all refer to a member of a cytokine that binds to IL-21 receptor and has potent regulatory effects on cells of the immune system, including natural killer (NK) cells and cytotoxic cells and binds to IL-21 receptor that can destroy virally infected or cancerous cells. Thus, without being bound by any particular theory of operation, it is believed that IL-21 can increase the survivability and/or anti-tumor effects of the TIL compositions provided herein.

[00647] In some embodiments, the IL-21 is a human IL-21. In some embodiments, the IL-21 associated with the modified TIL is a full length IL-21, a fragment or a variant of IL-21. In some embodiments, the IL-21 is a human IL-21 or a variant human IL-21. In exemplary embodiments, the IL-21 is a biological active human IL-21 variant. In some embodiments, the IL-21 includes a 1, 2, 3,4 ,5 ,6 7, 8, 9, or 10 mutations as compared to a wild-type IL-21.

TABLE 8 – IL-21 Related Sequences.

Description	Amino Acid Sequence
Human IL-21	QGQDRHMIRMRQLIDIVDQLKNYVNDLVPE FLPAPEDVETNCEWSAFSCFQKAQLKSANT GNNERIINVSIKKLKRKPPSTNAGRRQKHRL TCPSCDSYEKKPPKEFLERFKSLLQKMIHQH LSSRTHGSEDS (SEQ ID NO:271)

[00648] In some embodiments, the TIL compositions include an immunomodulatory fusion protein or nanoparticle composition that includes a IL-21 or a bioactive variant thereof. Exemplary fusion proteins that include IL-21 are depicted in Figures 36 and 37, and Tables 58 and 59.

[00649] In exemplary embodiments the TIL compositions provided herein includes a nucleic acid encoding an immunomodulatory fusion protein that includes an IL-21, wherein the nucleic acid is operably linked to a NFAT promoter, as described herein.

5. IL-2

[00650] In some embodiments, the modified TIL is associated with an IL-2 or a variant thereof. In exemplary embodiments, the IL-2 is included as part of an immunomodulatory fusion protein as described herein (e.g., a membrane anchored immunomodulatory fusion protein).

[00651] In certain embodiments, the cytokine-ABD includes an IL-2 molecule or fragment thereof. As used herein, "interleukin 2" "IL-2", "IL2," and "TCGF" (e.g., Genbank Accession numbers: NM_000586 and NP_000577 (human) all refer to a member of a cytokine that binds to IL-2 receptor. IL-2 enhances activation-induced cell death (AICD). IL-2 also promotes the differentiation of T cells into effector T cells and into memory T cells when the initial T cell is also stimulated by an antigen, thus helping the body fight off infections. Together with other polarizing cytokines, IL-2 stimulates naive CD4+ T cell differentiation into Th1 and Th2 lymphocytes and impedes differentiation into Th17 and follicular Th lymphocytes.. IL-2 also increases the cell killing activity of both natural killer cells and cytotoxic T cells. Thus, without being bound by any particular theory of operation, it is believed that IL-2 can increase the survivability and/or anti-tumor effects of the TIL compositions provided herein.

[00652] In some embodiments, the IL-2 is a human IL-2. In some embodiments, the IL-2 associated with the modified TIL is a full length IL-2, a fragment or a variant of IL-2. In some embodiments, the IL-2 is a human IL-2 or a variant human IL-2. In exemplary embodiments, the IL-2 is a biological active human IL-2 variant. In some embodiments, the IL-2 includes a 1, 2, 3,4 ,5 ,6 7, 8, 9, or 10 mutations as compared to a wild-type IL-2.

TABLE 9 – IL-2 Related Sequences.

Description	Amino Acid Sequence
Human IL-2	MYRMQLLSCIALSLALVTNSAPTSSSTKKTQ LQLEHLLLDLQMILNGINNYKNPKLTRMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNL AQSKNFHLRPRDLISNINVIVLELKGSETTFM CEYADETATIVEFLNRWITFCQSIISTLT (SEQ ID NO:272)

[00653] In some embodiments, the TIL compositions include an immunomodulatory fusion protein or nanoparticle composition that includes a IL-2 or a bioactive variant thereof. Exemplary fusion proteins that include IL-2 are depicted in Figures 36 and 37.

[00654] In exemplary embodiments the TIL compositions provided herein includes a nucleic acid encoding an immunomodulatory fusion protein that includes an IL-2, wherein the nucleic acid is operably linked to a NFAT promoter, as described herein.

6. CD40 Agonists

[00655] In some embodiments, the modified TIL is associated with CD40 agonist. In exemplary embodiments, the CD40 agonist is included as part of an immunomodulatory fusion protein as described herein (e.g., a membrane anchored immunomodulatory fusion protein).

[00656] Cluster of differentiation 40, CD40, is a costimulatory protein found on antigen-presenting cells (APCs) and is required for APC activation. The binding of CD40L (CD154) on T helper cells to CD40 activates antigen presenting cells (e.g., dendritic cells) and induces a variety of downstream effects. Without being by any particular theory of operation, it is believed that the addition of one or more immunomodulatory agents that activate CD40 on antigen presenting cells (i.e., CD40 agonists) can enhance the anti-tumor effects of the TIL compositions provided herein. CD40 agonists, include, for example, CD40L and antibody or antibody fragments thereof (e.g., an scFv) that agonistically binds CD40. In some embodiments, the TIL compositions include an immunomodulatory fusion protein or nanoparticle composition that includes a CD40L or a bioactive variant thereof. In some embodiments, the TIL composition includes an immunomodulatory fusion protein that includes an agonistic anti-CD40 binding domain (e.g., an scFv). Exemplary CD40 agonist sequences are depicted in the table below.

[00657] CD40 agonist activity can be measured using any suitable method known in the art. Ligation of CD40 on DC, for example, induces increased surface expression of costimulatory and MHC molecules, production of proinflammatory cytokines, and enhanced T cell triggering. CD40 ligation on resting B cells increases antigen-presenting function and proliferation. In exemplary embodiments, the CD40 agonist is capable of activating human dendritic cells.

[00658] In some embodiments, the TIL composition includes an agonistic anti-CD40 binding domain having the VH and VL sequences of an anti-CD40 scFv depicted in Table 10 or a bioactive variant thereof. In some embodiments, the anti-CD40 binding domain includes a VH sequence that is at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the VH sequence depicted in Table 10. In some embodiments, the agonistic anti-CD40 binding domain includes a VH sequence that includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions as compared to the VH sequence depicted in Table 10. In some embodiments, the anti-CD40 binding domain includes a VL sequence that is at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the VL sequence depicted in Table 10. In some embodiments, the anti-CD40 binding domain includes a VL sequence that includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions as compared to the VL sequence depicted in Table 10. In exemplary embodiments, the anti-CD40 binding domain is an anti-CD40 scFv selected from SEQ ID NOs:276, 279, 282, and 285 in Table 10.

[00659] In some embodiments, the anti-CD40 binding domain is a variant of an anti-CD40 scFv in Table 10 that is capable of binding to human CD40. In exemplary embodiments, the variant anti-CD40 scFv is least about 75%, 80%, 85%, 90%, 95%, or 99% identical to an anti-CD40 scFv selected from SEQ ID NOs:276, 279, 282, and 285 in Table 10.

[00660] Assessment of CD40 binding domain binding can be measured using any suitable assay known in the art, including, but not limited to: a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g., Octet assay) assay.

[00661] Additional CD40 binding domains (VH and VLs) that are useful as immunomodulatory agents include those described in US Patent Nos. US 6,838,261, US 6,843,989, US 7,338,660, US 8,7778,345, which are incorporated by reference herein,

particularly with respect to teachings of anti-CD40 antibodies and VH, VL and CDR sequences.

[00662] In some embodiments, the CD40 agonist is a CD40 ligand (CD40L). In exemplary embodiments, the CD40L is human CD40L (SEQ ID NO:270). In some embodiments, the CD40L is a variant of a human CD40L that is at least about 75%, 80%, 85%, 90%, 95%, or 99% identical to SEQ ID NO:253. In some embodiments, the CD40L is a variant of a human CD40L that includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions as compared to SEQ ID NO:273.

[00663] Exemplary fusion proteins that include CD40 agonists are depicted in Figures 36 and 37.

[00664] In exemplary embodiments the TIL compositions provided herein includes a nucleic acid encoding an immunomodulatory fusion protein that includes a CD40 agonist, wherein the nucleic acid is operably linked to a NFAT promoter, as described herein.

TABLE 10 – CD40 Agonist Related Sequences.

Description	Amino Acid Sequence
Human CD40L	MIETYNQTSPRSAATGLPISMKIFMYLLTVFL ITQMIGSALFAVYLHRRLDKIEDERNLHEDF VFMKTIQRCNTGERSLSLLNCEEIKSQFEGFV KDIMLNKEETKKENSFEMQKGDQNPQIAAH VISEASSKTTSVLQWAEKGYYTMSNNLVTL ENGKQLTVKRQGLYYIYAQVTFCSNREASS QAPFIASLCLKSPGRFERILLRAANTHSSAKP CGQQSIHLGGVFELQPGASVFVNVTDPSQVS HGTGFTSFGLLKL (SEQ ID NO: 273)
Anti-human CD40 VH (Sotigalimab)	QVQLVESGGGVVQPGRSLRLSCAASGFSFSS TYVCWVRQAPGKGLEWIACIYTGDGTNYSA SWAKGRFTISKDSSKNTVYLQMNSLRAEDT AVYFCARPDITYGFAINFWGPGTLVTVSS (SEQ ID NO: 274)
Anti-human CD40 VL (Sotigalimab)	DIQMTQSPSSLSASVGDRVTIKCQASQSISSR LAWYQQKPGKPPKLLIYRASTLASGVPSRFS GSGSGTDFTLTISSLQPEDVATYYCQCTGYGI SWPIGGGTKVEIK (SEQ ID NO: 275)
Anti-human CD40 scFv (Sotigalimab)	QVQLVESGGGVVQPGRSLRLSCAASGFSFSS TYVCWVRQAPGKGLEWIACIYTGDGTNYSA SWAKGRFTISKDSSKNTVYLQMNSLRAEDT AVYFCARPDITYGFAINFWGPGTLVTVSSGG GGSGGGGSGGGGSGGGGSDIQMTQSPSSLS ASVGDRVTIKCQASQSISSRLAWYQQKPGKP PKLLIYRASTLASGVPSRFSGSGSGTDFTLTIS

	SLQPEDVATYYCQCTGYGISWPIGGGTKVEIK (SEQ ID NO: 276)
Anti-human CD40 VH (Dacetuzumab)	QLVESGGGLVQPGGSLRLSCAASGYSFTGYYIHWVRQAPGKGLEWVARVIPNAGGTSYNQKFKGRFTLSVDNSKNTAYLQMNSLRAEDTAVYYCAREGIYWWGQGTLVTVSS (SEQ ID NO: 277)
Anti-human CD40 VL (Dacetuzumab)	DIQMTQSPSSLSASVGDRVTITCRSSQSLVHSNGNTFLHWYQQKPGKAPKLLIYTVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCSQTTHVPWTFGQGTKVEIK (SEQ ID NO: 278)
Anti-human CD40 scFv (Dacetuzumab)	QLVESGGGLVQPGGSLRLSCAASGYSFTGYYIHWVRQAPGKGLEWVARVIPNAGGTSYNQKFKGRFTLSVDNSKNTAYLQMNSLRAEDTAVYYCAREGIYWWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRSSQSLVHSNGNTFLHWYQQKPGKAPKLLIYTVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCSQTTHVPWTFGQGTKVEIK (SEQ ID NO: 279)
Anti-CD40 VH (Lucatutuzumab)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYEESNRYHADSVKGRFTISRDNSKITLYLQMNSLRTEDTAVYYCARDGGIAAPGPDYWGQGTLVTVSS (SEQ ID NO: 280)
Anti-CD40 VL (Lucatutuzumab)	DIVMTQSPLSLTVTPGEPASISCRSSQSLLYSNGYNYLDWYLQKPGQSPQVLISLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQARQTPFTFGPGTKVDIR (SEQ ID NO: 281)
Anti-CD40 scFv (Lucatutuzumab)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYEESNRYHADSVKGRFTISRDNSKITLYLQMNSLRTEDTAVYYCARDGGIAAPGPDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIVMTQSPLSLTVTPGEPASISCRSSQSLLYSNGYNYLDWYLQKPGQSPQVLISLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQARQTPFTFGPGTKVDIR (SEQ ID NO: 282)
Anti-CD40 VH (Selicrelumab)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPDSGGTNYAQKFQGRVTMTRDTSISTAYMELNRLRSDDTAVYYCARDQPLGYCTNGVCSYFDYWGQGTLVTVSS (SEQ ID NO: 283)
Anti-CD40 VL (Selicrelumab)	DIQMTQSPSSVSASVGDRVTITCRASQGIYSWLAWYQQKPGKAPNLLIYTASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANIFPLTFGGGTKVEIK (SEQ ID NO: 284)

Anti-CD40 scFv (Selicrelumab)	QVQLVQSGAEVKKPGASVKVSCKASGYTFT GYYMHWVRQAPGQGLEWMGWINPDSGGT NYAQKFQGRVTMTRDTSISTAYMELNRLRS DDTAVYYCARDQPLGYCTNGVCSYFDYWG QGTLVTVSSGGGGSGGGGSGGGGSGGGGSD IQMTQSPSSVSASVGDRVTITCRASQGIYSW LAWYQQKPGKAPNLLIYTASTLQSGVPSRFS GSGSGTDFTLTISSLQPEDFATYYCQQANIFP LTFGGGTKVEIK (SEQ ID NO: 285)

IV. Gene-Editing Processes

A. Overview: TIL Expansion + Gene-Editing + Transient Gene-Editing

[00665] In some embodiments of the present invention directed to methods for expanding TIL populations, the methods comprise one or more steps of gene-editing at least a portion of the TILs in order to enhance their therapeutic effect. As used herein, "gene-editing," "gene editing," and "genome editing" refer to a type of genetic modification in which DNA is permanently modified in the genome of a cell, *e.g.*, DNA is inserted, deleted, modified or replaced within the cell's genome. In some embodiments, gene-editing causes the expression of a DNA sequence to be silenced (sometimes referred to as a gene knockout) or inhibited/reduced (sometimes referred to as a gene knockdown). In other embodiments, gene-editing causes the expression of a DNA sequence to be enhanced (*e.g.*, by causing over-expression). In accordance with embodiments of the present invention, gene-editing technology is used to enhance the effectiveness of a therapeutic population of TILs.

[00666] A method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs may be carried out in accordance with any embodiment of the methods described herein (*e.g.*, an exemplary TIL expansion method known as process 2A is described below), wherein the method further comprises gene-editing at least a portion of the TILs. According to additional embodiments, a method for expanding TILs into a therapeutic population of TILs is carried out in accordance with any embodiment of the methods described in U.S. Pat. No. 10,517,894, U.S. Patent Application Publication No. 2020/0121719 A1, or U.S. Pat. No. 10,894,063, which are incorporated by reference herein in their entireties, wherein the method further comprises gene-editing at least a portion of the TILs. Thus, some embodiments of the present invention provides a therapeutic population of TILs that has been expanded in accordance with any embodiment described herein, wherein at least a portion of the therapeutic population has been gene-edited, *e.g.*, at least a portion of

the therapeutic population of TILs that is transferred to the infusion bag is permanently gene-edited.

[00667] In some embodiments of the present invention directed to methods for expanding TIL populations, the methods comprise one or more steps of introducing into at least a portion of the TILs nucleic acids, e.g., mRNAs, for transient expression of an immunomodulatory protein, e.g., an immunomodulatory fusion protein comprising an immunomodulatory protein fused to a membrane anchor, in order to produce modified TILs with (i) reduced dependence on cytokines in when expanded in culture and/or (ii) an enhanced therapeutic effect. As used herein, "transient gene-editing", "transient gene editing", "transient phenotypic alteration," "transient phenotypic modification", "temporary phenotypic alteration," "temporary phenotypic modification", "transient cellular change", "transient cellular modification", "temporary cellular alteration", "temporary cellular modification", "transient expression", "transient alteration of expression", "transient alteration of protein expression", "transient modification", "transitory phenotypic alteration", "non-permanent phenotypic alteration", "transiently modified", "temporarily modified", "non-permanently modified", "transiently altered", "temporarily altered", grammatical variations of any of the foregoing, and any expressions of similar meaning, refer to a type of cellular modification or phenotypic change in which nucleic acid (e.g., mRNA) is introduced into a cell, such as transfer of nucleic acid into a cell by electroporation, calcium phosphate transfection, viral transduction, etc., and expressed in the cell (e.g., expression of an immunomodulatory protein, such as an immunomodulatory fusion protein comprising an immunomodulatory protein fused to a membrane anchor) in order to effect a transient or non-permanent phenotypic change in the cell, such as the transient display of membrane-anchored immunomodulatory fusion protein on the cell surface. In accordance with embodiments of the present invention, transient phenotypic alteration technology is used to reduce dependence on cytokines in the expansion of TILs in culture and/or enhance the effectiveness of a therapeutic population of TILs.

[00668] In some embodiments, a microfluidic platform is used for intracellular delivery of nucleic acids encoding the immunomodulatory fusion proteins provided herein. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform. The SQZ platform is capable of delivering nucleic acids and proteins, to a variety of primary human cells, including T cells (Sharei *et al.* PNAS 2013, as well as Sharei et al. PLOS ONE 2015 and Greisbeck et al. J. Immunology vol. 195, 2015). In the SQZ platform, the cell

membranes of the cells for modification (e.g., TILs) are temporarily disrupted by microfluidic constriction, thereby allowing the delivery of nucleic acids encoding the immunomodulatory fusion proteins into the cells. Such methods as described in International Patent Application Publication Nos. WO 2013/059343A1, WO 2017/008063A1, or WO 2017/123663A1, or U.S. Patent Application Publication Nos. US 2014/0287509A1, US 2018/0201889A1, or US 2018/0245089A1 can be employed with the present invention for delivering nucleic acids encoding the subject immunomodulatory fusion proteins to a population of TILs. In some embodiments, the delivered nucleic acid allows for transient protein expression of the immunomodulatory fusion proteins in the modified TILs. In some embodiments, the SQZ platform is used for stable incorporation of the delivered nucleic acid encoding the immunomodulatory fusion protein into the TIL cell genome.

B. Timing of Gene-Editing / Transient Phenotypic Alteration During TIL Expansion

[00669] According to some embodiments, a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprises:

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3 (*e.g.*, OKT-3 may be present in the culture medium beginning on the start date of the expansion process), to produce a second population of TILs, wherein the first expansion is 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

(d) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally 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 performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

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

(g) at any time during the method prior to the transfer to the infusion bag in step (f), gene-editing at least a portion of the TIL cells to express an immunomodulatory composition comprising an immunomodulatory agent (e.g., a membrane anchored immunomodulatory fusion protein described herein) on the surface of the TIL cells. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist.

[00670] As stated in step (g) of the embodiments described above, the gene-editing process may be carried out at any time during the TIL expansion method prior to the transfer to the infusion bag in step (f), which means that the gene editing may be carried out on TILs before, during, or after any of the steps in the expansion method; for example, during any of steps (a)-(f) outlined in the method above, or before or after any of steps (a)-(e) outlined in the method above. According to certain embodiments, TILs are collected during the expansion method (*e.g.*, the expansion method is "paused" for at least a portion of the TILs), and the collected TILs are subjected to a gene-editing process, and, in some cases, subsequently reintroduced back into the expansion method (*e.g.*, back into the culture medium) to continue the expansion process, so that at least a portion of the therapeutic population of TILs that are eventually transferred to the infusion bag are permanently gene-edited. In some embodiments, the gene-editing process may be carried out before expansion by activating TILs, performing a gene-editing step on the activated TILs, and expanding the gene-edited TILs according to the processes described herein. In some embodiments, nucleic acids for gene editing are delivered to the TILs using a microfluidic platform. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform.

[00671] In some embodiments, the gene-editing process is carried out after the first TIL expansion step. In some embodiments, the gene-editing process is carried out after the first

TIL expansion step and before the second expansion step. In some embodiments, the gene-editing process is carried out after the TILs are activated. In some embodiments, the gene-editing process is carried out after the first expansion step and after the TILs are activated, but before the second expansion step. In some embodiments, the gene-editing process is carried out after the first expansion step and after the TILs are activated, and the TILs are rested after gene-editing and before the second expansion step. In some embodiments, the TILs are rested for about 1 to 2 days after gene-editing and before the second expansion step. In some embodiments, the TILs are activated by exposure to an anti-CD3 agonist and an anti-CD28 agonist. In some embodiments, the anti-CD3 agonist is an anti-CD3 agonist antibody and the anti-CD28 agonist is an anti-CD28 agonist antibody. In some embodiments, the anti-CD3 agonist antibody is OKT-3. In some embodiments, the TILs are activated by exposure to anti-CD3 agonist antibody- and anti-CD28 agonist antibody-conjugated beads. In some embodiments, the anti-CD3 agonist antibody- and anti-CD28 agonist antibody-conjugated beads are the TransAct™ product of Miltenyi. In some embodiments, the gene-editing process is carried out by viral transduction. In some embodiments, the gene-editing process is carried out by retroviral transduction. In some embodiments, the gene-editing process is carried out by lentiviral transduction. In some embodiments, the immunomodulatory composition is a membrane anchored immunomodulatory fusion protein. In some embodiments, the immunomodulatory fusion protein comprises IL-15. In some embodiments, the immunomodulatory fusion protein comprises IL-21. In some embodiments, the immunomodulatory composition comprises two or more different membrane bound fusion proteins. In some embodiments, the immunomodulatory composition comprises a first immunomodulatory protein comprising IL-15 and a second immunomodulatory fusion protein comprising IL-21. In some embodiments, the TILs are gene-edited to express the immunomodulatory composition under the control of an NFAT promoter. In some embodiments, the TILs are gene-edited to express an immunomodulatory fusion protein comprising IL-15 under the control of an NFAT promoter. In some embodiments, the TILs are gene-edited to express an immunomodulatory fusion protein comprising IL-21 under the control of an NFAT promoter. In some embodiments, the TILs are gene-edited to express a first immunomodulatory fusion protein comprising IL-15 and a second immunomodulatory fusion protein comprising IL-21 under the control of an NFAT promoter.

[00672] In some embodiments, the gene-editing process is carried out by viral transduction. In some embodiments, the gene-editing process is carried out by retroviral transduction. In some embodiments, the gene-editing process is carried out by lentiviral transduction.

[00673] According to some embodiments, a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprises:

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3 (*e.g.*, OKT-3 may be present in the culture medium beginning on the start date of the expansion process), to produce a second population of TILs, wherein the first expansion is 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

(d) gene-editing at least a portion of the TIL cells in the second population of TILs to express an immunomodulatory composition comprising an immunomodulatory agent (e.g., a membrane anchored immunomodulatory fusion protein described herein) on the surface of the TIL cells;

(e) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally 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 performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

[00674] (g) transferring the harvested TIL population from step (e) to an infusion bag, wherein the transfer from step (e) to (f) occurs without opening the system. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-

7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, the TILs are rested after the gene-editing step and before the second expansion step. In some embodiments, the TILs are rested for about 1 to 2 days after the gene-editing step and before the second expansion step. In some embodiments, the TILs are activated by exposure to an anti-CD3 agonist and an anti-CD28 agonist. In some embodiments, the anti-CD3 agonist is an anti-CD3 agonist antibody and the anti-CD28 agonist is an anti-CD28 agonist antibody. In some embodiments, the anti-CD3 agonist antibody is OKT-3. In some embodiments, the TILs are activated by exposure to anti-CD3 agonist antibody- and anti-CD28 agonist antibody-conjugated beads. In some embodiments, the anti-CD3 agonist antibody- and anti-CD28 agonist antibody-conjugated beads are the TransAct™ product of Miltenyi. In some embodiments, the gene-editing process is carried out by viral transduction. In some embodiments, the gene-editing process is carried out by retroviral transduction of the TILs, optionally for about 2 days. In some embodiments, the gene-editing process is carried out by lentiviral transduction of the TILs, optionally for about 2 days. In some embodiments, the immunomodulatory composition is a membrane anchored immunomodulatory fusion protein. In some embodiments, the immunomodulatory fusion protein comprises IL-15. In some embodiments, the immunomodulatory fusion protein comprises IL-21. In some embodiments, the immunomodulatory composition comprises two or more different membrane bound fusion proteins. In some embodiments, the immunomodulatory composition comprises a first immunomodulatory protein comprising IL-15 and a second immunomodulatory fusion protein comprising IL-21. In some embodiments, the TILs are gene-edited to express the immunomodulatory composition under the control of an NFAT promoter. In some embodiments, the TILs are gene-edited to express an immunomodulatory fusion protein comprising IL-15 under the control of an NFAT promoter. In some embodiments, the TILs are gene-edited to express an immunomodulatory fusion protein comprising IL-21 under the control of an NFAT promoter. In some embodiments, the TILs are gene-edited to express a first immunomodulatory fusion protein comprising IL-15 and a second immunomodulatory fusion protein comprising IL-21 under the control of an NFAT promoter.

[00675] It should be noted that alternative embodiments of the expansion process may differ from the method shown above; *e.g.*, alternative embodiments may not have the same steps (a)-(g), or may have a different number of steps. Regardless of the specific embodiment, the gene-editing process may be carried out at any time during the TIL expansion method. For example, alternative embodiments may include more than two expansions, and it is possible that gene-editing may be conducted on the TILs during a third or fourth expansion, etc.

[00676] According to other embodiments, a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprises:

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3 (*e.g.*, OKT-3 may be present in the culture medium beginning on the start date of the expansion process), to produce a second population of TILs, wherein the first expansion is 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

(d) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally 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 performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

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

(g) at any time during the method prior to the transfer to the infusion bag in step (f), introducing a transient phenotypic alteration in at least a portion of the TIL cells to express an

immunomodulatory composition comprising an immunomodulatory agent on the surface of the TIL cells (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, nucleic acids for transient phenotypic alteration are delivered to the TILs using a microfluidic platform. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform.

[00677] As stated in step (g) of the embodiments described above, the transient phenotypic alteration process may be carried out at any time during the TIL expansion method prior to the transfer to the infusion bag in step (f), which means that the transient phenotypic alteration may be carried out on TILs before, during, or after any of the steps in the expansion method; for example, during any of steps (a)-(f) outlined in the method above, or before or after any of steps (a)-(e) outlined in the method above. According to certain embodiments, TILs are collected during the expansion method (*e.g.*, the expansion method is "paused" for at least a portion of the TILs), and the collected TILs are subjected to a transient modification process, and, in some cases, subsequently reintroduced back into the expansion method (*e.g.*, back into the culture medium) to continue the expansion process, so that at least a portion of the therapeutic population of TILs that are eventually transferred to the infusion bag are transiently altered to express the immunomodulatory composition on the surface of the TIL cells. In some embodiments, the transient cellular modification process may be carried out before expansion by activating TILs, performing a transient phenotypic alteration step on the activated TILs, and expanding the modified TILs according to the processes described herein.

[00678] It should be noted that alternative embodiments of the expansion process may differ from the method shown above; *e.g.*, alternative embodiments may not have the same steps (a)-(g), or may have a different number of steps. Regardless of the specific embodiment, the transient cellular modification process may be carried out at any time during the TIL expansion method. For example, alternative embodiments may include more than two expansions, and it is possible that transient cellular modification process may be conducted on the TILs during a third or fourth expansion, etc.

[00679] According to some embodiments, the gene-editing process is carried out on TILs from one or more of the first population, the second population, and the third population. For example, gene-editing may be carried out on the first population of TILs, or on a portion of TILs collected from the first population, and following the gene-editing process those TILs may subsequently be placed back into the expansion process (*e.g.*, back into the culture medium). Alternatively, gene-editing may be carried out on TILs from the second or third population, or on a portion of TILs collected from the second or third population, respectively, and following the gene-editing process those TILs may subsequently be placed back into the expansion process (*e.g.*, back into the culture medium). According to other embodiments, gene-editing is performed while the TILs are still in the culture medium and while the expansion is being carried out, *i.e.*, they are not necessarily "removed" from the expansion in order to conduct gene-editing.

[00680] According to some embodiments, the transient cellular modification process is carried out on TILs from one or more of the first population, the second population, and the third population. For example, transient cellular modification may be carried out on the first population of TILs, or on a portion of TILs collected from the first population, and following the gene-editing process those transiently modified TILs may subsequently be placed back into the expansion process (*e.g.*, back into the culture medium). Alternatively, transient cellular modification may be carried out on TILs from the second or third population, or on a portion of TILs collected from the second or third population, respectively, and following the transient cellular modification process those modified TILs may subsequently be placed back into the expansion process (*e.g.*, back into the culture medium). According to other embodiments, transient cellular modification is performed while the TILs are still in the culture medium and while the expansion is being carried out, *i.e.*, they are not necessarily "removed" from the expansion in order to effect transient cellular modification.

[00681] According to other embodiments, the gene-editing process is carried out on TILs from the first expansion, or TILs from the second expansion, or both. For example, during the first expansion or second expansion, gene-editing may be carried out on TILs that are collected from the culture medium, and following the gene-editing process those TILs may subsequently be placed back into the expansion method, *e.g.*, by reintroducing them back into the culture medium.

[00682] According to other embodiments, the transient cellular modification process is carried out on TILs from the first expansion, or TILs from the second expansion, or both. For

example, during the first expansion or second expansion, transient cellular modification may be carried out on TILs that are collected from the culture medium, and following the transient cellular modification process those modified TILs may subsequently be placed back into the expansion method, *e.g.*, by reintroducing them back into the culture medium.

[00683] According to other embodiments, the gene-editing process is carried out on at least a portion of the TILs after the first expansion and before the second expansion. For example, after the first expansion, gene-editing may be carried out on TILs that are collected from the culture medium, and following the gene-editing process those TILs may subsequently be placed back into the expansion method, *e.g.*, by reintroducing them back into the culture medium for the second expansion.

[00684] According to other embodiments, the transient cellular modification process is carried out on at least a portion of the TILs after the first expansion and before the second expansion. For example, after the first expansion, transient cellular modification may be carried out on TILs that are collected from the culture medium, and following the transient cellular modification process those modified TILs may subsequently be placed back into the expansion method, *e.g.*, by reintroducing them back into the culture medium for the second expansion.

[00685] According to alternative embodiments, the gene-editing process is carried out before step (c) (*e.g.*, before, during, or after any of steps (a)-(b)), before step (d) (*e.g.*, before, during, or after any of steps (a)-(c)), before step (e) (*e.g.*, before, during, or after any of steps (a)-(d)), or before step (f) (*e.g.*, before, during, or after any of steps (a)-(e)).

[00686] According to alternative embodiments, the transient cellular modification process is carried out before step (c) (*e.g.*, before, during, or after any of steps (a)-(b)), before step (d) (*e.g.*, before, during, or after any of steps (a)-(c)), before step (e) (*e.g.*, before, during, or after any of steps (a)-(d)), or before step (f) (*e.g.*, before, during, or after any of steps (a)-(e)).

[00687] It should be noted with regard to OKT-3, according to certain embodiments, that the cell culture medium may comprise OKT-3 beginning on the start day (Day 0), or on Day 1 of the first expansion, such that the gene-editing or transient cellular modification is carried out on TILs after they have been exposed to OKT-3 in the cell culture medium on Day 0 and/or Day 1. According to other embodiments, the cell culture medium comprises OKT-3 during the first expansion and/or during the second expansion, and the gene-editing or transient cellular modification is carried out before the OKT-3 is introduced into the cell culture

medium. Alternatively, the cell culture medium may comprise OKT-3 during the first expansion and/or during the second expansion, and the gene-editing or transient cellular modification is carried out after the OKT-3 is introduced into the cell culture medium.

[00688] It should also be noted with regard to a 4-1BB agonist, according to certain embodiments, that the cell culture medium may comprise a 4-1BB agonist beginning on the start day (Day 0), or on Day 1 of the first expansion, such that the gene-editing or transient cellular modification is carried out on TILs after they have been exposed to a 4-1BB agonist in the cell culture medium on Day 0 and/or Day 1. According to other embodiments, the cell culture medium comprises a 4-1BB agonist during the first expansion and/or during the second expansion, and the gene-editing or transient cellular modification is carried out before the 4-1BB agonist is introduced into the cell culture medium. Alternatively, the cell culture medium may comprise a 4-1BB agonist during the first expansion and/or during the second expansion, and the gene-editing or transient cellular modification is carried out after the 4-1BB agonist is introduced into the cell culture medium.

[00689] It should also be noted with regard to IL-2, according to certain embodiments, that the cell culture medium may comprise IL-2 beginning on the start day (Day 0), or on Day 1 of the first expansion, such that the gene-editing or transient cellular modification is carried out on TILs after they have been exposed to IL-2 in the cell culture medium on Day 0 and/or Day 1. According to other embodiments, the cell culture medium comprises IL-2 during the first expansion and/or during the second expansion, and the gene-editing or transient cellular modification is carried out before the IL-2 is introduced into the cell culture medium. Alternatively, the cell culture medium may comprise IL-2 during the first expansion and/or during the second expansion, and the gene-editing or transient cellular modification is carried out after the IL-2 is introduced into the cell culture medium.

[00690] As discussed above, one or more of OKT-3, 4-1BB agonist and IL-2 may be included in the cell culture medium beginning on Day 0 or Day 1 of the first expansion. According to some embodiments, OKT-3 is included in the cell culture medium beginning on Day 0 or Day 1 of the first expansion, and/or a 4-1BB agonist is included in the cell culture medium beginning on Day 0 or Day 1 of the first expansion, and/or IL-2 is included in the cell culture medium beginning on Day 0 or Day 1 of the first expansion. According to other examples, the cell culture medium comprises OKT-3 and a 4-1BB agonist beginning on Day 0 or Day 1 of the first expansion. According to other examples, the cell culture medium comprises OKT-3, a 4-1BB agonist and IL-2 beginning on Day 0 or Day 1 of the first

expansion. Of course, one or more of OKT-3, 4-1BB agonist and IL-2 may be added to the cell culture medium at one or more additional time points during the expansion process, as set forth in various embodiments described herein.

[00691] According to some embodiments, a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprises:

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

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

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

(d) activating the second population of TILs by adding OKT-3 and culturing for about 1 to 2 days, wherein the transition from step (c) to step (d) occurs without opening the system;

(e) gene-editing at least a portion of the TIL cells in the second population of TILs to express an immunomodulatory composition comprising an immunomodulatory agent (e.g., a membrane anchored immunomodulatory fusion protein described herein) on the surface of the TIL cells;

(f) optionally resting the second population of TILs for about 1 day;

(g) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3 antibody, optionally an OX40 antibody, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7 to 11 days to obtain a third population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (f) to step (g) occurs without opening the system;

(h) harvesting the therapeutic population of TILs obtained from step (g) to provide a harvested TIL population, wherein the transition from step (g) to step (h) occurs without opening the system, wherein the harvested population of TILs is a therapeutic population of TILs; and

(i) transferring the harvested TIL population to an infusion bag, wherein the transfer from step (h) to (i) occurs without opening the system. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, the TILs are rested after the gene-editing step and before the second expansion step. In some embodiments, the TILs are rested for about 1 to 2 days after the gene-editing step and before the second expansion step. In some embodiments, the TILs are activated by exposure to an anti-CD3 agonist and an anti-CD28 agonist for about 2 days. In some embodiments, the anti-CD3 agonist is an anti-CD3 agonist antibody and the anti-CD28 agonist is an anti-CD28 agonist antibody. In some embodiments, the anti-CD3 agonist antibody is OKT-3. In some embodiments, the TILs are activated by exposure to anti-CD3 agonist antibody- and anti-CD28 agonist antibody-conjugated beads. In some embodiments, the anti-CD3 agonist antibody- and anti-CD28 agonist antibody-conjugated beads are the TransAct™ product of Miltenyi. In some embodiments, the gene-editing process is carried out by viral transduction. In some embodiments, the gene-editing process is carried out by retroviral transduction of the TILs, optionally for about 2 days. In some embodiments, the gene-editing process is carried out by lentiviral transduction of the TILs, optionally for about 2 days. In some embodiments, the immunomodulatory composition is a membrane anchored immunomodulatory fusion protein. In some embodiments, the immunomodulatory fusion protein comprises IL-15. In some embodiments, the immunomodulatory fusion protein comprises IL-21. In some embodiments, the immunomodulatory composition comprises two or more different membrane bound fusion proteins. In some embodiments, the immunomodulatory composition comprises a first immunomodulatory protein comprising IL-15 and a second immunomodulatory fusion protein comprising IL-21. In some embodiments, the TILs are gene-edited to express the immunomodulatory composition under the control of an NFAT promoter. In some embodiments, the TILs are gene-edited to express an immunomodulatory fusion protein comprising IL-15 under the control of an NFAT promoter. In some embodiments, the TILs are gene-edited to express an immunomodulatory fusion protein comprising IL-21 under the control of an NFAT promoter. In some embodiments, the TILs are gene-edited to express a

first immunomodulatory fusion protein comprising IL-15 and a second immunomodulatory fusion protein comprising IL-21 under the control of an NFAT promoter.

[00692] According to some embodiments, a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprises:

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 and optionally comprising OKT-3 and/or a 4-1BB agonist antibody for about 3 to 11 days to produce a second population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area;

(d) stimulating the second population of TILs by adding OKT-3 and culturing for about 1 to 3 days, wherein the transition from step (c) to step (d) occurs without opening the system;

(e) sterile electroporating the second population of TILs to effect transfer of at least one gene editor into a portion of cells of the second population of TILs;

(f) resting the second population of TILs for about 1 day;

(g) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3 antibody, optionally an OX40 antibody, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7 to 11 days to obtain a third population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (f) to step (g) occurs without opening the system;

(h) harvesting the therapeutic population of TILs obtained from step (g) to provide a harvested TIL population, wherein the transition from step (g) to step (h) occurs without opening the system, wherein the harvested population of TILs is a therapeutic population of TILs; and

(i) transferring the harvested TIL population to an infusion bag, wherein the transfer from step (h) to (i) occurs without opening the system,

wherein the sterile electroporation of the at least one gene editor into the portion of cells of the second population of TILs modifies a plurality of cells in the portion to express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist.

[00693] According to other embodiments, a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprises:

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 and optionally comprising OKT-3 and/or a 4-1BB agonist antibody for about 3 to 11 days to produce a second population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area;

(d) stimulating the second population of TILs by adding OKT-3 and culturing for about 1 to 3 days, wherein the transition from step (c) to step (d) occurs without opening the system;

(e) sterile electroporating the second population of TILs to effect transfer of at least one nucleic acid molecule into a portion of cells of the second population of TILs;

(f) resting the second population of TILs for about 1 day;

(g) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3 antibody, optionally an OX40 antibody, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7 to 11 days to obtain a third population of TILs, wherein the second expansion is performed in a closed container providing a second

gas-permeable surface area, and wherein the transition from step (f) to step (g) occurs without opening the system;

(h) harvesting the therapeutic population of TILs obtained from step (g) to provide a harvested TIL population, wherein the transition from step (g) to step (h) occurs without opening the system, wherein the harvested population of TILs is a therapeutic population of TILs; and

(i) transferring the harvested TIL population to an infusion bag, wherein the transfer from step (h) to (i) occurs without opening the system,

wherein the sterile electroporation of the at least one nucleic acid molecule into the portion of cells of the second population of TILs modifies a plurality of cells in the portion to transiently express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist.

[00694] According to some embodiments, a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprises:

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 and optionally comprising OKT-3 and/or a 4-1BB agonist antibody for about 3 to 11 days to produce a second population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area;

(d) stimulating the second population of TILs by adding OKT-3 and culturing for about 1 to 3 days, wherein the transition from step (c) to step (d) occurs without opening the system;

(e) sterile electroporating the second population of TILs to effect transfer of at least one gene editor into a portion of cells of the second population of TILs;

(f) resting the second population of TILs for about 1 day;

(g) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3 antibody, optionally an OX40 antibody, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7 to 11 days to obtain a third population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (f) to step (g) occurs without opening the system;

(h) harvesting the therapeutic population of TILs obtained from step (g) to provide a harvested TIL population, wherein the transition from step (g) to step (h) occurs without opening the system, wherein the harvested population of TILs is a therapeutic population of TILs; and

(i) transferring the harvested TIL population to an infusion bag, wherein the transfer from step (h) to (i) occurs without opening the system,

wherein the sterile electroporation of the at least one gene editor into the portion of cells of the second population of TILs modifies a plurality of cells in the portion to express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist.

[00695] According to some embodiments, a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprises:

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 and optionally comprising OKT-3 and/or a 4-1BB agonist antibody for about 3 to 11 days to produce a second population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area;

(d) stimulating the second population of TILs by adding OKT-3 and culturing for about 1 to 3 days, wherein the transition from step (c) to step (d) occurs without opening the system;

(e) temporarily disrupting the cell membranes of the second population of TILs to effect transfer of at least one gene editor into a portion of cells of the second population of TILs;

(f) resting the second population of TILs for about 1 day;

(g) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3 antibody, optionally an OX40 antibody, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7 to 11 days to obtain a third population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (f) to step (g) occurs without opening the system;

(h) harvesting the therapeutic population of TILs obtained from step (g) to provide a harvested TIL population, wherein the transition from step (g) to step (h) occurs without opening the system, wherein the harvested population of TILs is a therapeutic population of TILs; and

(i) transferring the harvested TIL population to an infusion bag, wherein the transfer from step (h) to (i) occurs without opening the system,

wherein the at least one gene editor delivered into the portion of cells of the second population of TILs modifies a plurality of cells in the portion to express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group

consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, a microfluidic platform is used to temporarily disrupt the cell membranes of the second population of TILs. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform.

[00696] According to other embodiments, a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprises:

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 and optionally comprising OKT-3 and/or a 4-1BB agonist antibody for about 3 to 11 days to produce a second population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area;

(d) stimulating the second population of TILs by adding OKT-3 and culturing for about 1 to 3 days, wherein the transition from step (c) to step (d) occurs without opening the system;

(e) temporarily disrupting the cell membranes of the second population of TILs to effect transfer of at least one nucleic acid molecule into a portion of cells of the second population of TILs;

(f) resting the second population of TILs for about 1 day;

(g) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3 antibody, optionally an OX40 antibody, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7 to 11 days to obtain a third population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (f) to step (g) occurs without opening the system;

(h) harvesting the therapeutic population of TILs obtained from step (g) to provide a harvested TIL population, wherein the transition from step (g) to step (h) occurs without opening the system, wherein the harvested population of TILs is a therapeutic population of TILs; and

(i) transferring the harvested TIL population to an infusion bag, wherein the transfer from step (h) to (i) occurs without opening the system,

wherein the at least one nucleic acid molecule delivered into the portion of cells of the second population of TILs modifies a plurality of cells in the portion to transiently express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18 , IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, a microfluidic platform is used to temporarily disrupt the cell membranes of the second population of TILs. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform.

[00697] According to some embodiments, a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprises:

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 and optionally comprising OKT-3 and/or a 4-1BB agonist antibody for about 3 to 11 days to produce a second population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area;

(d) stimulating the second population of TILs by adding OKT-3 and culturing for about 1 to 3 days, wherein the transition from step (c) to step (d) occurs without opening the system;

(e) temporarily disrupting the cell membranes of the second population of TILs to effect transfer of at least one gene editor into a portion of cells of the second population of TILs;

(f) resting the second population of TILs for about 1 day;

(g) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3 antibody, optionally an OX40 antibody, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7 to 11 days to obtain a third population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (f) to step (g) occurs without opening the system;

(h) harvesting the therapeutic population of TILs obtained from step (g) to provide a harvested TIL population, wherein the transition from step (g) to step (h) occurs without opening the system, wherein the harvested population of TILs is a therapeutic population of TILs; and

(i) transferring the harvested TIL population to an infusion bag, wherein the transfer from step (h) to (i) occurs without opening the system,

wherein the at least one gene editor delivered into the portion of cells of the second population of TILs modifies a plurality of cells in the portion to express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, a microfluidic platform is used to temporarily disrupt the cell membranes of the second population of TILs. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform.

[00698] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) culturing the first population of TILs in a first cell culture medium comprising IL-2 for about 3-9 days to produce a second population of TILs;

(c) activating the second population of TILs using anti-CD3 and anti-CD28 beads or antibodies for 1-7 days, to produce a third population of TILs;

(d) sterile electroporating the third population of TILs to effect transfer of at least one gene editor into a portion of cells of the third population of TILs to produce a fourth population of TILs; and

(e) culturing the fourth population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the sterile electroporation of the at least one gene editor into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist.

[00699] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) culturing the first population of TILs in a first cell culture medium comprising IL-2 for about 3-9 days to produce a second population of TILs;

(c) activating the second population of TILs using anti-CD3 and anti-CD28 beads or antibodies for 1-7 days, to produce a third population of TILs;

(d) gene-editing at least a portion of the TIL cells in the second population of TILs to express an immunomodulatory composition comprising an immunomodulatory agent (e.g., a membrane anchored immunomodulatory fusion protein described herein) on the surface of the TIL cells; and

(e) culturing the fourth population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs.

In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, the TILs are rested after the gene-editing step and before the second expansion step. In some embodiments, the TILs are rested for about 1 to 2 days after the gene-editing step and before the second expansion step. In some embodiments, the TILs are activated by exposure to an anti-CD3 agonist and an anti-CD28 agonist for about 2 days. In some embodiments, the anti-CD3 agonist is an anti-CD3 agonist antibody and the anti-CD28 agonist is an anti-CD28 agonist antibody. In some embodiments, the anti-CD3 agonist antibody is OKT-3. In some embodiments, the TILs are activated by exposure to anti-CD3 agonist antibody- and anti-CD28 agonist antibody-conjugated beads. In some embodiments, the anti-CD3 agonist antibody- and anti-CD28 agonist antibody-conjugated beads are the TransAct™ product of Miltenyi. In some embodiments, the gene-editing process is carried out by viral transduction. In some embodiments, the gene-editing process is carried out by retroviral transduction of the TILs, optionally for about 2 days. In some embodiments, the gene-editing process is carried out by lentiviral transduction of the TILs, optionally for about 2 days. In some embodiments, the immunomodulatory composition is a membrane anchored immunomodulatory fusion protein. In some embodiments, the immunomodulatory fusion protein comprises IL-15. In some embodiments, the immunomodulatory fusion protein comprises IL-21. In some embodiments, the immunomodulatory composition comprises two or more different membrane bound fusion proteins. In some embodiments, the immunomodulatory composition comprises a first immunomodulatory protein comprising IL-15 and a second immunomodulatory fusion protein comprising IL-21. In some embodiments, the TILs are

gene-edited to express the immunomodulatory composition under the control of an NFAT promoter. In some embodiments, the TILs are gene-edited to express an immunomodulatory fusion protein comprising IL-15 under the control of an NFAT promoter. In some embodiments, the TILs are gene-edited to express an immunomodulatory fusion protein comprising IL-21 under the control of an NFAT promoter. In some embodiments, the TILs are gene-edited to express a first immunomodulatory fusion protein comprising IL-15 and a second immunomodulatory fusion protein comprising IL-21 under the control of an NFAT promoter.

[00700] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) culturing the first population of TILs in a first cell culture medium comprising IL-2 for about 3-9 days to produce a second population of TILs;

(c) activating the second population of TILs using anti-CD3 and anti-CD28 beads or antibodies for 1-7 days, to produce a third population of TILs;

(d) sterile electroporating the third population of TILs to effect transfer of at least one nucleic acid molecule into a portion of cells of the third population of TILs to produce a fourth population of TILs; and

(e) culturing the fourth population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the at least one nucleic acid molecule delivered into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to transiently express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist.

In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist.

[00701] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) digesting in an enzyme media the tumor tissue to produce a tumor digest;

(c) culturing the first population of TILs in a first cell culture medium comprising IL-2 for about 3-9 days to produce a second population of TILs;

(d) activating the second population of TILs using anti-CD3 and anti-CD28 beads or antibodies for 1-7 days, to produce a third population of TILs;

(e) gene-editing at least a portion of the TIL cells in the second population of TILs to express an immunomodulatory composition comprising an immunomodulatory agent (e.g., a membrane anchored immunomodulatory fusion protein described herein) on the surface of the TIL cells; and

(f) culturing the fourth population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs.

In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, the TILs are rested after the gene-editing step and before the second expansion step. In some embodiments, the TILs are rested for about 1 to 2 days after the gene-editing step and before the second expansion step. In some embodiments, the TILs are activated by exposure to an anti-CD3 agonist and an anti-CD28 agonist for about 2 days. In some embodiments, the anti-CD3 agonist is an anti-CD3 agonist antibody and the anti-CD28 agonist is an anti-CD28 agonist antibody. In some embodiments, the anti-CD3 agonist antibody is OKT-3. In some embodiments, the TILs are activated by exposure to anti-CD3 agonist antibody- and anti-

CD28 agonist antibody-conjugated beads. In some embodiments, the anti-CD3 agonist antibody- and anti-CD28 agonist antibody-conjugated beads are the TransAct™ product of Miltenyi. In some embodiments, the gene-editing process is carried out by viral transduction. In some embodiments, the gene-editing process is carried out by retroviral transduction of the TILs, optionally for about 2 days. In some embodiments, the gene-editing process is carried out by lentiviral transduction of the TILs, optionally for about 2 days. In some embodiments, the immunomodulatory composition is a membrane anchored immunomodulatory fusion protein. In some embodiments, the immunomodulatory fusion protein comprises IL-15. In some embodiments, the immunomodulatory fusion protein comprises IL-21. In some embodiments, the immunomodulatory composition comprises two or more different membrane bound fusion proteins. In some embodiments, the immunomodulatory composition comprises a first immunomodulatory protein comprising IL-15 and a second immunomodulatory fusion protein comprising IL-21. In some embodiments, the TILs are gene-edited to express the immunomodulatory composition under the control of an NFAT promoter. In some embodiments, the TILs are gene-edited to express an immunomodulatory fusion protein comprising IL-15 under the control of an NFAT promoter. In some embodiments, the TILs are gene-edited to express an immunomodulatory fusion protein comprising IL-21 under the control of an NFAT promoter. In some embodiments, the TILs are gene-edited to express a first immunomodulatory fusion protein comprising IL-15 and a second immunomodulatory fusion protein comprising IL-21 under the control of an NFAT promoter.

[00702] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) digesting in an enzyme media the tumor tissue to produce a tumor digest;

(c) culturing the first population of TILs in a first cell culture medium comprising IL-2 for about 3-9 days to produce a second population of TILs;

(d) activating the second population of TILs using anti-CD3 and anti-CD28 beads or antibodies for 1-7 days, to produce a third population of TILs;

(e) sterile electroporating the third population of TILs to effect transfer of at least one gene editor into a portion of cells of the third population of TILs to produce a fourth population of TILs; and

(f) culturing the fourth population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the sterile electroporation of the at least one gene editor into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist.

[00703] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) digesting in an enzyme media the tumor tissue to produce a tumor digest;

(c) culturing the first population of TILs in a first cell culture medium comprising IL-2 for about 3-9 days to produce a second population of TILs;

(d) activating the second population of TILs using anti-CD3 and anti-CD28 beads or antibodies for 1-7 days, to produce a third population of TILs;

(e) sterile electroporating the third population of TILs to effect transfer of at least one nucleic acid molecule into a portion of cells of the third population of TILs to produce a fourth population of TILs; and

(f) culturing the fourth population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the at least one nucleic acid molecule delivered into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to transiently express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist.

[00704] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) culturing the first population of TILs in a first cell culture medium comprising IL-2 for about 3-9 days to produce a second population of TILs;

(c) activating the second population of TILs using anti-CD3 and anti-CD28 beads or antibodies for 1-7 days, to produce a third population of TILs;

(d) temporarily disrupting the cell membranes of the third population of TILs to effect transfer of at least one gene editor into a portion of cells of the third population of TILs to produce a fourth population of TILs; and

(e) culturing the fourth population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the transfer of the at least one gene editor into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to express an immunomodulatory composition on the surface of the cells. In some embodiments, the

immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, a microfluidic platform is used to temporarily disrupt the cell membranes of the second population of TILs. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform.

[00705] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) culturing the first population of TILs in a first cell culture medium comprising IL-2 for about 3-9 days to produce a second population of TILs;

(c) activating the second population of TILs using anti-CD3 and anti-CD28 beads or antibodies for 1-7 days, to produce a third population of TILs;

(d) temporarily disrupting the cell membranes of the third population of TILs to effect transfer of at least one nucleic acid molecule into a portion of cells of the third population of TILs to produce a fourth population of TILs; and

(e) culturing the fourth population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the transfer of the at least one nucleic acid molecule into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to transiently express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the

immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, a microfluidic platform is used to temporarily disrupt the cell membranes of the second population of TILs. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform.

[00706] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) digesting in an enzyme media the tumor tissue to produce a tumor digest;

(c) culturing the first population of TILs in a first cell culture medium comprising IL-2 for about 3-9 days to produce a second population of TILs;

(d) activating the second population of TILs using anti-CD3 and anti-CD28 beads or antibodies for 1-7 days, to produce a third population of TILs;

(e) temporarily disrupting the cell membranes of the third population of TILs to effect transfer of at least one gene editor into a portion of cells of the third population of TILs to produce a fourth population of TILs; and

(f) culturing the fourth population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the transfer of the at least one gene editor into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the

immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, a microfluidic platform is used to temporarily disrupt the cell membranes of the second population of TILs. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform.

[00707] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) digesting in an enzyme media the tumor tissue to produce a tumor digest;

(c) culturing the first population of TILs in a first cell culture medium comprising IL-2 for about 3-9 days to produce a second population of TILs;

(d) activating the second population of TILs using anti-CD3 and anti-CD28 beads or antibodies for 1-7 days, to produce a third population of TILs;

(e) temporarily disrupting the cell membranes of the third population of TILs to effect transfer of at least one nucleic acid molecule into a portion of cells of the third population of TILs to produce a fourth population of TILs; and

(f) culturing the fourth population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the transfer of the at least one nucleic acid molecule into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to transiently express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist.

[00708] In some embodiments, any of the foregoing methods is modified such that the step of culturing the fourth population of TILs is replaced with the steps of:

(f) culturing the fourth population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 1-7 days, to produce a culture of a fifth population of TILs; and

(g) splitting the culture of the fifth population of TILs into a plurality of subcultures, culturing each of the plurality of subcultures in a third cell culture medium comprising IL-2 for about 3-7 days, and combining the plurality of subcultures to provide an expanded number of TILs.

[00709] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days or 7 days.

[00710] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 2-7 days.

[00711] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 3-7 days.

[00712] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 4-7 days.

[00713] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 5-7 days.

[00714] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 6-7 days.

[00715] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 1-6 days.

[00716] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 1-5 days.

[00717] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 1-4 days.

[00718] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 1-3 days.

[00719] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 1-2 days.

[00720] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 2-6 days.

[00721] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 3-6 days.

[00722] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 4-6 days.

[00723] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 5-6 days.

[00724] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 3-5 days.

[00725] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 3-4 days.

[00726] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 2-5 days.

[00727] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 2-4 days.

[00728] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 2-3 days.

[00729] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 4-5 days.

[00730] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 1 day.

[00731] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 2 days.

[00732] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 3 days.

[00733] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 4 days.

[00734] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 5 days.

[00735] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 6 days.

[00736] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of activating the second population of TILs is performed for about 7 days.

[00737] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) culturing the first population of TILs in a first cell culture medium comprising IL-2 and OKT-3 for about 3-9 days to produce a second population of TILs;

(c) sterile electroporating the second population of TILs to effect transfer of at least one gene editor into a portion of cells of the second population of TILs to produce a third population of TILs; and

(d) culturing the third population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the sterile electroporation of the at least one gene editor into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist.

[00738] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) culturing the first population of TILs in a first cell culture medium comprising IL-2 and OKT-3 for about 3-9 days to produce a second population of TILs;

(c) sterile electroporating the second population of TILs to effect transfer of at least one nucleic acid molecule into a portion of cells of the second population of TILs to produce a third population of TILs; and

(d) culturing the third population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the sterile electroporation of the at least one nucleic acid molecule into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to transiently express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist.

[00739] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) digesting in an enzyme media the tumor tissue to produce a tumor digest;

(c) culturing the first population of TILs in a first cell culture medium comprising IL-2 and OKT-3 for about 3-9 days to produce a second population of TILs;

(d) sterile electroporating the second population of TILs to effect transfer of at least one gene editor into a portion of cells of the second population of TILs to produce a third population of TILs; and

(e) culturing the third population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the sterile electroporation of the at least one gene editor into the portion of cells of the second population of TILs modifies a plurality of cells in the portion to express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the cytokine is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the cytokine is selected from the group consisting of IL-2, IL-12, IL-15, IL-18 and IL-21. In some embodiments, the cytokine is selected from the group consisting of IL-12, IL-15, IL-18, IL-21 and a CD40 agonist.

[00740] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) digesting in an enzyme media the tumor tissue to produce a tumor digest;

(c) culturing the first population of TILs in a first cell culture medium comprising IL-2 and OKT-3 for about 3-9 days to produce a second population of TILs;

(d) sterile electroporating the second population of TILs to effect transfer of at least one nucleic acid molecule into a portion of cells of the second population of TILs to produce a third population of TILs; and

(e) culturing the third population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the sterile electroporation of the at least one nucleic acid molecule into the portion of cells of the second population of TILs modifies a plurality of cells in the portion to transiently express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g.,

CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist.

In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) culturing the first population of TILs in a first cell culture medium comprising IL-2 and OKT-3 for about 3-9 days to produce a second population of TILs;

(c) temporarily disrupting the cell membranes of the second population of TILs to effect transfer of at least one gene editor into a portion of cells of the second population of TILs to produce a third population of TILs; and

(d) culturing the third population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the transfer of the at least one gene editor into the portion of cells of the second population of TILs modifies a plurality of cells in the portion to express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, a microfluidic platform is used to temporarily disrupt the cell membranes of the second population of TILs. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform.

[00741] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) culturing the first population of TILs in a first cell culture medium comprising IL-2 and OKT-3 for about 3-9 days to produce a second population of TILs;

(c) temporarily disrupting the cell membranes of the second population of TILs to effect transfer of at least one nucleic acid molecule into a portion of cells of the second population of TILs to produce a third population of TILs; and

(d) culturing the third population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the transfer of the at least one gene editor into the portion of cells of the second population of TILs modifies a plurality of cells in the portion to transiently express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, a microfluidic platform is used to temporarily disrupt the cell membranes of the second population of TILs. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform.

[00742] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) digesting in an enzyme media the tumor tissue to produce a tumor digest;

(c) culturing the first population of TILs in a first cell culture medium comprising IL-2 and OKT-3 for about 3-9 days to produce a second population of TILs;

(d) temporarily disrupting the cell membranes of the second population of TILs to effect transfer of at least one gene editor into a portion of cells of the second population of TILs to produce a third population of TILs; and

(e) culturing the third population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the transfer of the at least one gene editor into the portion of cells of the second population of TILs modifies a plurality of cells in the portion to express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, a microfluidic platform is used to temporarily disrupt the cell membranes of the second population of TILs. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform.

[00743] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) digesting in an enzyme media the tumor tissue to produce a tumor digest;

(c) culturing the first population of TILs in a first cell culture medium comprising IL-2 and OKT-3 for about 3-9 days to produce a second population of TILs;

(d) temporarily disrupting the cell membranes of the second population of TILs to effect transfer of at least one nucleic acid molecule into a portion of cells of the second population of TILs to produce a third population of TILs; and

(e) culturing the third population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the transfer of the at least one nucleic acid molecule into the portion of cells of the second population of TILs modifies a plurality of cells in the portion to transiently express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, a microfluidic platform is used to temporarily disrupt the cell membranes of the second population of TILs. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform.

[00744] In some embodiments, the step of culturing the third population of TILs is performed by culturing the third population of TILs in the second cell culture medium for a first period of about 1-7 days, at the end of the first period the culture is split into a plurality of subcultures, each of the plurality of subcultures is cultured in a third culture medium comprising IL-2 for a second period of about 3-7 days, and at the end of the second period the plurality of subcultures are combined to provide the expanded number of TILs.

[00745] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days or 11 days.

[00746] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs or the first expansion step is performed for about 4-11 days.

[00747] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs or the first expansion step is performed for about 5-11 days.

[00748] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs or the first expansion step is performed for about 6-11 days.

[00749] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs or the first expansion step is performed for about 7-11 days.

[00750] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs or the first expansion step is performed for about 8-11 days.

[00751] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs or the first expansion step is performed for about 9-11 days.

[00752] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs or the first expansion step is performed for about 10-11 days.

[00753] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs or the first expansion step is performed for about 4-10 days.

[00754] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs or the first expansion step is performed for about 5-10 days.

[00755] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs or the first expansion step is performed for about 6-10 days.

[00756] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs or the first expansion step is performed for about 7-10 days.

[00757] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs or the first expansion step is performed for about 8-10 days.

[00758] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs or the first expansion step is performed for about 9-10 days.

[00759] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 4-9 days.

[00760] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 5-9 days.

[00761] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 6-9 days.

[00762] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 7-9 days.

[00763] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 8-9 days.

[00764] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 3-8 days.

[00765] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 3-7 days.

[00766] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 3-6 days.

[00767] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 3-5 days.

[00768] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 3-4 days.

[00769] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 4-8 days.

[00770] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 4-7 days.

[00771] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 4-6 days.

[00772] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 4-6 days.

[00773] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 5-8 days.

[00774] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 5-7 days.

[00775] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 5-6 days.

[00776] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 6-8 days.

[00777] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 6-7 days.

[00778] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 7-8 days.

[00779] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 4-5 days.

[00780] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 3 days.

[00781] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 4 days.

[00782] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 5 days.

[00783] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 6 days.

[00784] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 7 days.

[00785] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 8 days.

[00786] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 9 days.

[00787] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 10 days.

[00788] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the first population of TILs in the first cell culture medium is performed for about 11 days.

[00789] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

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

(c) culturing the second population of TILs in a second cell culture medium comprising IL-2 and OKT-3 for 2-4 days to produce a third population of TILs;

(d)) sterile electroporating the third population of TILs to effect transfer of at least one gene editor into a portion of cells of the third population of TILs to produce a fourth population of TILs; and

(e) culturing the fourth population of TILs in a third cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the sterile electroporation of the at least one gene editor into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some

embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist.

[00790] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

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

(c) culturing the second population of TILs in a second cell culture medium comprising IL-2 and OKT-3 for 2-4 days to produce a third population of TILs;

(d)) sterile electroporating the third population of TILs to effect transfer of at least one nucleic acid molecule into a portion of cells of the third population of TILs to produce a fourth population of TILs; and

(e) culturing the fourth population of TILs in a third cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the sterile electroporation of the at least one nucleic acid molecule into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to transiently express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist.

[00791] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) digesting in an enzyme media the tumor tissue to produce a tumor digest;

(c) culturing the first population of TILs in a first cell culture medium comprising IL-2 for about 3 days to produce a second population of TILs;

(d) culturing the second population of TILs in a second cell culture medium comprising IL-2 and OKT-3 for 2-4 days to produce a third population of TILs;

(e) sterile electroporating the third population of TILs to effect transfer of at least one gene editor into a portion of cells of the third population of TILs to produce a fourth population of TILs; and

(f) culturing the fourth population of TILs in a third cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the sterile electroporation of the at least one gene editor into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist.

[00792] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) digesting in an enzyme media the tumor tissue to produce a tumor digest;

(c) culturing the first population of TILs in a first cell culture medium comprising IL-2 for about 3 days to produce a second population of TILs;

(d) culturing the second population of TILs in a second cell culture medium comprising IL-2 and OKT-3 for 2-4 days to produce a third population of TILs;

(e) sterile electroporating the third population of TILs to effect transfer of at least one nucleic acid molecule into a portion of cells of the third population of TILs to produce a fourth population of TILs; and

(f) culturing the fourth population of TILs in a third cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the sterile electroporation of the at least one nucleic acid molecule into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to transiently express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist.

[00793] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

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

(c) culturing the second population of TILs in a second cell culture medium comprising IL-2 and OKT-3 for 2-4 days to produce a third population of TILs;

(d)) temporarily disrupting the cell membranes of the third population of TILs to effect transfer of at least one gene editor into a portion of cells of the third population of TILs to produce a fourth population of TILs; and

(e) culturing the fourth population of TILs in a third cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the transfer of the at least one gene editor into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, a microfluidic platform is used to temporarily disrupt the cell membranes of the second population of TILs. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform.

[00794] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

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

(c) culturing the second population of TILs in a second cell culture medium comprising IL-2 and OKT-3 for 2-4 days to produce a third population of TILs;

(d)) temporarily disrupting the cell membranes of the third population of TILs to effect transfer of at least one nucleic acid molecule into a portion of cells of the third population of TILs to produce a fourth population of TILs; and

(e) culturing the fourth population of TILs in a third cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the transfer of the at least one nucleic acid molecule into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to transiently express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, a microfluidic platform is used to temporarily disrupt the cell membranes of the second population of TILs. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform.

[00795] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) digesting in an enzyme media the tumor tissue to produce a tumor digest;

(c) culturing the first population of TILs in a first cell culture medium comprising IL-2 for about 3 days to produce a second population of TILs;

(d) culturing the second population of TILs in a second cell culture medium comprising IL-2 and OKT-3 for 2-4 days to produce a third population of TILs;

(e) temporarily disrupting the cell membranes of the third population of TILs to effect transfer of at least one gene editor into a portion of cells of the third population of TILs to produce a fourth population of TILs; and

(f) culturing the fourth population of TILs in a third cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the transfer of the at least one gene editor into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, a microfluidic platform is used to temporarily disrupt the cell membranes of the second population of TILs. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform.

[00796] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

(b) digesting in an enzyme media the tumor tissue to produce a tumor digest;

(c) culturing the first population of TILs in a first cell culture medium comprising IL-2 for about 3 days to produce a second population of TILs;

(d) culturing the second population of TILs in a second cell culture medium comprising IL-2 and OKT-3 for 2-4 days to produce a third population of TILs;

(e) temporarily disrupting the cell membranes of the third population of TILs to effect transfer of at least one nucleic acid molecule into a portion of cells of the third population of TILs to produce a fourth population of TILs; and

(f) culturing the fourth population of TILs in a third cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs,

wherein the transfer of the at least one nucleic acid molecule into the portion of cells of the third population of TILs modifies a plurality of cells in the portion to transiently express an immunomodulatory composition on the surface of the cells. In some embodiments, the immunomodulatory composition comprises an immunomodulatory agent fused to a membrane anchor (e.g., a membrane anchored immunomodulatory fusion protein described herein). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist (e.g., CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21 and a CD40 agonist. In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-12, IL-15, IL-18, IL-21, and a CD40 agonist. In some embodiments, a microfluidic platform is used to temporarily disrupt the cell membranes of the second population of TILs. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform.

[00797] In some embodiments, the step of culturing the fourth population of TILs is performed by culturing the fourth population of TILs in the third cell culture medium for a first period of about 1-7 days, at the end of the first period the culture is split into a plurality of subcultures, each of the plurality of subcultures is cultured in a fourth culture medium comprising IL-2 for a second period of about 3-7 days, and at the end of the second period the plurality of subcultures are combined to provide the expanded number of TILs.

[00798] In some embodiments, in the step of culturing the first population of TILs in the first culture medium the first culture medium further comprises anti-CD3 and anti-CD28 beads or antibodies.

[00799] In some embodiments, the anti-CD3 and anti-CD28 beads or antibodies comprise the OKT-3 in the first culture medium.

[00800] In some embodiments, in the step of culturing the second population of TILs in the second culture medium the second culture medium further comprises anti-CD3 and anti-CD28 beads or antibodies.

[00801] In some embodiments, the anti-CD3 and anti-CD28 beads or antibodies comprise the OKT-3 in the second culture medium.

[00802] According to some embodiments, the foregoing method further comprises cryopreserving the harvested TIL population using a cryopreservation medium. In some embodiments, the cryopreservation medium is a dimethylsulfoxide-based cryopreservation medium. In other embodiments, the cryopreservation medium is CS10.

[00803] In some embodiments, the invention provides the method described in any preceding paragraph above modified as applicable such that the step of culturing the second population of TILs in the second culture medium is performed for about 2-3 days.

[00804] In some embodiments, the invention provides the method described in any preceding paragraph above modified as applicable such that the step of culturing the second population of TILs in the second culture medium is performed for about 3-4 days.

[00805] In some embodiments, the invention provides the method described in any preceding paragraph above modified as applicable such that the step of culturing the second population of TILs in the second culture medium is performed for about 2 days.

[00806] In some embodiments, the invention provides the method described in any preceding paragraph above modified as applicable such that the step of culturing the second population of TILs in the second culture medium is performed for about 3 days.

[00807] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the second population of TILs in the second culture medium is performed for about 4 days.

[00808] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs, as applicable, in the second or third cell culture medium, applicable, is performed for about 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days or 15 days.

[00809] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 6-15 days.

[00810] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 7-15 days.

[00811] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 8-15 days.

[00812] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 9-15 days.

[00813] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 10-15 days.

[00814] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 11-15 days.

[00815] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 12-15 days.

[00816] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 13-15 days.

[00817] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 14-15 days.

[00818] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 5-14 days.

[00819] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 6-14 days.

[00820] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 7-14 days.

[00821] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 8-14 days.

[00822] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 9-14 days.

[00823] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 10-14 days.

[00824] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 11-14 days.

[00825] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 12-14 days.

[00826] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 13-14 days.

[00827] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 5-13 days.

[00828] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 5-12 days.

[00829] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 5-11 days.

[00830] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 5-10 days.

[00831] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 5-9 days.

[00832] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 5-8 days.

[00833] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 5-7 days.

[00834] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 5-6 days.

[00835] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 6-13 days.

[00836] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 6-12 days.

[00837] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 6-11 days.

[00838] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 6-10 days.

[00839] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 6-9 days.

[00840] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 6-8 days.

[00841] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 6-7 days.

[00842] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 7-13 days.

[00843] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 7-12 days.

[00844] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 7-11 days.

[00845] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 7-10 days.

[00846] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 7-9 days.

[00847] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 7-8 days.

[00848] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 8-13 days.

[00849] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 8-12 days.

[00850] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 8-11 days.

[00851] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 8-10 days.

[00852] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 8-9 days.

[00853] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 9-13 days.

[00854] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 9-12 days.

[00855] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 9-11 days.

[00856] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 9-10 days.

[00857] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 10-13 days.

[00858] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 10-12 days.

[00859] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 10-11 days.

[00860] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 11-13 days.

[00861] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 11-12 days.

[00862] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 12-13 days.

[00863] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 5 days.

[00864] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 6 days.

[00865] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 7 days.

[00866] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 8 days.

[00867] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 9 days.

[00868] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 10 days.

[00869] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 11 days.

[00870] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 12 days.

[00871] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 13 days.

[00872] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 14 days.

[00873] In some embodiments, the invention provides the method described in any of the preceding paragraphs as applicable above modified such that the step of culturing the third or fourth population of TILs in the second or third cell culture medium is performed for about 15 days.

[00874] According to some embodiments, any of the foregoing methods may be used to provide an autologous harvested TIL population for the treatment of a human subject with cancer.

C. Gene Editing Methods

[00875] As discussed above, embodiments of the present invention provide tumor infiltrating lymphocytes (TILs) that have been genetically modified via gene-editing to enhance their therapeutic effect (*e.g.*, expression of an immunomodulatory fusion protein on its cell surface). Embodiments of the present invention embrace genetic editing through nucleotide insertion (RNA or DNA) into a population of TILs for both promotion of the expression of one or more proteins and inhibition of the expression of one or more proteins, as well as combinations thereof. Embodiments of the present invention also provide methods for expanding TILs into a therapeutic population, wherein the methods comprise gene-editing the TILs. There are several gene-editing technologies that may be used to genetically modify a population of TILs, which are suitable for use in accordance with the present invention.

[00876] In some embodiments, a method of genetically modifying a population of TILs includes the step of stable incorporation of genes for production of one or more proteins. In some embodiments, a method of genetically modifying a population of TILs includes the step of retroviral transduction. In some embodiments, a method of genetically modifying a population of TILs includes the step of lentiviral transduction. Lentiviral transduction systems are known in the art and are described, e.g., in Levine, et al., Proc. Nat'l Acad. Sci. 2006, 103, 17372-77; Zufferey, et al., Nat. Biotechnol. 1997, 15, 871-75; Dull, et al., J. Virology 1998, 72, 8463-71, and U.S. Patent No. 6,627,442, the disclosures of each of which are incorporated by reference herein. In some embodiments, a method of genetically modifying a population of TILs includes the step of gamma-retroviral transduction. Gamma-retroviral transduction systems are known in the art and are described, e.g., Cepko and Pear, Cur. Prot. Mol. Biol. 1996, 9.9.1-9.9.16, the disclosure of which is incorporated by reference herein. In some embodiments, a method of genetically modifying a population of TILs includes the step of transposon-mediated gene transfer. Transposon-mediated gene transfer systems are known in the art and include systems wherein the transposase is provided as DNA expression vector or as an expressible RNA or a protein such that long-term expression of the transposase does not occur in the transgenic cells, for example, a transposase provided as an mRNA (*e.g.*, an mRNA comprising a cap and poly-A tail). Suitable transposon-

mediated gene transfer systems, including the salmonid-type Tel-like transposase (SB or Sleeping Beauty transposase), such as SB10, SB11, and SB100x, and engineered enzymes with increased enzymatic activity, are described in, *e.g.*, Hackett, *et al.*, *Mol. Therapy* **2010,** *18,* 674-83 and U.S. Patent No. 6,489,458, the disclosures of each of which are incorporated by reference herein.

[00877] In some embodiments, a method of genetically modifying a population of TILs includes the step of stable incorporation of genes for production or inhibition (*e.g.*, silencing) of one or more proteins. In some embodiments, a method of genetically modifying a population of TILs includes the step of electroporation. Electroporation methods are known in the art and are described, *e.g.*, in Tsong, *Biophys. J.* **1991,** *60,* 297-306, and U.S. Patent Application Publication No. 2014/0227237 A1, the disclosures of each of which are incorporated by reference herein. Other electroporation methods known in the art, such as those described in U.S. Patent Nos. 5,019,034; 5,128,257; 5,137,817; 5,173,158; 5,232,856; 5,273,525; 5,304,120; 5,318,514; 6,010,613 and 6,078,490, the disclosures of which are incorporated by reference herein, may be used. In some embodiments, the electroporation method is a sterile electroporation method. In some embodiments, the electroporation method is a pulsed electroporation method. In some embodiments, the electroporation method is a pulsed electroporation method comprising the steps of treating TILs with pulsed electrical fields to alter, manipulate, or cause defined and controlled, permanent or temporary changes in the TILs, comprising the step of applying a sequence of at least three single, operator-controlled, independently programmed, DC electrical pulses, having field strengths equal to or greater than 100 V/cm, to the TILs, wherein the sequence of at least three DC electrical pulses has one, two, or three of the following characteristics: (1) at least two of the at least three pulses differ from each other in pulse amplitude; (2) at least two of the at least three pulses differ from each other in pulse width; and (3) a first pulse interval for a first set of two of the at least three pulses is different from a second pulse interval for a second set of two of the at least three pulses. In some embodiments, the electroporation method is a pulsed electroporation method comprising the steps of treating TILs with pulsed electrical fields to alter, manipulate, or cause defined and controlled, permanent or temporary changes in the TILs, comprising the step of applying a sequence of at least three single, operator-controlled, independently programmed, DC electrical pulses, having field strengths equal to or greater than 100 V/cm, to the TILs, wherein at least two of the at least three pulses differ from each other in pulse amplitude. In some embodiments, the electroporation method is a pulsed

electroporation method comprising the steps of treating TILs with pulsed electrical fields to alter, manipulate, or cause defined and controlled, permanent or temporary changes in the TILs, comprising the step of applying a sequence of at least three single, operator-controlled, independently programmed, DC electrical pulses, having field strengths equal to or greater than 100 V/cm, to the TILs, wherein at least two of the at least three pulses differ from each other in pulse width. In some embodiments, the electroporation method is a pulsed electroporation method comprising the steps of treating TILs with pulsed electrical fields to alter, manipulate, or cause defined and controlled, permanent or temporary changes in the TILs, comprising the step of applying a sequence of at least three single, operator-controlled, independently programmed, DC electrical pulses, having field strengths equal to or greater than 100 V/cm, to the TILs, wherein a first pulse interval for a first set of two of the at least three pulses is different from a second pulse interval for a second set of two of the at least three pulses. In some embodiments, the electroporation method is a pulsed electroporation method comprising the steps of treating TILs with pulsed electrical fields to induce pore formation in the TILs, comprising the step of applying a sequence of at least three DC electrical pulses, having field strengths equal to or greater than 100 V/cm, to TILs, wherein the sequence of at least three DC electrical pulses has one, two, or three of the following characteristics: (1) at least two of the at least three pulses differ from each other in pulse amplitude; (2) at least two of the at least three pulses differ from each other in pulse width; and (3) a first pulse interval for a first set of two of the at least three pulses is different from a second pulse interval for a second set of two of the at least three pulses, such that induced pores are sustained for a relatively long period of time, and such that viability of the TILs is maintained. In some embodiments, a method of genetically modifying a population of TILs includes the step of calcium phosphate transfection. Calcium phosphate transfection methods (calcium phosphate DNA precipitation, cell surface coating, and endocytosis) are known in the art and are described in Graham and van der Eb, *Virology* **1973,** *52,* 456-467; Wigler, *et al.*, *Proc. Natl. Acad. Sci.* **1979,** *76,* 1373-1376; and Chen and Okayarea, *Mol. Cell. Biol.* **1987,** *7,* 2745-2752; and in U.S. Patent No. 5,593,875, the disclosures of each of which are incorporated by reference herein. In some embodiments, a method of genetically modifying a population of TILs includes the step of liposomal transfection. Liposomal transfection methods, such as methods that employ a 1:1 (w/w) liposome formulation of the cationic lipid *N*-[1-(2,3-dioleyloxy)propyl]-*n,n,n*-trimethylammonium chloride (DOTMA) and dioleoyl phophotidylethanolamine (DOPE) in filtered water, are known in the art and are described in Rose, *et al.*, *Biotechniques* **1991,** *10,* 520-525 and Felgner, *et al.*, *Proc. Natl. Acad. Sci. USA*,

1987, *84,* 7413-7417 and in U.S. Patent Nos. 5,279,833; 5,908,635; 6,056,938; 6,110,490; 6,534,484; and 7,687,070, the disclosures of each of which are incorporated by reference herein. In some embodiments, a method of genetically modifying a population of TILs includes the step of transfection using methods described in U.S. Patent Nos. 5,766,902; 6,025,337; 6,410,517; 6,475,994; and 7,189,705; the disclosures of each of which are incorporated by reference herein.

[00878] According to some embodiments, the gene-editing process may comprise the use of a programmable nuclease that mediates the generation of a double-strand or single-strand break at one or more immune checkpoint genes. Such programmable nucleases enable precise genome editing by introducing breaks at specific genomic loci, *i.e.*, they rely on the recognition of a specific DNA sequence within the genome to target a nuclease domain to this location and mediate the generation of a double-strand break at the target sequence. A double-strand break in the DNA subsequently recruits endogenous repair machinery to the break site to mediate genome editing by either non-homologous end-joining (NHEJ) or homology-directed repair (HDR). Thus, the repair of the break can result in the introduction of insertion/deletion mutations that disrupt (*e.g.*, silence, repress, or enhance) the target gene product.

[00879] Major classes of nucleases that have been developed to enable site-specific genomic editing include zinc finger nucleases (ZFNs), transcription activator-like nucleases (TALENs), and CRISPR-associated nucleases (*e.g.*, CRISPR/Cas9). These nuclease systems can be broadly classified into two categories based on their mode of DNA recognition: ZFNs and TALENs achieve specific DNA binding via protein-DNA interactions, whereas CRISPR systems, such as Cas9, are targeted to specific DNA sequences by a short RNA guide molecule that base-pairs directly with the target DNA and by protein-DNA interactions. *See, e.g.,* Cox *et al.*, *Nature Medicine,* 2015, Vol. 21, No. 2.

[00880] Non-limiting examples of gene-editing methods that may be used in accordance with TIL expansion methods of the present invention include CRISPR methods, TALE methods, and ZFN methods, embodiments of which are described in more detail below. According to some embodiments, a method for expanding TILs into a therapeutic population may be carried out in accordance with any embodiment of the methods described herein (*e.g.*, process 2A) or as described in PCT/US2017/058610, PCT/US2018/012605, or PCT/US2018/012633, wherein the method further comprises gene-editing at least a portion of the TILs by one or more of a CRISPR method, a TALE method or a ZFN method, in order to

generate TILs that can provide an enhanced therapeutic effect. According to some embodiments, gene-edited TILs can be evaluated for an improved therapeutic effect by comparing them to non-modified TILs *in vitro, e.g.,* by evaluating *in vitro* effector function, cytokine profiles, etc. compared to unmodified TILs.

[00881] In some embodiments of the present invention, electroporation is used for delivery of a gene editing system, such as CRISPR, TALEN, and ZFN systems. In some embodiments of the present invention, the electroporation system is a flow electroporation system. An example of a suitable flow electroporation system suitable for use with some embodiments of the present invention is the commercially-available MaxCyte STX system. There are several alternative commercially-available electroporation instruments which may be suitable for use with the present invention, such as the AgilePulse system or ECM 830 available from BTX-Harvard Apparatus, Cellaxess Elektra (Cellectricon), Nucleofector (Lonza/Amaxa), GenePulser MXcell (BIORAD), iPorator-96 (Primax) or siPORTer96 (Ambion). In some embodiments of the present invention, the electroporation system forms a closed, sterile system with the remainder of the TIL expansion method. In some embodiments of the present invention, the electroporation system is a pulsed electroporation system as described herein, and forms a closed, sterile system with the remainder of the TIL expansion method.

[00882] In some embodiments, a microfluidic platform is used for delivery of the gene editing system. In some embodiments, the microfluidic platform is a SQZ vector-free microfluidic platform.

D. Transient Cellular Modification

[00883] In some embodiments, the expanded TILs of the present invention are further manipulated before, during, or after an expansion step, including during closed, sterile manufacturing processes, each as provided herein, in order to alter protein expression in a transient manner. In some embodiments, the present invention includes transient cellular modification through nucleotide insertion, such as through ribonucleic acid (RNA) insertion, including insertion of messenger RNA (mRNA), into a population of TILs for promotion of the expression of one or more proteins or inhibition of the expression of one or more proteins, as well as simultaneous combinations of both promotion of one set of proteins with inhibition of another set of proteins.

[00884] In some embodiments, the expanded TILs of the present invention undergo transient alteration of protein expression. In some embodiments, the transient alteration of protein expression occurs in the bulk TIL population prior to first expansion. In some embodiments, the transient alteration of protein expression occurs after the first expansion. In some embodiments, the transient alteration of protein expression occurs in the bulk TIL population prior to second expansion. In some embodiments, the transient alteration of protein expression occurs after the second expansion.

[00885] In some embodiments, the transient alteration of protein expression results in transient expression of an immunomodulatory composition. In some embodiments, the immunomodulatory composition is an immunomodulatory fusion protein. In some embodiments, the immunomodulatory fusion protein comprises a membrane anchor fused to an immunomodulatory agent. In some embodiments, the immunomodulatory agent is selected from the group consisting of: IL-2, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21, and a CD40 agonist (e.g., a CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is selected from the group consisting of IL-2, IL-12, IL-15, IL-18 and IL-21. In some embodiments, the immunomodulatory agent is an interleukin selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21, and a CD40 agonist (e.g., a CD40L or an agonistic CD40 binding domain). In some embodiments, the immunomodulatory agent is an interleukin selected from the group consisting of IL-2, IL-12, IL-15, IL-18, IL-21, and a CD40 agonist (e.g., a CD40L or an agonistic CD40 binding domain).

[00886] As discussed herein, embodiments of the present invention provide tumor infiltrating lymphocytes (TILs) that have been transiently modified via transient alteration of protein expression to enhance their therapeutic effect. Embodiments of the present invention embrace transient modification through nucleotide insertion (e.g., RNA) into a population of TILs for expression of an immunomodulatory composition. Embodiments of the present invention also provide methods for expanding TILs into a therapeutic population, wherein the methods comprise transient modification of the TILs. There are several gene-editing technologies that may be used to transiently modify a population of TILs, which are suitable for use in accordance with the present invention.

[00887] In some embodiments, a method of transiently altering protein expression in a population of TILs includes contacting the TILs with nucleic acid (e.g., mRNA) encoding the immunomodulatory composition and then subjecting the cells to the step of electroporation.

Electroporation methods are known in the art and are described, *e.g.*, in Tsong, *Biophys. J.* 1991, *60,* 297-306, and U.S. Patent Application Publication No. 2014/0227237 A1, the disclosures of each of which are incorporated by reference herein. Other electroporation methods known in the art, such as those described in U.S. Patent Nos. 5,019,034; 5,128,257; 5,137,817; 5,173,158; 5,232,856; 5,273,525; 5,304,120; 5,318,514; 6,010,613 and 6,078,490, the disclosures of which are incorporated by reference herein, may be used. In some embodiments, the electroporation method is a sterile electroporation method. In some embodiments, the electroporation method is a pulsed electroporation method. In some embodiments, the electroporation method is a pulsed electroporation method comprising the steps of treating TILs with pulsed electrical fields to alter, manipulate, or cause defined and controlled, permanent or temporary changes in the TILs, comprising the step of applying a sequence of at least three single, operator-controlled, independently programmed, DC electrical pulses, having field strengths equal to or greater than 100 V/cm, to the TILs, wherein the sequence of at least three DC electrical pulses has one, two, or three of the following characteristics: (1) at least two of the at least three pulses differ from each other in pulse amplitude; (2) at least two of the at least three pulses differ from each other in pulse width; and (3) a first pulse interval for a first set of two of the at least three pulses is different from a second pulse interval for a second set of two of the at least three pulses. In some embodiments, the electroporation method is a pulsed electroporation method comprising the steps of treating TILs with pulsed electrical fields to alter, manipulate, or cause defined and controlled, permanent or temporary changes in the TILs, comprising the step of applying a sequence of at least three single, operator-controlled, independently programmed, DC electrical pulses, having field strengths equal to or greater than 100 V/cm, to the TILs, wherein at least two of the at least three pulses differ from each other in pulse amplitude. In some embodiments, the electroporation method is a pulsed electroporation method comprising the steps of treating TILs with pulsed electrical fields to alter, manipulate, or cause defined and controlled, permanent or temporary changes in the TILs, comprising the step of applying a sequence of at least three single, operator-controlled, independently programmed, DC electrical pulses, having field strengths equal to or greater than 100 V/cm, to the TILs, wherein at least two of the at least three pulses differ from each other in pulse width. In some embodiments, the electroporation method is a pulsed electroporation method comprising the steps of treating TILs with pulsed electrical fields to alter, manipulate, or cause defined and controlled, permanent or temporary changes in the TILs, comprising the step of applying a sequence of at least three single, operator-controlled, independently

programmed, DC electrical pulses, having field strengths equal to or greater than 100 V/cm, to the TILs, wherein a first pulse interval for a first set of two of the at least three pulses is different from a second pulse interval for a second set of two of the at least three pulses. In some embodiments, the electroporation method is a pulsed electroporation method comprising the steps of treating TILs with pulsed electrical fields to induce pore formation in the TILs, comprising the step of applying a sequence of at least three DC electrical pulses, having field strengths equal to or greater than 100 V/cm, to TILs, wherein the sequence of at least three DC electrical pulses has one, two, or three of the following characteristics: (1) at least two of the at least three pulses differ from each other in pulse amplitude; (2) at least two of the at least three pulses differ from each other in pulse width; and (3) a first pulse interval for a first set of two of the at least three pulses is different from a second pulse interval for a second set of two of the at least three pulses, such that induced pores are sustained for a relatively long period of time, and such that viability of the TILs is maintained.

[00888] In some embodiments, a method of transiently altering protein expression in population of TILs includes the step of calcium phosphate transfection. Calcium phosphate transfection methods (calcium phosphate nucleic acid precipitation, cell surface coating, and endocytosis) are known in the art and are described in Graham and van der Eb, *Virology* 1973, *52,* 456-467; Wigler, *et al.*, *Proc. Natl. Acad. Sci.* 1979, *76,* 1373-1376; and Chen and Okayarea, *Mol. Cell. Biol.* 1987, *7,* 2745-2752; and in U.S. Patent No. 5,593,875, the disclosures of each of which are incorporated by reference herein. In some embodiments, a method of transiently altering protein expression in a population of TILs includes the step of liposomal transfection. Liposomal transfection methods, such as methods that employ a 1:1 (w/w) liposome formulation of the cationic lipid *N*-[1-(2,3-dioleyloxy)propyl]-*n,n,n*-trimethylammonium chloride (DOTMA) and dioleoyl phophotidylethanolamine (DOPE) in filtered water, are known in the art and are described in Rose, *et al.*, *Biotechniques* **1991**, *10,* 520-525 and Felgner, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1987**, *84,* 7413-7417 and in U.S. Patent Nos. 5,279,833; 5,908,635; 6,056,938; 6,110,490; 6,534,484; and 7,687,070, the disclosures of each of which are incorporated by reference herein. In some embodiments, a method of transiently altering protein expression in a population of TILs includes the step of transfection using methods described in U.S. Patent Nos. 5,766,902; 6,025,337; 6,410,517; 6,475,994; and 7,189,705; the disclosures of each of which are incorporated by reference herein. The TILs may be a first population, a second population and/or a third population of TILs as described herein.

[00889] In some embodiments, a SQZ vector-free microfluidic platform is used for transiently altering protein expression. See, e.g., International Patent Application Publication Nos. WO 2013/059343A1, WO 2017/008063A1, or WO 2017/123663A1, or U.S. Patent Application Publication Nos. US 2014/0287509A1, US 2018/0201889A1, or US 2018/0245089A1, all of which are incorporated by reference herein in their entireties, and particularly for disclosures of microfluidic platforms for nucleic acid delivery. In the SQZ platform, the cell membranes of the TILs for modification are temporarily disrupted by microfluidic constriction, thereby allowing the delivery of nucleic acids encoding the transiently expressed protein. The TILs may be a first population, a second population and/or a third population of TILs as described herein.

E. Immune Checkpoints

[00890] According to particular embodiments of the present invention, a TIL population is gene-edited to express one or more immunomodulatory compositions at the cell surface of TIL cells in the TIL population and to genetically modify one or more immune checkpoint genes in the TIL population. Stated another way, in addition to modification of a TIL population to express one or more immunomodulatory compositions at the cell surface, a DNA sequence within the TIL that encodes one or more of the TIL's immune checkpoints is permanently modified, *e.g.*, inserted, deleted or replaced, in the TIL's genome. Immune checkpoints are molecules expressed by lymphocytes that regulate an immune response via inhibitory or stimulatory pathways. In the case of cancer, immune checkpoint pathways are often activated to inhibit the anti-tumor response, *i.e.*, the expression of certain immune checkpoints by malignant cells inhibits the anti-tumor immunity and favors the growth of cancer cells. *See, e.g.,* Marin-Acevedo *et al., Journal of Hematology & Oncology* (2018) 11:39. Thus, certain inhibitory checkpoint molecules serve as targets for immunotherapies of the present invention. According to particular embodiments, TILs are gene-edited to block or stimulate certain immune checkpoint pathways and thereby enhance the body's immunological activity against tumors.

[00891] As used herein, an immune checkpoint gene comprises a DNA sequence encoding an immune checkpoint molecule. According to particular embodiments of the present invention, gene-editing TILs during the TIL expansion method causes expression of one or more immune checkpoint genes to be silenced or reduced in at least a portion of the therapeutic population of TILs. For example, gene-editing may cause the expression of an

inhibitory receptor, such as PD-1 or CTLA-4, to be silenced or reduced in order to enhance an immune reaction.

[00892] The most broadly studied checkpoints include programmed cell death receptor-1 (PD-1) and cytotoxic T lymphocyte-associated molecule-4 (CTLA-4), which are inhibitory receptors on immune cells that inhibit key effector functions (*e.g.*, activation, proliferation, cytokine release, cytotoxicity, etc.) when they interact with an inhibitory ligand. Numerous checkpoint molecules, in addition to PD-1 and CTLA-4, have emerged as potential targets for immunotherapy, as discussed in more detail below.

[00893] Non-limiting examples of immune checkpoint genes that may be silenced or inhibited by permanently gene-editing TILs of the present invention include PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, PKA, CBL-B, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, BTLA, CD160, TIGIT, TET2, BAFF (BR3), CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3, TOX, SOCS1, ANKRD11, and BCOR. For example, immune checkpoint genes that may be silenced or inhibited in TILs of the present invention may be selected from the group comprising PD-1, CTLA-4, LAG-3, TIM-3, Cish, CBL-B, TIGIT, TET2, TGFβ, and PKA. BAFF (BR3) is described in Bloom, *et al.*, *J. Immunother.*, **2018,** in press. According to another example, immune checkpoint genes that may be silenced or inhibited in TILs of the present invention may be selected from the group comprising PD-1, LAG-3, TIM-3, CTLA-4, TIGIT, TET2, CISH, TGFβR2, PRA, CBLB, BAFF (BR3), and combinations thereof.

[00894] According to some embodiments, a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprises:

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 and optionally comprising OKT-3 and/or a 4-1BB agonist antibody for about 3 to 11 days to produce a second population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area;

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NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

WHAT IS CLAIMED IS:

1. A method of treating a cancer in a patient or subject in need thereof comprising administering a population of tumor infiltrating lymphocytes (TILs), optionally wherein the patient or subject has received at least one prior therapy,

wherein a portion of the TILs are modified TILs such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

2. 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) 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;

(b) adding the first population of TILs into a closed system;

(c) 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 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

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

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

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

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

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

(i) modifying a portion of the TILs at any time prior to the administering (h) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

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 a first population of TILs from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments;

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

(c) 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 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

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

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

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

(g) cryopreserving the infusion bag comprising the harvested TIL population from step

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(f) using a cryopreservation process;

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

(i) modifying a portion of the TILs at any time prior to the administering (h) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

4. 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) adding the first population of TILs into a closed system;

(c) 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 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

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

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

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

(g) cryopreserving the infusion bag comprising the harvested TIL population from step

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(f) using a cryopreservation process;

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

(i) modifying a portion of the TILs at any time prior to the administering (h) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

5. 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 and adding the tumor fragments into a closed system;

(c) 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 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

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

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

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

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wherein the transfer from step (e) to (f) occurs without opening the system;

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

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

(i) modifying a portion of the TILs at any time prior to the administering (h) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

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) 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;

(c) contacting the first population of TILs 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 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) modifying a portion of the TILs at any time prior to the administering (g) such that

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each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

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) 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;

(c) contacting the tumor fragments 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 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) modifying a portion of the TILs at any time prior to the administering (g) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

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

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(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;

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

(c) performing a priming first expansion by culturing the PD-l enriched TIL population 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;

(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 therapeutic 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;

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

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

(g) modifying a portion of the TILs at any time during the method such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

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;

(b) adding the first population of TILs into a closed system;

(c) 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 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

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

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

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

(g) modifying a portion of the TILs at any time prior to the transfer to the infusion bag in step (f) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

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;

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

(c) 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 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, and wherein the transition from step (b) to step (c) occurs

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without opening the system;

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

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

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

(g) modifying a portion of the TILs at any time prior to the transfer to the infusion bag in step (f) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

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) adding the first population of TILs into a closed system;

(c) 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 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

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

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

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

(g) modifying a portion of the TILs at any time prior to the transfer in step (f) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

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 a 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) adding the tumor fragments into a closed system;

(c) 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 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, and wherein the transition from step (b) to step (c) occurs without opening the system;

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

(e) harvesting the third population of TILs obtained from step (d), wherein the transition

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from step (d) to step (e) occurs without opening the system;

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

(g) modifying a portion of the TILs at any time prior to the transfer in step (f) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

13. The method of any of claims 9-12, wherein the first expansion is divided into a first step and a second step, wherein the method further comprises performing the first step of the first expansion by culturing the first population of TILs in a cell culture medium containing IL-2 to produce TILs that egress from the tumor fragments or sample, separating TILs that remain in the tumor fragments or sample from TILs that egressed from the tumor fragments or sample, optionally digesting the tumor fragments or sample to produce a tumor digest, and performing the second step of the first expansion by culturing in the cell culture medium of the TILs remaining in the tumor fragments or sample or tumor digest to produce the second population of TILs.

14. 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) contacting the first population of TILs with a first cell culture medium;

(c) 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;

(d) 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;

(e) harvesting the third population of TILs; and

(f) modifying a portion of the TILs at any time prior to the harvesting in step (f) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

15. 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 a cancer in a 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 of the tumor that contains a mixture of tumor and TIL cells;

(b) fragmenting the tumor into tumor fragments;

(c) contacting the tumor fragments 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 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) modifying a portion of the TILs at any time prior to the harvesting in step (f) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

16. 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;

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

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

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

(e) modifying a portion of the TILs at any time prior to or after the harvesting in step (d) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

17. The method of claim 16, wherein in step (b) the cell culture medium further comprises antigen-presenting cells (APCs), and wherein the number of APCs in the culture medium in step (c) is greater than the number of APCs in the culture medium in step (b).

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

(a) performing a priming first expansion by culturing a first population of TILs, said first population of TILs obtainable by processing a tumor sample from a tumor resected from a cancer in a subject into multiple tumor fragments, in a cell culture medium comprising IL-2, optionally OKT-3, and optionally antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for first period of about 1 to 7/8 days to obtain the second population of TILs, wherein the second

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population of TILs is greater in number than the first population of TILs;

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

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

(d) modifying a portion of the TILs at any time prior to or after the harvesting in step (c) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

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 7/8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

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

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

(d) modifying a portion of the TILs at any time prior to or after the harvesting in step (c) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

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

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in step (c) is greater than the number of APCs in the culture medium in step (b).

21. The method of any of claims 14-18, wherein the priming first expansion is divided into a first step and a second step, wherein the method further comprises performing the first step of the priming first expansion by culturing the first population of TILs in a cell culture medium containing IL-2 to produce TILs that egress from the tumor fragments or sample, separating TILs that remain in the tumor fragments or sample from TILs that egressed from the tumor fragments or sample, optionally digesting the tumor fragments or sample to produce a tumor digest, and performing the second step of the priming first expansion by culturing in the cell culture medium the TILs remaining in the tumor fragments or sample or tumor digest to produce the second population of TILs.

22. 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 sample obtained from one or more small biopsies, core biopsies, or needle biopsies of a tumor from a cancer in a subject by culturing the tumor sample in a first cell culture medium comprising IL-2 for about 3 days;

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

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

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

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

(f) modifying a portion of the TILs at any time prior to transfer to the infusion bag in step (e) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

23. 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 sample obtained from one or more small biopsies, core biopsies, or needle biopsies of a tumor from a cancer in a subject by culturing the tumor sample in a first cell culture medium comprising IL-2 for about 3 days;

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

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

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

(e) modifying a portion of the TILs at any time prior to or after the harvesting in step (f) such that each of the modified TILs comprises an immunomodulatory composition associated with its surface membrane.

24. The method of any one of claims 1-18 and 21-23, wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, triple negative breast cancer, cancer caused by human papilloma virus, head and neck cancer (including head and neck squamous cell carcinoma (HNSCC)), renal cancer, and renal cell carcinoma.

25. A method of expanding T cells comprising:

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

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

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

(d) modifying a portion of the T cells at any time prior to or after the harvesting in step (c) such that each of the modified T cells comprises an immunomodulatory composition associated with its surface membrane.

26. 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;

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

(d) modifying a portion of the T cells at any time prior to or after the harvesting in step (e) such that each of the modified T cells comprises an immunomodulatory composition associated with its surface membrane.

27. A method for expanding peripheral blood lymphocytes (PBLs) from peripheral blood, the method comprising the steps of:

(a) obtaining a sample of peripheral blood mononuclear cells (PBMCs) from peripheral blood of a patient;

(b) culturing said PBMCs in a culture comprising a first cell culture medium with IL-2, anti-CD3/anti-CD28 antibodies and a first combination of antibiotics, for a period of

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time selected from the group consisting of: about 9 days, about 10 days, about 11 days, about 12 days, about 13 days and about 14 days, thereby effecting expansion of peripheral blood lymphocytes (PBLs) from said PBMCs;

(c) harvesting the PBLs from the culture in step (b); and

(d) modifying a portion of the PBLs at any time prior to or after the harvesting in step (c) such that each of the modified PBLs comprises an immunomodulatory composition associated with its surface membrane.

28. The method of claim 27, wherein the patient is pre-treated with ibrutinib or another interleukin-2 inducible T cell kinase (ITK) inhibitor.

29. The method of claim 26 or 28, wherein the patient is refractory to treatment with ibrutinib or such other ITK inhibitor.

30. The method of any one of claims 2-29, wherein immunomodulatory composition comprises one or more membrane anchored immunomodulatory fusion proteins each comprising one or more immunomodulatory agents and a cell membrane anchor moiety.

31. The method of claim 30, wherein the one or more immunomodulatory agents comprise one or more cytokines.

32. The method of claim 31, wherein the one or more cytokines comprise IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, or GCSF or a variant thereof.

33. The method of claim 32, wherein the one or more cytokines comprise IL-12.

34. The method of claim 33, wherein the IL-12 comprises a human IL-12 p35 subunit attached to a human IL-12 p40 subunit.

35. The method of claim 34, wherein the human IL-12 p35 subunit has the amino acid sequence of SEQ ID NO:247 and the human IL-12 p40 subunit has the amino acid sequence of SEQ ID NO:248.

36. The method of claim 32, wherein the one or more cytokines comprise IL-15.

37. The method of claim 36, wherein the IL-15 is human IL-15.

38. The method of claim 37, wherein the human IL-15 has the amino acid sequence of SEQ ID NO:258.

39. The method of claim 32, wherein the one or more cytokines comprise IL-18.

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40. The method of claim 39, wherein the IL-18 is human IL-18.

41. The method of claim 40, wherein the human IL-18 has the amino acid sequence of SEQ ID NO:269 or SEQ ID NO:270.

42. The method of claim 32, wherein the one or more cytokines comprise IL-21.

43. The method of claim 42, wherein the IL-21 is human IL-21.

44. The method of claim 43, wherein the human IL-21 has the amino acid sequence of SEQ ID NO:271.

45. The method of claim 30, wherein the one or more immunomodulatory agents comprise a CD40 agonist.

46. The method of claim 45, wherein the CD40 agonist is an anti-CD40 binding domain or CD40L.

47. The method of claim 46, wherein the CD40 agonist is a CD40 binding domain comprising a variable heavy domain (VH) and a variable light domain (VL).

48. The method of claim 47, wherein the VH and VL of the CD40 binding domain are selected from the following:

    a. a VH having the amino acid sequence of SEQ ID NO: 274, and a VL having the amino acid sequence of SEQ ID NO:275;

    b. a VH having the amino acid sequence of SEQ ID NO: 277, and a VL having the amino acid sequence of SEQ ID NO:278;

    c. a VH having the amino acid sequence of SEQ ID NO: 280, and a VL having the amino acid sequence of SEQ ID NO:281; and

    d. a VH having the amino acid sequence of SEQ ID NO: 283, and a VL having the amino acid sequence of SEQ ID NO:284.

49. The method of claim 47 or 48, wherein the CD40 binding domain is an scFv.

50. The method of claim 46, wherein the CD40 agonist is a human CD40L having the amino acid sequence of SEQ ID NO: 273.

51. The method of any one of claims 30 to 50, wherein the membrane anchored immunomodulatory fusion protein is according to the formula, from N- to C-terminus: S-IA-L-C, wherein S is a signal peptide, IA is an immunomodulatory agent, L is a linker

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and C is a cell membrane anchor moiety.

52. The method of any one of claims 30-51, wherein the cell membrane anchor moiety comprises a CD8a transmembrane-intracellular domain, a B7-1 transmembrane domain, a B7-2 transmembrane domain, or a CD8a transmembrane domain.

53. The method of claim 52, wherein the cell membrane anchor moiety comprises a B7-1 transmembrane domain.

54. The method of claim 53, wherein the cell membrane anchor moiety has the amino acid sequence of SEQ ID NO:239.

55. The method of any one of claims 30-54, wherein the immunomodulatory composition comprises two or more different membrane anchored immunomodulatory fusion proteins, wherein each of the different membrane anchored immunomodulatory fusion proteins each comprises a different immunomodulatory agent.

56. The method of claim 55, wherein the different immunomodulatory agents are selected from: IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, GCSF or a variant thereof, and a CD40 agonist.

57. The method of claim 56, wherein the different immunomodulatory agents are selected from: IL-12 and IL-15, IL-15 and IL-18, CD40L and IL-15, IL-15 and IL-21, and IL-2 and IL-12.

58. The method of any one of claims 30-57, wherein the modifying comprises introducing a heterologous nucleic acid encoding the fusion protein into the portion of TILs and expressing the fusion protein on the surface of the modified TILs.

59. The method of claim 58, wherein the heterologous nucleic acid is introduced into the genome of the modified TIL using one or more methods selected from a CRISPR method, a TALE method, a zinc finger method, and a combination thereof.

60. The method of any one of claims 2-29, wherein immunomodulatory composition comprises a fusion protein comprising one or more immunomodulatory agents linked to a TIL surface antigen binding domain.

61. The method of claim 60, wherein the one or more immunomodulatory agents comprise one or more cytokines.

62. The method of claim 61, wherein the one or more cytokines comprises IL-2, IL-6, IL-7,

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IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, or GCSF or a variant thereof.

63. The method of claim 62 , wherein the one or more cytokines comprise IL-12.

64. The method of claim 62, wherein the one or more cytokines comprise IL-15.

65. The method of claim 62, wherein the one or more cytokines comprise IL-21.

66. The method of any one of claims 60-65, wherein the TIL surface antigen binding domain comprises an antibody variable heavy domain and variable light domain.

67. The method of any one of claims 60-66, wherein the TIL surface antigen binding domain comprises an antibody or fragment thereof.

68. The method of any one of claims 43-50, wherein the TIL surface antigen binding domain exhibits an affinity for one or more of following TIL surface antigens: CD45, CD4, CD8, CD3, CDlla, CDllb, CDllc, CD18, CD25, CD127, CD19, CD20, CD22, HLA-DR, CD197, CD38, CD27, CD196, CXCR3, CXCR4, CXCR5, CD84, CD229, CCR1, CCR5, CCR4, CCR6, CCR8, CCR10, CD 16, CD56, CD 137, OX40, or GITR.

69. The method of any one of claims 60-68, wherein the modifying comprises incubating the fusion protein with the portion of TILs under conditions to permit the binding of the fusion protein to the portion of TILs.

70. The method of any one of claims 2-29, wherein immunomodulatory composition comprises a nanoparticle comprising a plurality of immunomodulatory agents.

71. The method of claim 70, wherein the plurality of immunomodulatory agents are covalently linked together by degradable linkers.

72. The method of claim 71, wherein the nanoparticle comprises at least one polymer, cationic polymer, or cationic block co-polymer on the nanoparticle surface.

73. The method of any one of claims 70-72, wherein the one or more cytokines comprises IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFNgamma, TNFa, IFN alpha, IFN beta, GM-CSF, or GCSF or a variant thereof.

74. The method of claim 73, wherein the one or more cytokines comprises IL-12.

75. The method of claim 73, wherein the one or more cytokines comprises IL-15.

76. The method of claim 73, wherein the one or more cytokines comprises IL-21.

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77. The method of any one of claims 70-76, wherein the nanoparticle is a liposome, a protein nanogel, a nucleotide nanogel, a polymer nanoparticle, or a solid nanoparticle.

78. The method of claim 77, wherein the nanoparticle is a nanogel.

79. The method of any one of claims 70-78, wherein the nanoparticle further comprises an antigen binding domain that binds to one or more of the following antigens: CD45, CDlla (integrin alpha- L), CD 18 (integrin beta-2), CD1lb, CD1lc, CD25, CD8, or CD4.

80. The method of any one of claims 70-79, wherein the modifying comprises attaching the immunomodulatory composition to the surface of the portion of TILs.

81. The method according to any of claims 2-5 or 9-13, wherein the modifying is carried out on TILs from the first expansion, or TILs from the second expansion, or both.

82. The method according to any of claims 6-8 or 14-23, wherein the modifying is carried out on TILs from the priming first expansion, or TILs from the rapid second expansion, or both.

83. The method according to any of claims 2-5 or 9-13, wherein the modifying is carried out after the first expansion and before the second expansion.

84. The method according to any of claims 6-8 or 14-23, wherein the modifying is carried out after the priming first expansion and before the rapid second expansion, or both.

85. The method according to any of claims 2-5 or 9-13, wherein the modifying is carried out after the second expansion.

86. The method according to any of claims 6-8 or 14-23, wherein the modifying is carried out after the rapid second expansion.

87. The method according to any of claims 2-63, wherein the modifying is carried out after the harvesting.

88. The method of any one of claims 2-5 or 9-13, wherein the first expansion is performed over a period of about 11 days.

89. The method of any one of claims 6-8 or 14-23, wherein the priming first expansion is performed over a period of about 11 days.

90. The method of any one of claims 2-5 or 9-13, wherein the IL-2 is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL in the cell culture medium in the first expansion.

91. The method of any one of claims 6-8 or 14-23, wherein the IL-2 is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL in the cell culture medium in the priming first expansion.

92. The method of any one of claims 2-5 or 9-13, wherein in the second expansion step, the IL-2 is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL and the OKT-3 antibody is present at an initial concentration of about 30 ng/mL.

93. The method of any one of claims 6-8 or 14-23, wherein in the rapid second expansion step, the IL-2 is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL and the OKT-3 antibody is present at an initial concentration of about 30 ng/mL.

94. The method of claims 2-5 or 9-13, wherein the first expansion is performed using a gas permeable container.

95. The method of any one of claims 6-8 or 14-23, wherein the priming first expansion is performed using a gas permeable container.

96. The method of any one of claims 2-5 or 9-13, wherein the second expansion is performed using a gas permeable container.

97. The method of claims 6-8 or 14-23, wherein the rapid second expansion is performed using a gas permeable container.

98. The method of any one of claim 2-5 or 9-13, wherein the cell culture medium of the first expansion further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

99. The method of claim 6-8 or 14-23, wherein the cell culture medium of the priming first expansion further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

100. The method of any one of any one of claims 2-5 or 9-13, wherein the cell culture medium of the second expansion further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

101. The method of any one of claims 6-8 or 14-23, wherein the cell culture medium of the rapid second expansion further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

102. The method of any one of claims 1-8, further comprising the step of treating the

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patient with a non-myeloablative lymphodepletion regimen prior to administering the TILs to the patient.

103. The method of claim 102, wherein the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for three days.

104. The method of claim 102, wherein the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day and fludarabine at a dose of 25 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for three days.

105. The method of claim 102, wherein the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day and fludarabine at a dose of 25 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for one day.

106. The method of any one of claims 103-105, wherein the cyclophosphamide is administered with mesna.

107. The method of any one of claims 1-7 or 102-106, further comprising the step of treating the patient with an IL-2 regimen starting on the day after the administration of TILs to the patient.

108. The method of any one of claims 1-7 or 102-106, further comprising the step of treating the patient with an IL-2 regimen starting on the same day as administration of TILs to the patient.

109. The method of claim 107 or 108, wherein the IL-2 regimen is a high-dose IL-2 regimen comprising 600,000 or 720,000 IU/kg of aldesleukin, or a biosimilar or variant thereof, administered as a 15-minute bolus intravenous infusion every eight hours until tolerance.

110. The method according to any one of claims 1-7 or 102-109, wherein a therapeutically effective population of TILs is administered and comprises from about 2.3×10^{10} to about 13.7×10^{10} TILs.

111. The method of any one of 6-8 or 14-23, wherein the priming first expansion and rapid second expansion are performed over a period of 21 days or less.

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112. The method of any one of claims 6-8 or 14-23, wherein the priming first expansion and rapid second expansion are performed over a period of 16 or 17 days or less.

113. The method of any one of claims 6-8 or 14-23, wherein the priming first expansion is performed over a period of 7 or 8 days or less.

114. The method of any one of claims 6-8 or 14-23, wherein the rapid second expansion is performed over a period of 11 days or less.

115. The method of any one of claims 2-5 or 9-13, the first expansion in step (c) and the second expansion in step (d) are each individually performed within a period of 11 days.

116. The method of any one of claims 2-5 or 9-13, wherein steps (a) through (f) are performed in about 10 days to about 22 days.

117. The method according to any of claims 2 to 116, wherein the modified TILs further comprise a genetic modification that causes expression of one or more immune checkpoint genes to be silenced or reduced in at least a portion of the therapeutic population of TILs.

118. The method according to claim 117, wherein said one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, PKA, CBL-B, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, BTLA, CD160, TIGIT, TET2, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3, TOX, SOCS1, ANKRD11, and BCOR.

119. The method according to claim 117, wherein said one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, and PKA.

120. The method according to any of claims 2 to 119, wherein the modified TILs further comprises a genetic modification that causes expression of one or more immune checkpoint genes to be enhanced in at least a portion of the therapeutic population of TILs, the immune checkpoint gene(s) being selected from the group comprising CCR2, CCR4, CCR5, CXCR2, CXCR3, CX3CR1, IL-2, IL-4, IL-7, IL-10, IL-15, IL-21, the NOTCH 1/2 intracellular domain (ICD), and/or the NOTCH ligand mDLL1.

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121. The method according to any of claims 117-120, wherein the genetic modification is produced using a programmable nuclease that mediates the generation of a double-strand or single-strand break at said one or more immune checkpoint genes.

122. The method according to any of claims 117-120, wherein the genetic modification is produced using one or more methods selected from a CRISPR method, a TALE method, a zinc finger method, and a combination thereof.

123. The method of claim 122, wherein the genetic modification is produced using a CRISPR method.

124. The method of claim 123, wherein the CRISPR method is a CRISPR/Cas9 method.

125. The method of claim 122, wherein genetic modification is produced using a TALE method.

126. The method of claim 122, wherein the genetic modification is produced using a zinc finger method.

127. The method of any of claims 1-23 or 81-116, wherein the modified TILs are modified to transiently express the immunomodulatory composition on the cell surface.

128. The method of claim 127, wherein the immunomodulatory composition comprises one or more membrane anchored immunomodulatory fusion proteins, wherein each fusion protein comprises one or more immunomodulatory agents and a cell membrane anchor moiety.

129. The method of claim 128, wherein the one or more immunomodulatory agents comprise one or more cytokines.

130. The method of claim 129, wherein the one or more cytokines comprise IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, or GCSF or a variant thereof.

131. The method of claim 130, wherein the one or more cytokine comprise IL-2.

132. The method of claim 131, wherein the IL-2 is human IL-2.

133. The method of claim 132, wherein the human IL-2 has the amino acid sequence of SEQ ID NO:272.

134. The method of claim 130, wherein the one or more cytokines comprise IL-12.

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135. The method of claim 134, wherein the IL-12 comprises a human IL-12 p35 subunit attached to a human IL-12 p40 subunit.

136. The method of claim 135, wherein the human IL-12 p35 subunit has the amino acid sequence of SEQ ID NO:267 and the human IL-12 p40 subunit has the amino acid sequence of SEQ ID NO:268.

137. The method of claim 130, wherein the one or more cytokines comprise IL-15.

138. The method of claim 137, wherein the IL-15 is human IL-15.

139. The method of claim 138, wherein the human IL-15 has the amino acid sequence of SEQ ID NO:258.

140. The method of claim 130, wherein the one or more cytokines comprise IL-18.

141. The method of claim 140, wherein the IL-18 is human IL-18.

142. The method of claim 141, wherein the human IL-18 has the amino acid sequence of SEQ ID NO:269 or SEQ ID NO:270.

143. The method of claim 130, wherein the one or more cytokines comprise IL-21.

144. The method of claim 143, wherein the IL-21 is human IL-21.

145. The method of claim 144, wherein the human IL-21 has the amino acid sequence of SEQ ID NO:271.

146. The method of claim 128, wherein the one or more immunomodulatory agents comprises a CD40 agonist.

147. The method of claim 146, wherein the CD40 agonist is an anti-CD40 binding domain or CD40L.

148. The method of claim 147, wherein the CD40 agonist is a CD40 binding domain comprising a variable heavy domain (VH) and a variable light domain (VL).

149. The method of claim 148, wherein the VH and VL of the CD40 binding domain are selected from the following:

a. a VH having the amino acid sequence of SEQ ID NO: 274, and a VL having the amino acid sequence of SEQ ID NO:275;

b. a VH having the amino acid sequence of SEQ ID NO: 277, and a VL having the amino acid sequence of SEQ ID NO:278;

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c. a VH having the amino acid sequence of SEQ ID NO: 280, and a VL having the amino acid sequence of SEQ ID NO:281; and

d. a VH having the amino acid sequence of SEQ ID NO: 283, and a VL having the amino acid sequence of SEQ ID NO:284.

150. The method of claim 148 or 149, wherein the CD40 binding domain is an scFv.

151. The method of claim 46, wherein the CD40 agonist is a human CD40L having the amino acid sequence of SEQ ID NO: 273.

152. The method of any one of claims 128 to 151, wherein the one or more membrane anchored immunomodulatory fusion proteins are independently according to the formula, from N- to C-terminus: S-IA-L-C, wherein S is a signal peptide, IA is an immunomodulatory agent, L is a linker and C is a cell membrane anchor moiety.

153. The method of any one of claims 128-152, wherein the cell membrane anchor moiety comprises a CD8a transmembrane-intracellular domain, a B7-1 transmembrane domain, a B7-2 transmembrane domain, or a CD8a transmembrane domain.

154. The method of claim 153, wherein the cell membrane anchor moiety comprises a B7-1 transmembrane domain.

155. The method of claim 154, wherein the cell membrane anchor moiety has the amino acid sequence of SEQ ID NO:239.

156. The method of any one of claims 128-155, wherein the immunomodulatory composition comprises two or more different membrane anchored immunomodulatory fusion proteins, wherein each of the different membrane anchored immunomodulatory fusion proteins each comprises a different immunomodulatory agent.

157. The method of claim 156, wherein the different immunomodulatory agents are selected from: IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, GCSF or a variant thereof, and a CD40 agonist.

158. The method of claim 157, wherein the different immunomodulatory agents are selected from: IL-12 and IL-15, IL-15 and IL-18, CD40L and IL-15, IL-15 and IL-21, and IL-2 and IL-12.

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159. The method of any one of claims 128-158, wherein the modifying comprises introducing a heterologous nucleic acid encoding the fusion protein into the portion of TILs and expressing the fusion protein on the surface of the modified TILs.

160. The method of claim 159, wherein the heterologous nucleic acid is introduced into the genome of the modified TIL using one or more methods selected from a CRISPR method, a TALE method, a zinc finger method, and a combination thereof.

161. The method of any of claims 128-158, wherein the modified TILs are modified by transfecting the TILs with a nucleic acid encoding the fusion protein.

162. The method of claim 161, wherein the nucleic acid is an RNA.

163. The method of claim 162, wherein the RNA is a mRNA.

164. The method of claim 163, wherein the TILs are transfected with the mRNA by electroporation.

165. The method of claim 164, wherein the TILs are transfected with the mRNA by electroporation after the first expansion and before the second expansion.

166. The method of claim 164, wherein the TILs are transfected with the mRNA by electroporation before the first expansion.

167. The method of claim 161, wherein the modified TILs are transfected with the nucleic acid encoding the fusion protein using a microfluidic device to temporarily disrupt the cell membranes of the TILs, thereby allowing transfection of the nucleic acid.

168. The method of any of claims 163-167, wherein the method further comprises activating the TILs by incubation with an anti-CD3 agonist before transfecting the TILs with the mRNA.

169. The method of claim 168, wherein the anti-CD3 agonist is OKT-3.

170. The method of claim 168 or 169, wherein the TILs are activated by incubating the TILs with the anti-CD3 agonist for about 1 to 3 days before transfecting the TILs with the mRNA.

171. A composition comprising the modified TILs of any one of claims 1 to 131.

172. A pharmaceutical composition comprising the modified TILs of any one of claims 1 to 131 and a pharmaceutically-acceptable carrier.

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173. The method of claim 30, wherein the one or more membrane anchored immunomodulatory fusion proteins comprise IL-2.

174. The method of claim 30, wherein the one or more membrane anchored immunomodulatory fusion proteins comprise IL-15.

175. The method of claim 30, wherein the one or more membrane anchored immunomodulatory fusion proteins comprise IL-18.

176. The method of claim 30, wherein the one or more membrane anchored immunomodulatory fusion proteins comprise IL-21.

177. The method of claim 30, wherein the modified TILs comprise a first membrane anchored immunomodulatory fusion protein and a second membrane anchored immunomodulatory fusion protein.

178. The method of claim 177, wherein the first membrane anchored immunomodulatory fusion protein comprises IL-15 and the second membrane anchored immunomodulatory fusion protein comprises IL-21.

179. The method of claim 177 or 178, wherein the first membrane anchored immunomodulatory fusion protein and the second immunomodulatory fusion protein are expressed under the control of an NFAT promoter in the modified TILs.

180. The method of claim 30, wherein the one or more membrane anchored immunomodulatory fusion proteins are independently according to the formula, from N- to C-terminus: S-IA-L-C, wherein S is a signal peptide, IA is an immunomodulatory agent, L is a linker and C is a cell membrane anchor moiety.

181. The method of claim 180, wherein IA is a cytokine.

182. The method of claim 180, wherein IA is selected from the group consisting of: IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, or GCSF or a variant thereof.

183. The method of claim 180, wherein IA is IL-2.

184. The method of claim 180, wherein IA is IL-12.

185. The method of claim 180, wherein IA is IL-15.

186. The method of claim 180, wherein IA is IL-21.

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187. The method of claim 30, wherein the one or more membrane anchored immunomodulatory fusion proteins are independently according to the formula, from N- to C-terminus: S1-IA1-L1-C1-L2-S2-IA2-L3-C2, wherein S1 and S2 are each independently a signal peptide, IA1 and IA2 are each independently an immunomodulatory agent, L1-L3 are each independently a linker, and C1 and C2 are each independently a cell membrane anchor moiety.

188. The method of claim 187, wherein S1 and S2 are the same.

189. The method of claim 187 or 188, wherein C1 and C2 are the same.

190. The method of any of 187-189, wherein L2 is a cleavable linker.

191. The method of claim 190, wherein L2 is a furin cleavable linker.

192. The method of any of claims 187-191, wherein IA1 and IA2 are each independently a cytokine.

193. The method of any of claims 187-191, wherein IA1 and IA2 are each independently selected from the group consisting of: IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, or GCSF or a variant thereof.

194. The method of any of claims 187-191, wherein IA1 and IA2 are each independently selected from the group consisting of IL-2 and IL-12, with the proviso that one of IA1 and IA2 is IL-2 and the other is IL-12.

195. The method of any of claims 187-191, wherein IA1 and IA2 are each independently selected from the group consisting of IL-15 and IL-21, with the proviso that one of IA1 and IA2 is IL-15 and the other is IL-21.

196. The method of any of claims 1-126 or 173-195, wherein the modified TILs are genetically modified to express the immunomodulatory composition on the cell surface.

197. The method of claim 196, wherein the immunomodulatory composition comprises one or more membrane anchored immunomodulatory fusion proteins each comprising one or more immunomodulatory agents and a cell membrane anchor moiety.

198. The method of claim 197, wherein the one or more membrane anchored immunomodulatory fusion proteins comprise IL-2.

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199. The method of claim 197, wherein the one or more membrane anchored immunomodulatory fusion proteins comprise IL-15.

200. The method of claim 197, wherein the one or more membrane anchored immunomodulatory fusion proteins comprise IL-18.

201. The method of claim 197, wherein the one or more membrane anchored immunomodulatory fusion proteins comprise IL-21.

202. The method of claim 197, wherein the modified TILs comprise a first membrane anchored immunomodulatory fusion protein and a second membrane anchored immunomodulatory fusion protein.

203. The method of claim 202, wherein the first membrane anchored immunomodulatory fusion protein comprises IL-15 and the second membrane anchored immunomodulatory fusion protein comprises IL-21.

204. The method of claim 202 or 203, wherein the first membrane anchored immunomodulatory fusion protein and the second immunomodulatory fusion protein are expressed under the control of an NFAT promoter in the modified TILs.

205. The method of claim 197, wherein the one or more membrane anchored immunomodulatory fusion proteins are independently according to the formula, from N- to C-terminus: S-IA-L-C, wherein S is a signal peptide, IA is an immunomodulatory agent, L is a linker and C is a cell membrane anchor moiety.

206. The method of claim 205, wherein IA is a cytokine.

207. The method of claim 205, wherein IA is selected from the group consisting of: IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, or GCSF or a variant thereof.

208. The method of claim 205, wherein IA is IL-2.

209. The method of claim 205, wherein IA is IL-12.

210. The method of claim 205, wherein IA is IL-15.

211. The method of claim 205, wherein IA is IL-21.

212. The method of any of claims 205-211, wherein L is a CD8a transmembrane-intracellular domain, a B7-1 transmembrane domain, a B7-2 transmembrane domain, or a CD8a transmembrane domain.

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213. The method of any of claims 205-211, wherein L is a B7-1 transmembrane domain.

214. The method of any of claims 205-211, wherein L has the amino acid sequence of SEQ ID NO:239.

215. The method of claim 197, wherein the one or more membrane anchored immunomodulatory fusion proteins are independently according to the formula, from N- to C-terminus: S1-IA1-L1-C1-L2-S2-IA2-L3-C2, wherein S1 and S2 are each independently a signal peptide, IA1 and IA2 are each independently an immunomodulatory agent, L1-L3 are each independently a linker, and C1 and C2 are each independently a cell membrane anchor moiety.

216. The method of claim 215, wherein S1 and S2 are the same.

217. The method of claim 215 or 216, wherein C1 and C2 are the same.

218. The method of any of 215-217, wherein L2 is a cleavable linker.

219. The method of claim 218, wherein L2 is a furin cleavable linker.

220. The method of any of claims 215-219, wherein IA1 and IA2 are each independently a cytokine.

221. The method of any of claims 215-219, wherein IA1 and IA2 are each independently selected from the group consisting of: IL-2, IL-6, IL-7, IL-9, IL-12, IL-15, IL-18, IL-21, IL-23, IL-27, IFN gamma, TNFa, IFN alpha, IFN beta, GM-CSF, or GCSF or a variant thereof.

222. The method of any of claims 215-219, wherein IA1 and IA2 are each independently selected from the group consisting of IL-2 and IL-12, with the proviso that one of IA1 and IA2 is IL-2 and the other is IL-12.

223. The method of any of claims 215-219, wherein IA1 and IA2 are each independently selected from the group consisting of IL-15 and IL-21, with the proviso that one of IA1 and IA2 is IL-15 and the other is IL-21.

224. The method of any of claims 215-223, wherein C1 and C2 are each independently a CD8a transmembrane-intracellular domain, a B7-1 transmembrane domain, a B7-2 transmembrane domain, or a CD8a transmembrane domain.

225. The method of any of claims 215-223, wherein C1 and C2 are each a B7-1 transmembrane domain.

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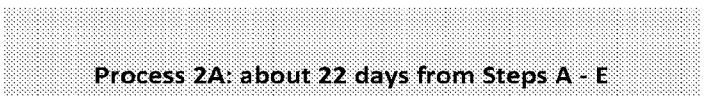
226. The method of any of claims 215-223, wherein C1 and C2 each have the amino acid sequence of SEQ ID NO:239.

227. The method of any of claims 197-226, wherein the modified TILs express the one or more membrane anchored immunomodulatory fusion proteins under the control of an NFAT promoter.

228. The method of any of claims 197-227, wherein the modified TILs are transduced with a retroviral vector to express the one or more membrane anchored immunomodulatory fusion proteins.

229. The method of any of claims 197-227, wherein the modified TILs are transduced with a lentiviral vector to express the one or more membrane anchored immunomodulatory fusion proteins.

Figure 1



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 F**

Final Formulation and/or Transfer to Infusion Bag

(optionally cryopreserve)

Figure 2A

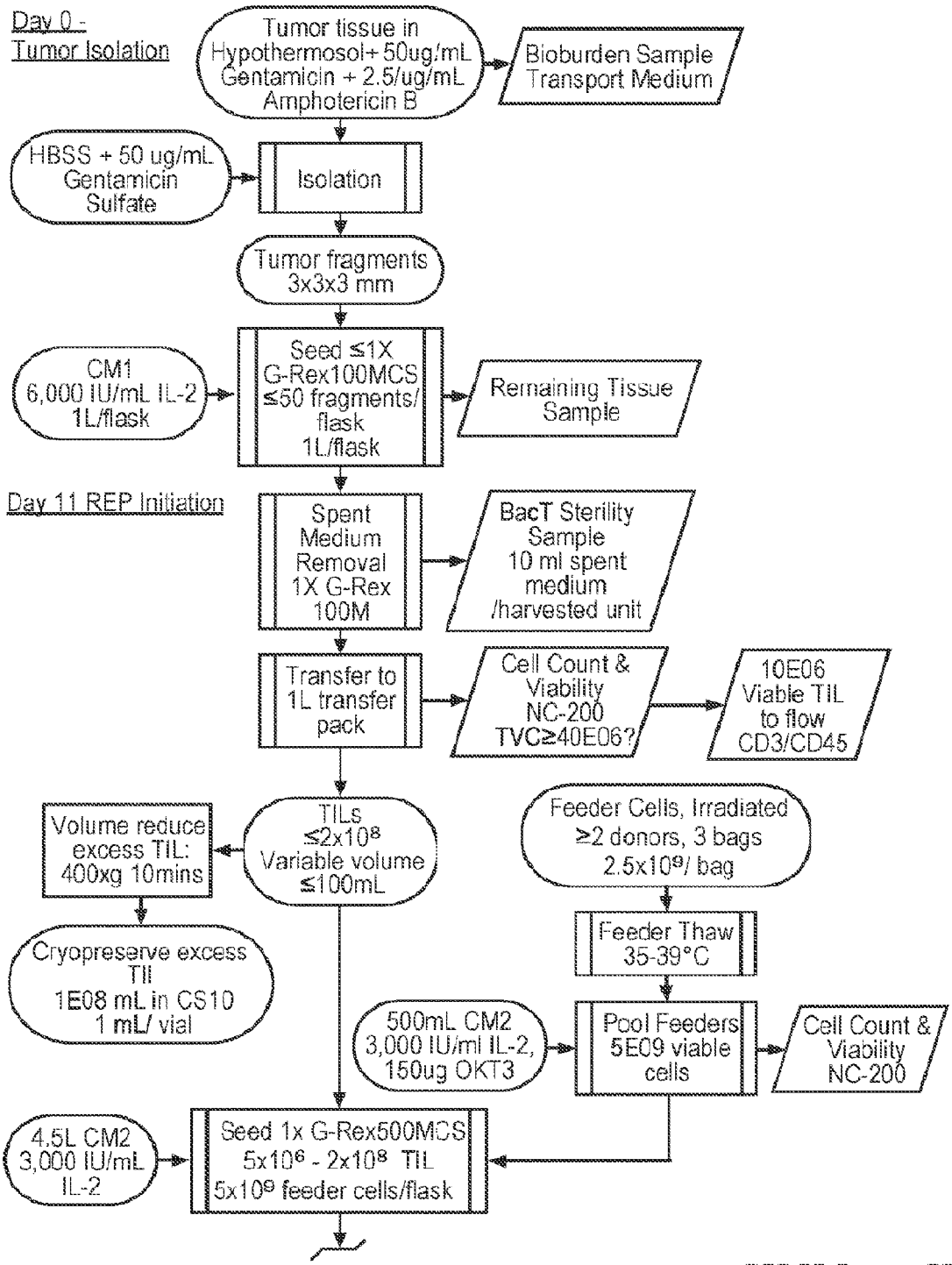


Figure 2B

REP PD Process PFD
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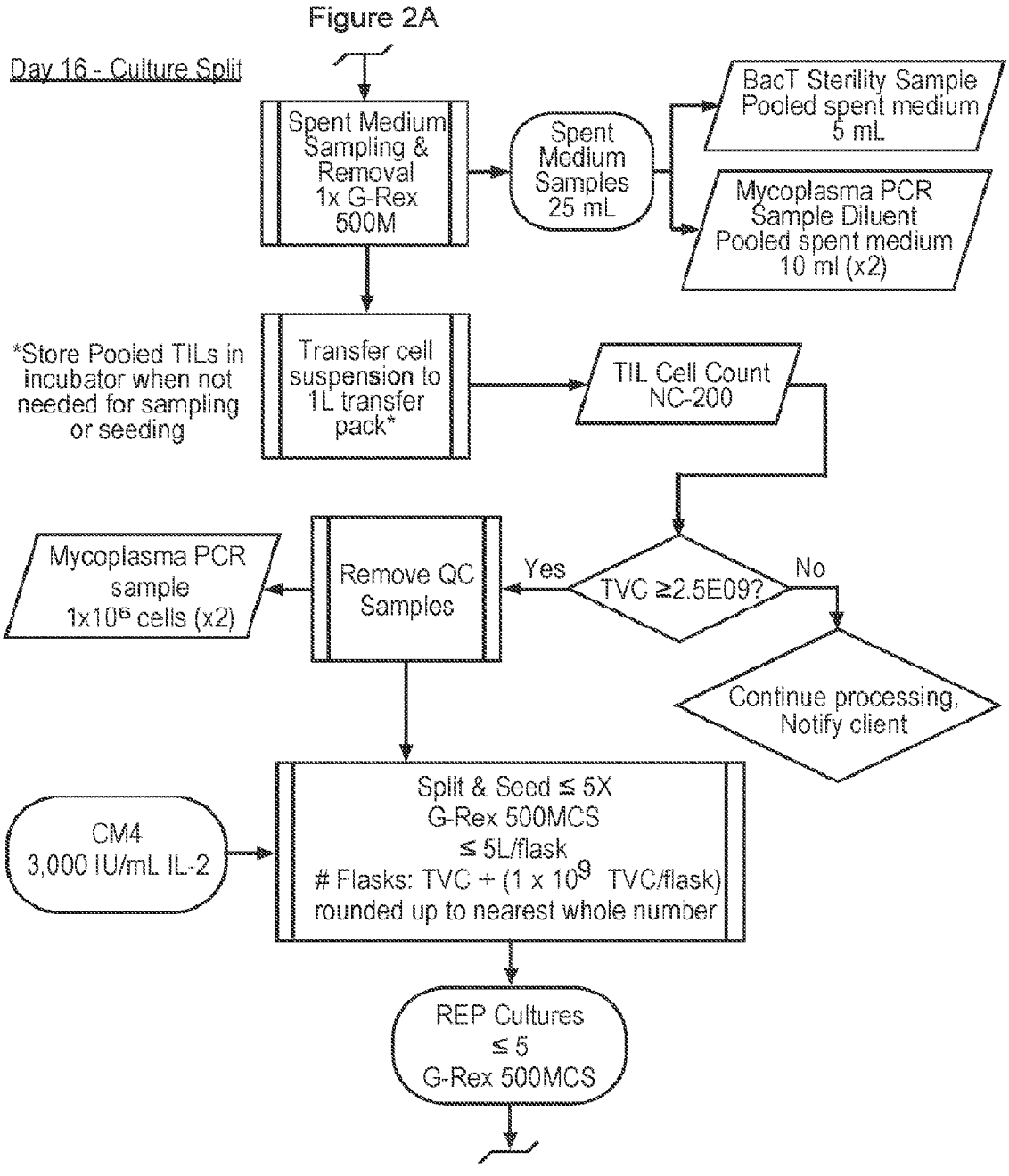
Figure 2B



REP PD Process
PFD Page 2/3

Figure 2C

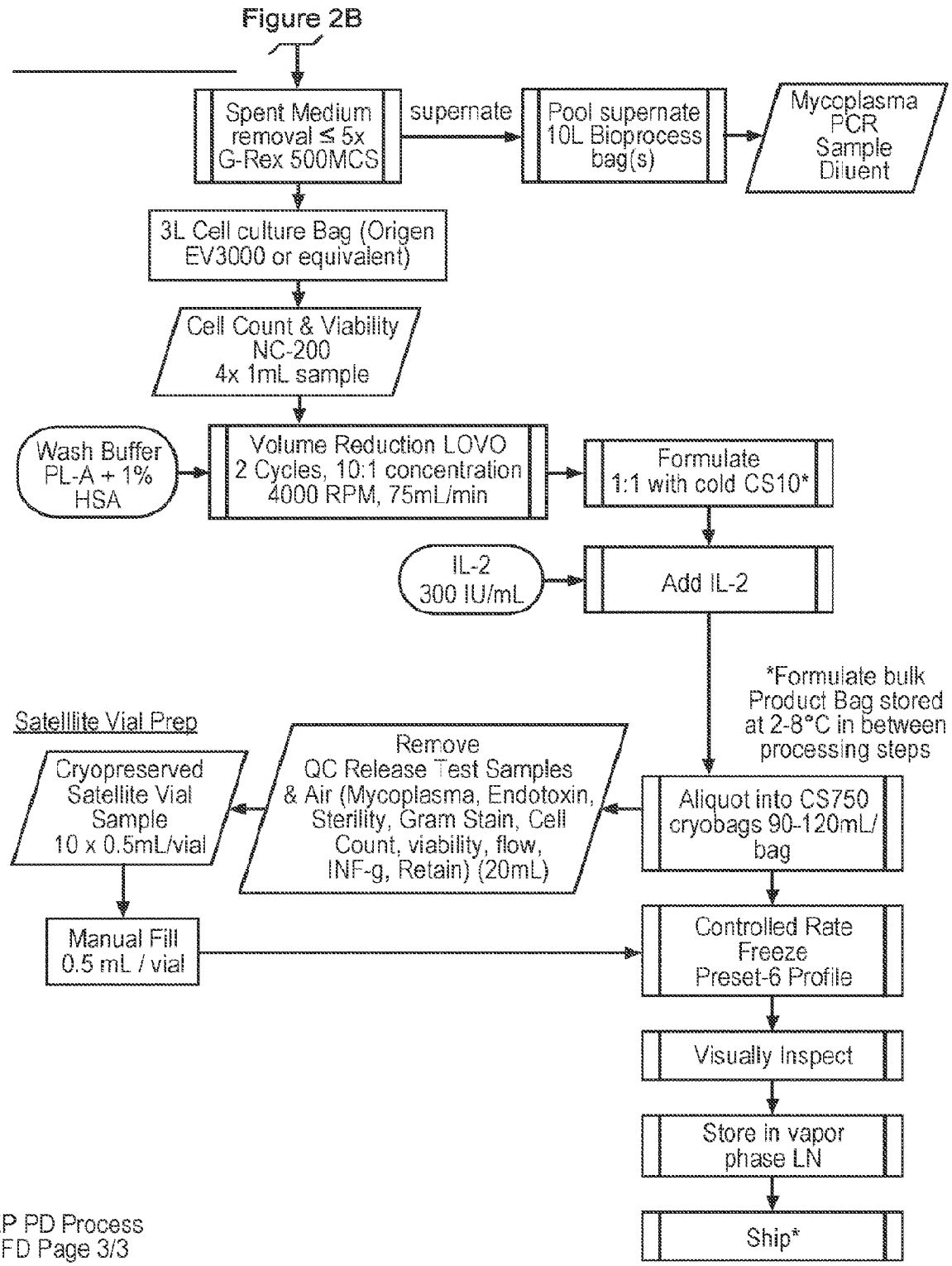


Figure 2B
Spent Medium removal ≤ 5x G-Rex 500MCS
supernate
Pool supernate 10L Bioprocess bag(s)
Mycoplasma PCR Sample Diluent
3L Cell culture Bag (Origen EV3000 or equivalent)
Cell Count & Viability NC-200 4x 1mL sample
Wash Buffer PL-A + 1% HSA
Volume Reduction LOVO 2 Cycles, 10:1 concentration 4000 RPM, 75mL/min
Formulate 1:1 with cold CS10*
IL-2 300 IU/mL
Add IL-2
*Formulate bulk Product Bag stored at 2-8°C in between processing steps
Satelllite Vial Prep
Cryopreserved Satellite Vial Sample 10 x 0.5mL/vial
Remove QC Release Test Samples & Air (Mycoplasma, Endotoxin, Sterility, Gram Stain, Cell Count, viability, flow, INF-g, Retain) (20mL)
Aliquot into CS750 cryobags 90-120mL/ bag
Manual Fill 0.5 mL / vial
Controlled Rate Freeze Preset-6 Profile
Visually Inspect
Store in vapor phase LN
Ship*
REP PD Process PFD Page 3/3

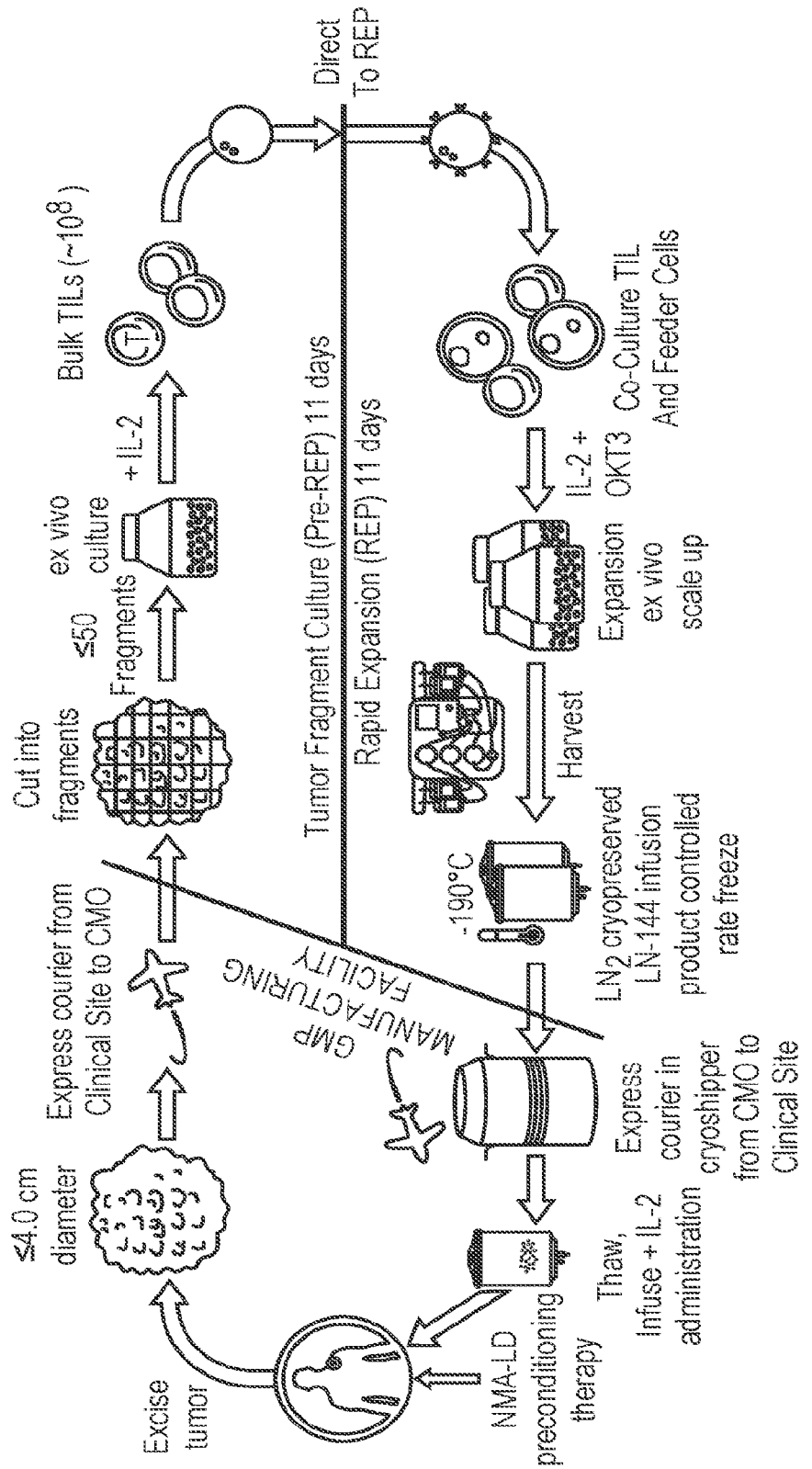

≤4.0 cm diameter
Excise tumor
Express courier from Clinical Site to CMO
Cut into fragments
≤50 Fragments
ex vivo culture
+ IL-2
Bulk TILs (~10^8)
Direct To REP
Tumor Fragment Culture (Pre-REP) 11 days
Rapid Expansion (REP) 11 days
GMP MANUFACTURING FACILITY
NMA-LD preconditioning therapy
Thaw, Infuse + IL-2 administration
Express courier in cryoshipper from CMO to Clinical Site
-190°C
LN2 cryopreserved LN-144 infusion product controlled rate freeze
Harvest
IL-2 + OKT3 Expansion ex vivo scale up
Co-Culture TIL And Feeder Cells

Figure 3

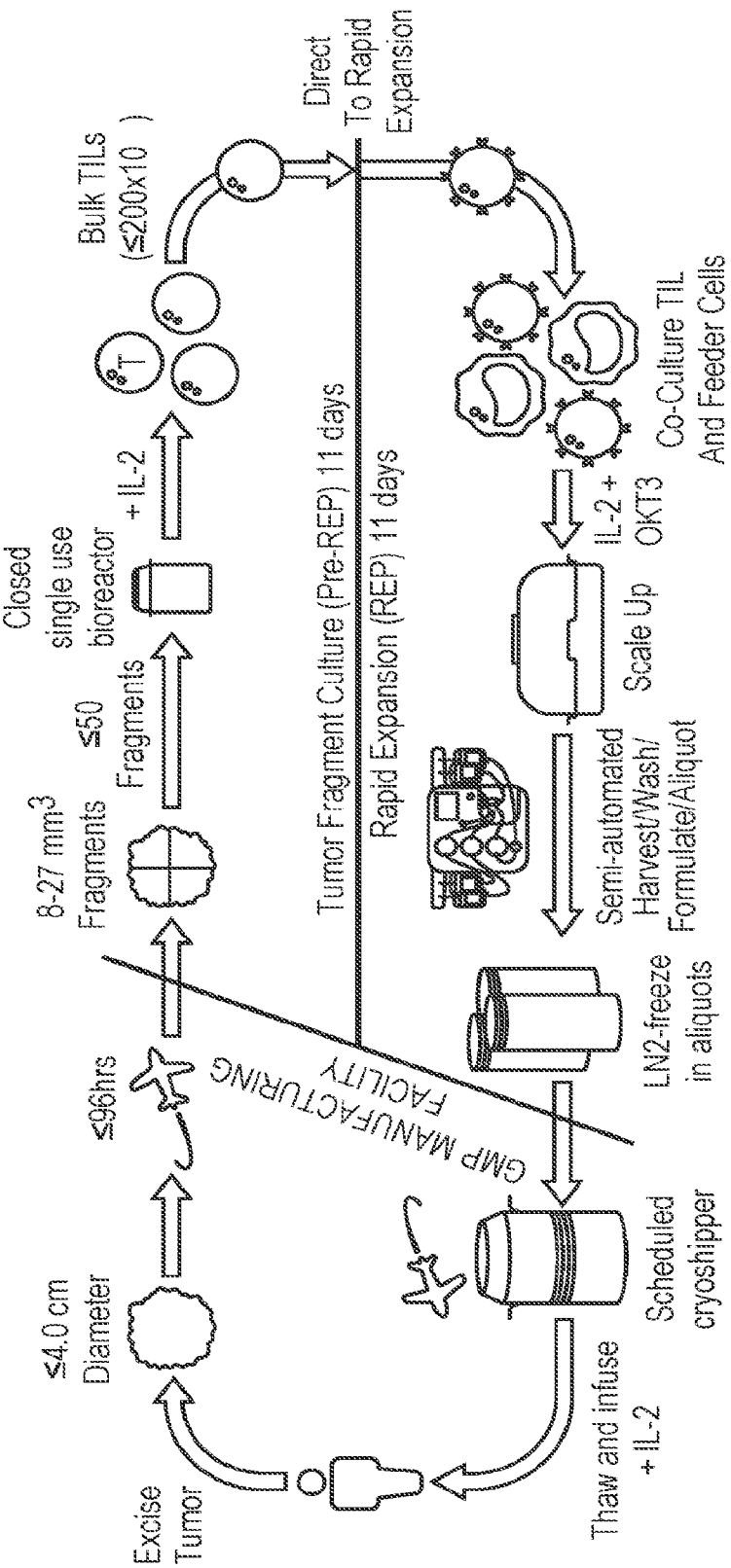

Excise Tumor
≤4.0 cm Diameter
≤96hrs
8-27 mm3 Fragments
≤50 Fragments
Closed single use bioreactor
+ IL-2
Bulk TILs (≤200x10)
Direct To Rapid Expansion
Tumor Fragment Culture (Pre-REP) 11 days
Rapid Expansion (REP) 11 days
GMP MANUFACTURING FACILITY
Thaw and infuse + IL-2
Scheduled cryoshipper
LN2-freeze in aliquots
Semi-automated Harvest/Wash/ Formulate/Aliquot
Scale Up
IL-2 + OKT3
Co-Culture TIL And Feeder Cells

Figure 4

Figure 5

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, and 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 6

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 >40×10^6 TIL	• Pre-REP TIL directly move to REP on day 11 • REP requires 25-200×10^6 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 • 5×10^6 TIL and 5×10^8 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 • 25-200×10^6 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	• Cyropreserved 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 7

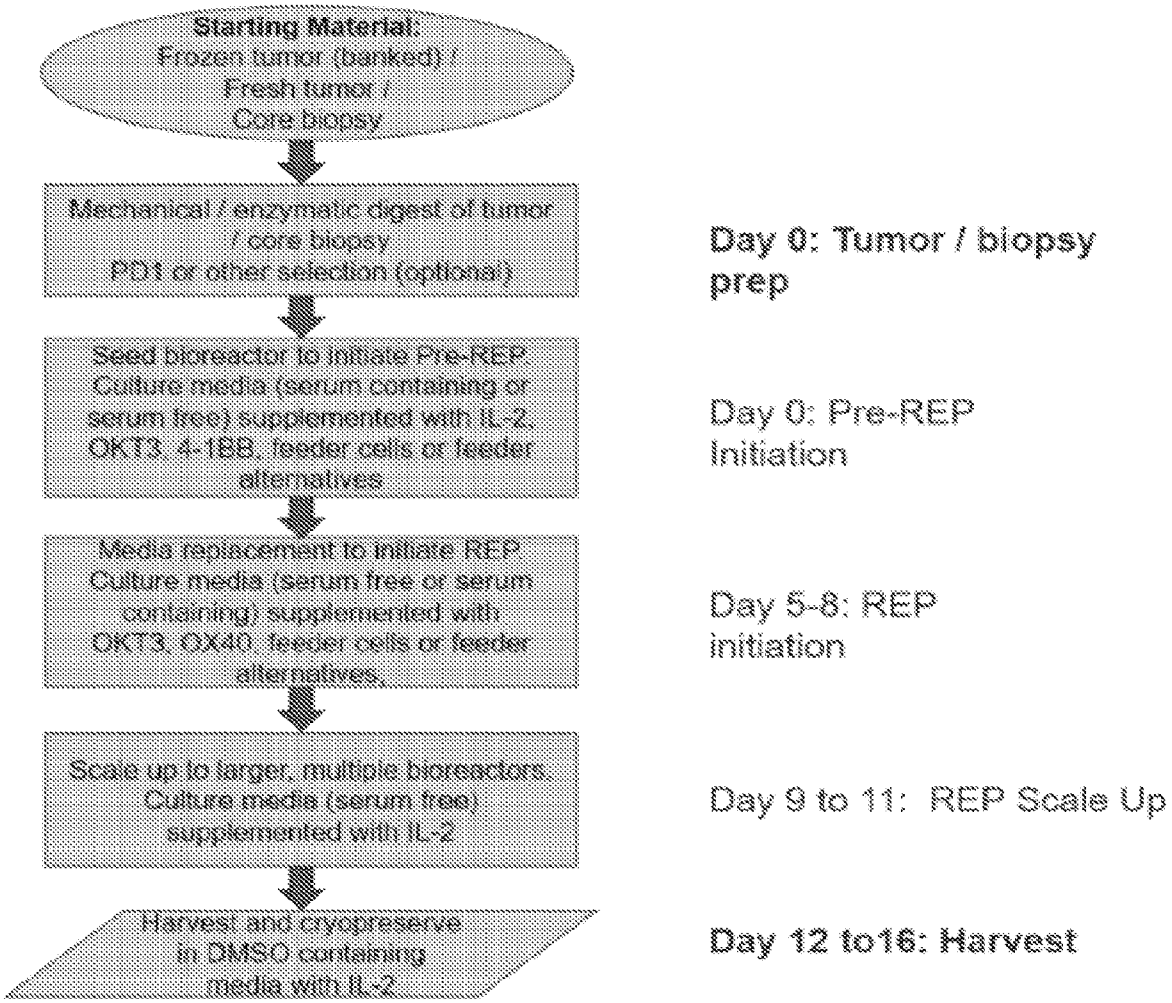

Starting Material:
Frozen tumor (banked) /
Fresh tumor /
Core biopsy
Mechanical / enzymatic digest of tumor / core biopsy
PD1 or other selection (optional)
Seed bioreactor to initiate Pre-REP. Culture media (serum containing or serum free) supplemented with IL-2, OKT3, 4-1BB, feeder cells or feeder alternatives
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 to16: Harvest

Figure 8A

Process 2A: about 22 days from Steps A - E	Process GEN 3: about 14-18 days from Steps A - E
STEP A Obtain Patient Tumor Sample (optionally can be frozen before Step B)	**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 8B

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 8C

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 8D

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 9

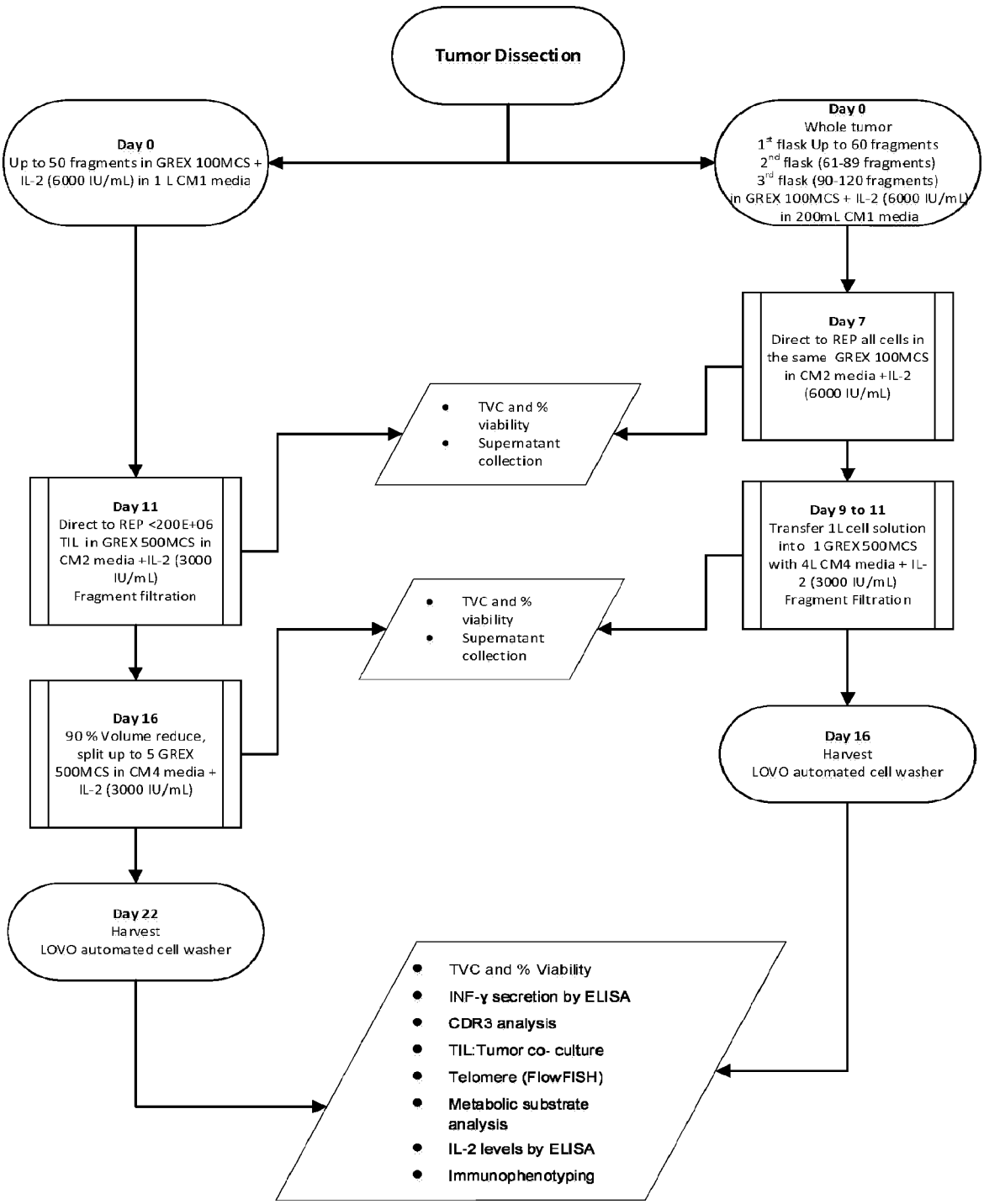

Tumor Dissection
Day 0
Up to 50 fragments in GREX 100MCS + IL-2 (6000 IU/mL) in 1 L CM1 media
Day 0
Whole tumor
1st flask Up to 60 fragments
2nd flask (61-89 fragments)
3rd flask (90-120 fragments)
in GREX 100MCS + IL-2 (6000 IU/mL) in 200mL CM1 media
Day 7
Direct to REP all cells in the same GREX 100MCS in CM2 media +IL-2 (6000 IU/mL)
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 GREX500MCS 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 10

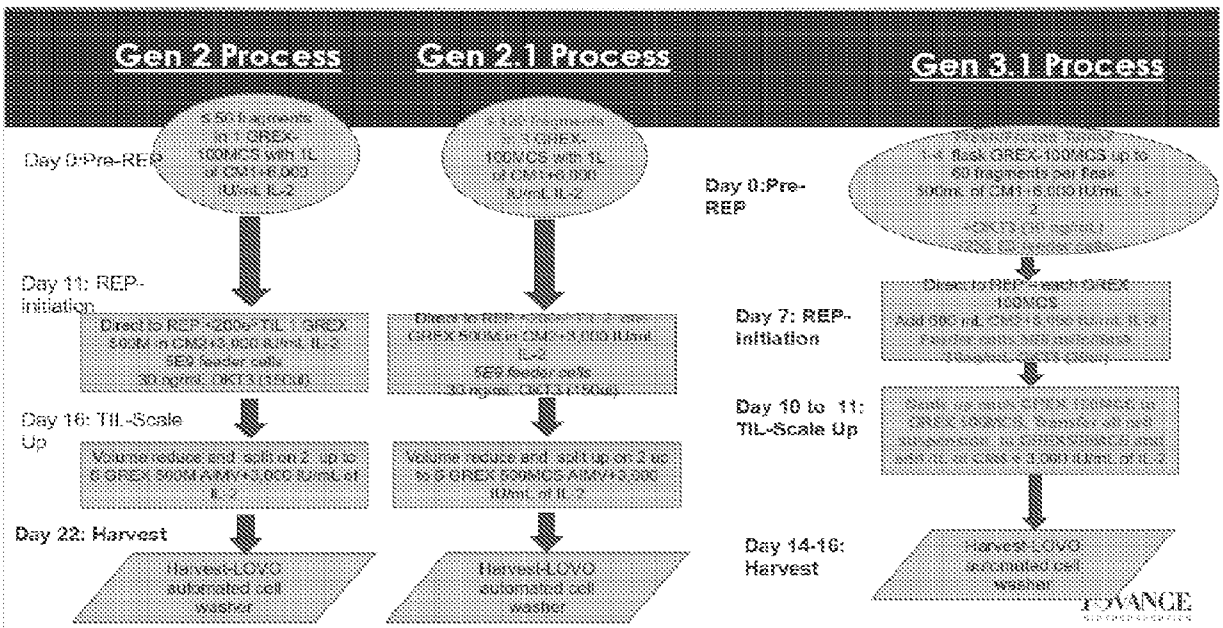

Gen 2 Process
Gen 2.1 Process
Gen 3.1 Process
Day 0:Pre-REP
Day 11: REP-Initiation
Day 16: TIL-Scale Up
Day 22: Harvest
Harvest-LOVO automated cell washer
Day 0:Pre-REP
Day 7: REP-Initiation
Day 10 to 11: TIL-Scale Up
Day 14-16: Harvest
Harvest-LOVO automated cell washer

Figure 11

STEP	Gen 2	Gen 2.1	Gen 3.0 Optimized
Pre REP- day 0	5 fragments/ 1 G-Rex 100MCS - **11 days**	5-180 fragments/ 1 G-Rex Pre-formulated CM1 warmed media 100MCS - **11 days**	*Fresh or Frozen Tumor* Whole tumor with 5-30 fragments up to 60 fragments per 1 G-Rex 100MCS (up to 4 G-Rex) pre-formulated 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 CM1 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 12

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 13

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 14

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 15

Process	Gen 2	Gen 3
L4054	Standard Media	Standard Media
L4056	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 16

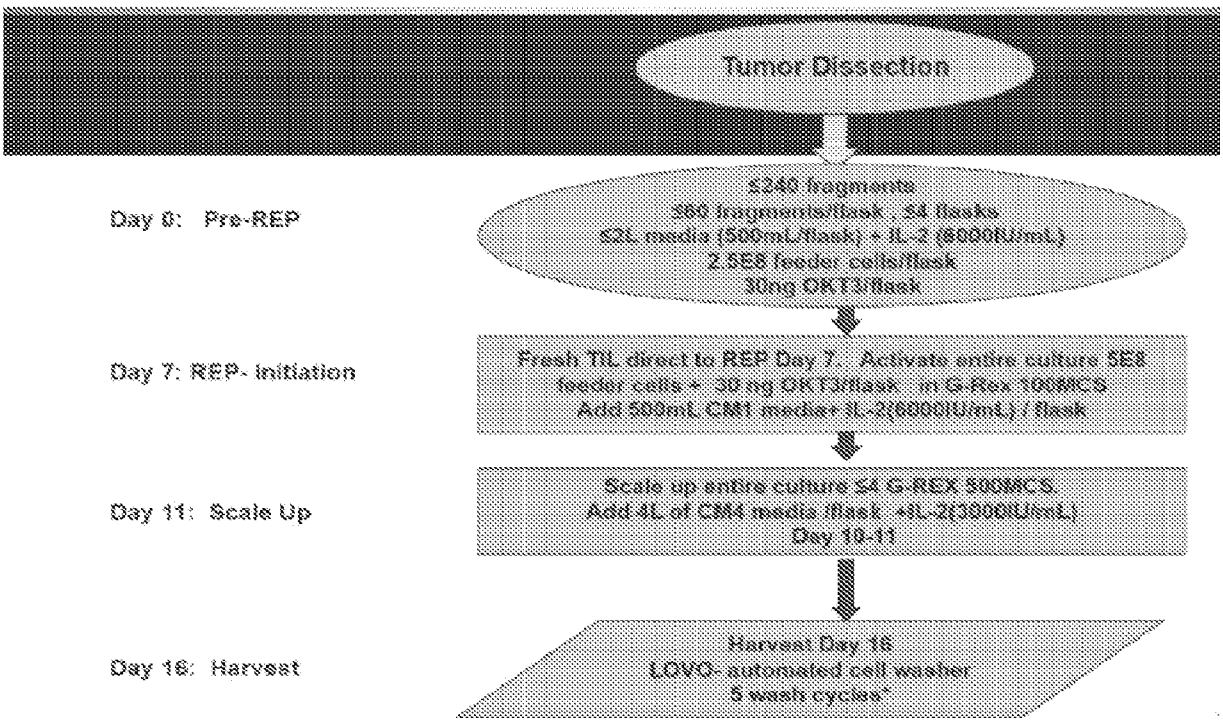

Tumor Dissection
Day 0: Pre-REP
≤240 fragments
≤60 fragments/flask ; ≤4 flasks
≤2L media (500mL/flask) + IL-2 (6000IU/mL)
2.5E8 feeder cells/flask
30ng OKT3/flask
Day 7: REP- Initiation
Fresh TIL direct to REP Day 7. Activate entire culture 5E8
feeder cells + 30 ng OKT3/flask in G-Rex 100MCS
Add 500mL 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 17

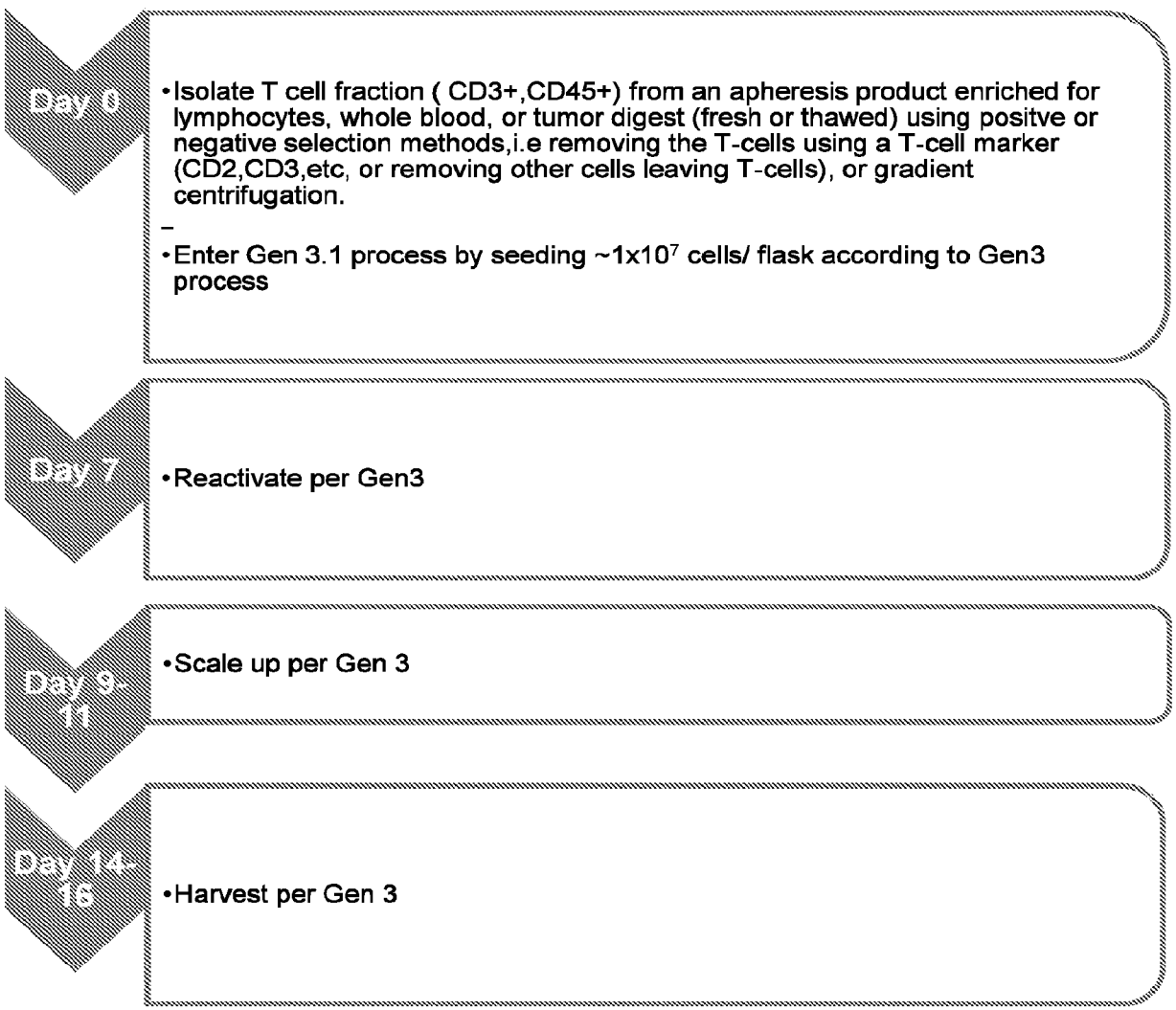

Day 0
•Isolate T cell fraction (CD3+,CD45+) from an apheresis product enriched for lymphocytes, whole blood, or tumor digest (fresh or thawed) using positve or negative selection methods,i.e removing the T-cells using a T-cell marker (CD2,CD3,etc, or removing other cells leaving T-cells), or gradient centrifugation.
–
•Enter Gen 3.1 process by seeding ~1x10^7 cells/ flask according to Gen3 process
Day 7
•Reactivate per Gen3
Day 9-11
•Scale up per Gen 3
Day 14-16
•Harvest per Gen 3

Figure 18

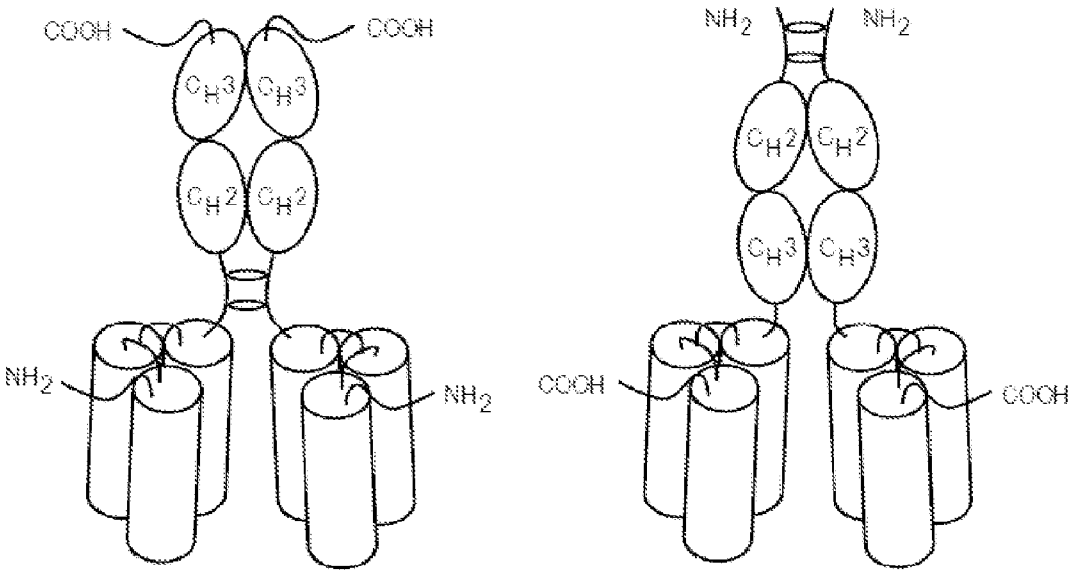

COOH
COOH
C_H3
C_H3
C_H2
C_H2
NH_2
NH_2
Structure I-A
NH_2
NH_2
C_H2
C_H2
C_H3
C_H3
COOH
COOH
Structure I-B

Figure 19

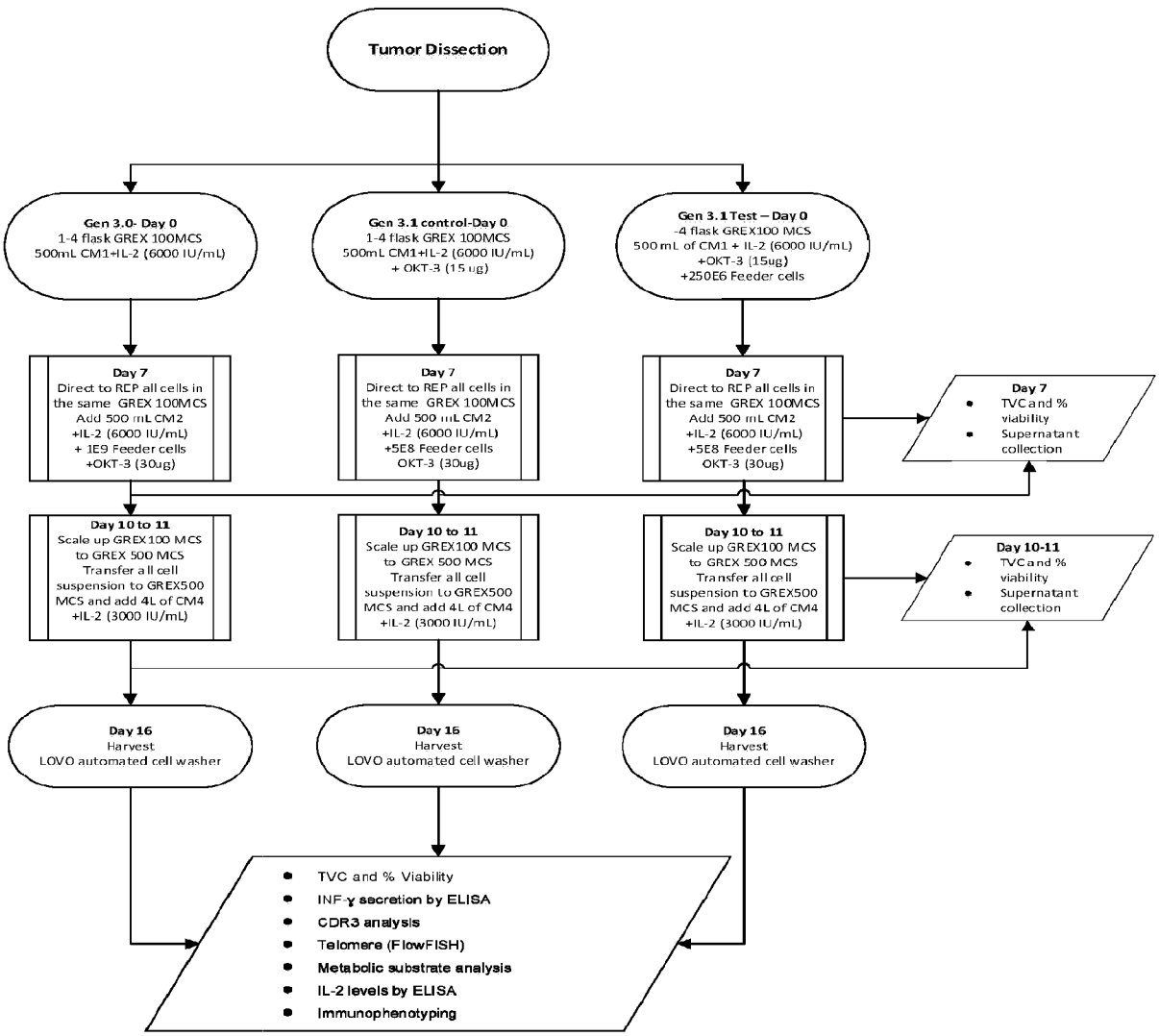

Tumor Dissection
Gen 3.0- Day 0
1-4 flask GREX 100MCS
500mL CM1+IL-2 (6000 IU/mL)
Gen 3.1 control-Day 0
1-4 flask GREX 100MCS
500mL CM1+IL-2 (6000 IU/mL)
+ OKT-3 (15 ug)
Gen 3.1 Test – Day 0
-4 flask GREX100 MCS
500 mL of CM1 + IL-2 (6000 IU/mL)
+OKT-3 (15ug)
+250E6 Feeder cells
Day 7
Direct to REP all cells in the same GREX 100MCS
Add 500 mL CM2
+IL-2 (6000 IU/mL)
+ 1E9 Feeder cells
+OKT-3 (30ug)
Day 7
Direct to REP all cells in the same GREX 100MCS
Add 500 mL CM2
+IL-2 (6000 IU/mL)
+5E8 Feeder cells
OKT-3 (30ug)
Day 7
Direct to REP all cells in the same GREX 100MCS
Add 500 mL CM2
+IL-2 (6000 IU/mL)
+5E8 Feeder cells
OKT-3 (30ug)
Day 7
TVC and % viability
Supernatant collection
Day 10 to 11
Scale up GREX100 MCS to GREX 500 MCS
Transfer all cell suspension to GREX500 MCS and add 4L of CM4
+IL-2 (3000 IU/mL)
Day 10 to 11
Scale up GREX100 MCS to GREX 500 MCS
Transfer all cell suspension to GREX500 MCS and add 4L of CM4
+IL-2 (3000 IU/mL)
Day 10 to 11
Scale up GREX100 MCS to GREX 500 MCS
Transfer all cell suspension to GREX500 MCS and add 4L of CM4
+IL-2 (3000 IU/mL)
Day 10-11
TVC and % viability
Supernatant collection
Day 16
Harvest
LOVO automated cell washer
Day 16
Harvest
LOVO automated cell washer
Day 16
Harvest
LOVO automated cell washer
TVC and % Viability
INF-γ secretion by ELISA
CDR3 analysis
Telomere (FlowFISH)
Metabolic substrate analysis
IL-2 levels by ELISA
Immunophenotyping

Figure 20

Process Day	Conditions	Gen 3.1 Test
Day 0- pre REP initiation	Media CM1	500 mL
	IL-2 (6000 IU/mL)	+
	OKT-3 (15 ug)	+
	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 (30 ug) 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 21

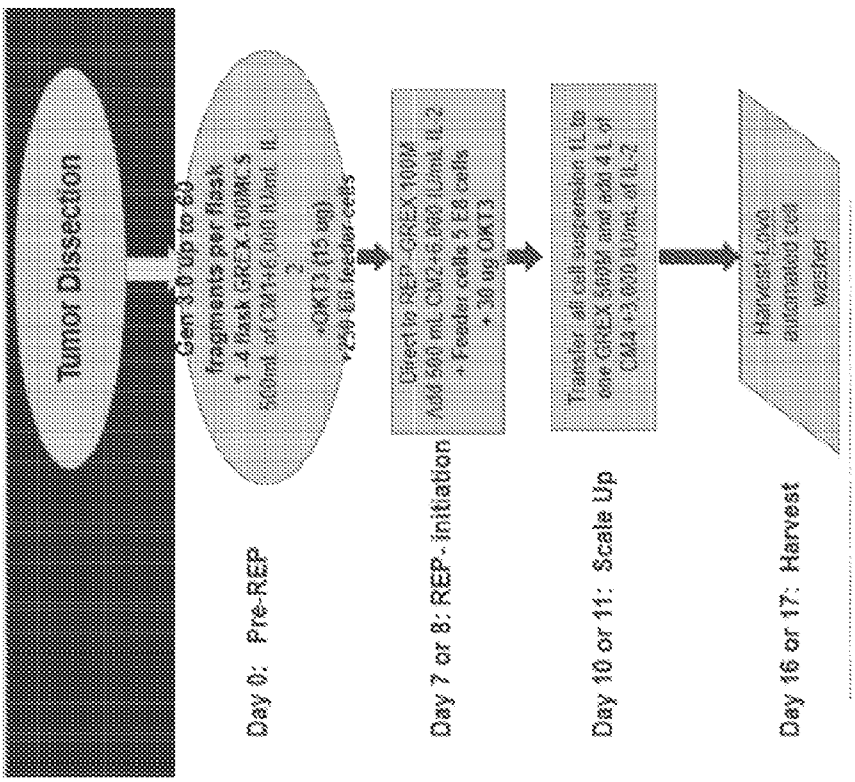

Tumor Dissection
Day 0: Pre-REP
Day 7 or 8: REP- initiation
Day 10 or 11: Scale Up
Day 16 or 17: Harvest

Figure 22

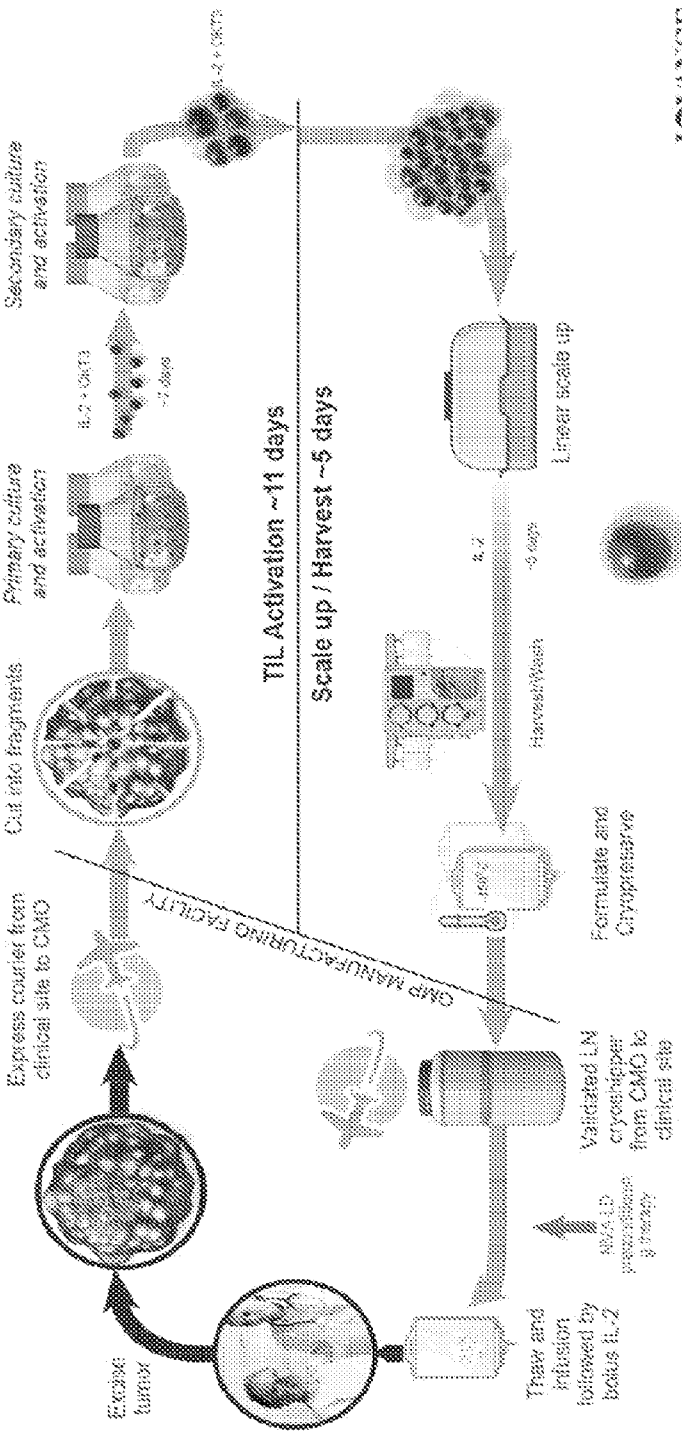

Excise tumor
Express courier from clinical site to CMO
Cut into fragments
Primary culture and activation
Secondary culture and activation
GMP MANUFACTURING FACILITY
TIL Activation ~11 days
Scale up / Harvest ~5 days
Thaw and infusion followed by bolus IL-2
Validated LN cryoshipper from CMO to clinical site
Formulate and Cryopreserve
Linear scale up

Figure 23A

	Gen2	Gen3
Total Culture Time (Vial thaw to Harvest)	22d	16-17d
Expansion Flask	500 fragments in 1 flask	500 fragments/flask in up to 4 flasks
Media volume	1L - Single addition	1L - 2 x 500mL additions
Target bioREF cell numbers	2.00e10 TIL	All cells carried through continuous process
Sampling	No screen	No screen
Selection of flasks	No selection	Bac-T sterility, visual inspection for contaminants
REP Scale up		
Feeder		Reduced by 240%
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
IL2	High dose	High dose
Number of flasks	1-5	1-4
Harvest Volume / solution	Closed 10:1	Closed 10:1
Concentrate/Wash	LOVO 10:1	LOVO 10:1
Formulation	1:1 HSAB:5% DMSO	1:1 CS10:5% DMSO
Shipment	Vapor phase LN	Vapor phase LN
Infusion	Thawed IV gravity	Thawed IV gravity

Figure 23B

	Gen2	Gen3
Total Culture Time	22d	16d
Fragment Culture		
Fragments/flask	560 fragments in 1 flask	560 fragments/flask in up to 4 flasks
Media Volume	1L - Single addition	1L – 2 x 500mL additions
Target mature cell numbers	≥20e6 TIL	All cells carried through continuous process
Screening	No screen	No screen
Selection of flasks	No selection	Bac-T sterility, visual inspection for contaminants
REP Scale up		
Factors		Reduced by 240%
Media	Contains HSAB	Defined
Scale up	Rocked culture Volume reduce to 400mL on Day 5 split up to 5 flasks (2000 cm2)	Flasks sealed linearly and treated as subcomponents
OKT3	150ng	≤180ng at max scale
IL2	High dose	High dose
Number of flasks	1-5	1-4
Harvest/Volume reduction	Closed 101	Closed 101
Concentration/Wash	LOVO 100:1	LOVO 100:1
Formulation	1:1 CS10 (6% DMSO)	1:1 CS10 (6% DMSO)
Shipment	Vapor phase LN	Vapor phase LN
Infusion	Thawed IV gravity	Thawed IV gravity

Figure 24

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
Surgery
Ship
Infuse
0
7
11
17
21
24
Culture Initiation
In-Process Testing
(Visual inspection, Sterility, Myco, Phenotype, IFNg)
Harvest
Sterility, TVC, Viability, Mycoplasma, Phenotype, Gram Stain, Endotoxin, IFN-g)
TIL Activation
Scale Up

Figure 25

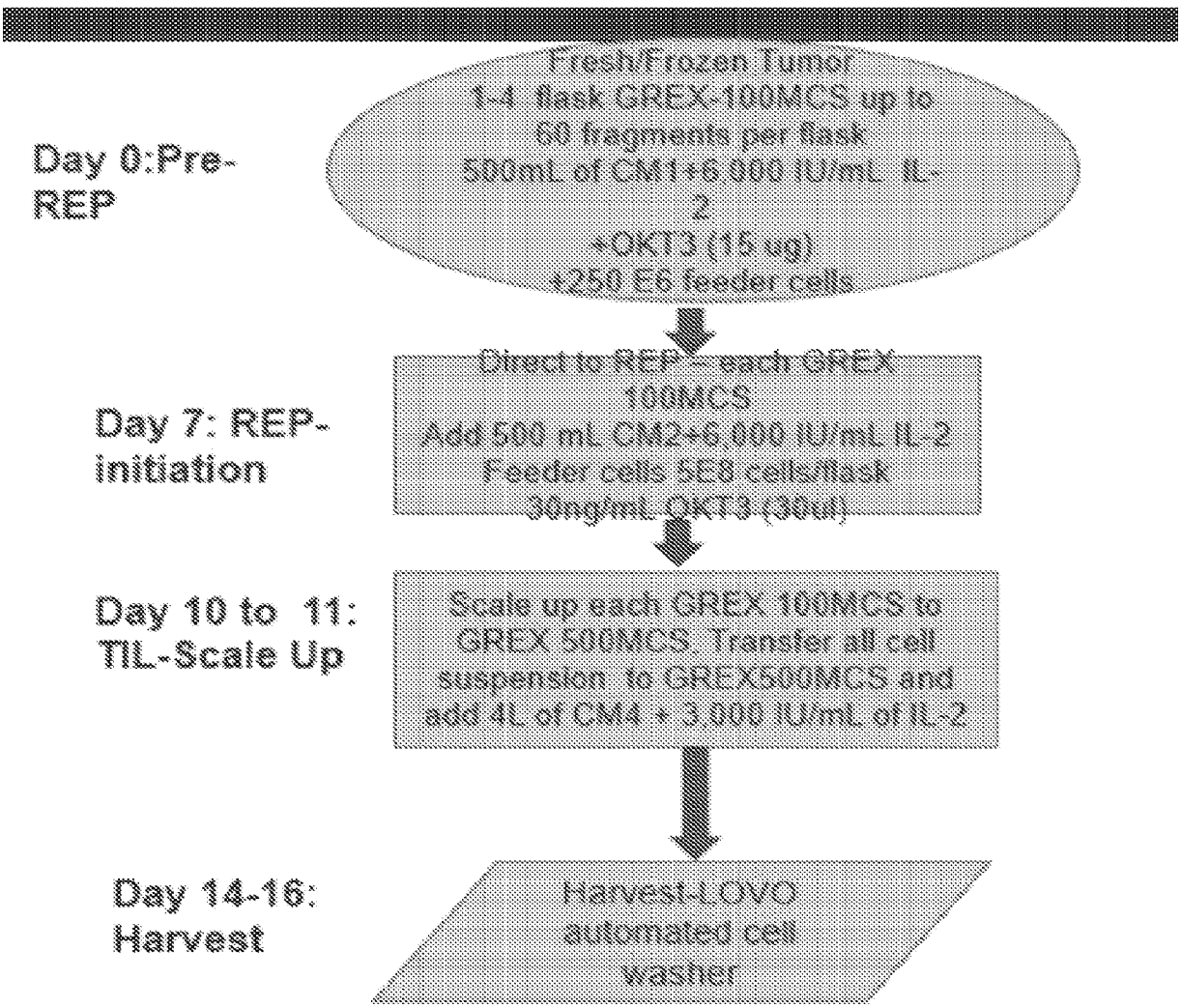

Day 0:Pre-REP
Fresh/Frozen Tumor
1-4 flask GREX-100MCS up to 60 fragments per flask
500mL of CM1+6 000 IU/mL IL-2
+OKT3 (15 ng)
+250 E6 feeder cells
Day 7: REP-initiation
Direct to REP – each GREX 100MCS
Add 500 mL CM2+6,000 IU/mL IL-2
Feeder cells 5E8 cells/flask
30ng/mL OKT3 (30ul)
Day 10 to 11: TIL-Scale 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 26A

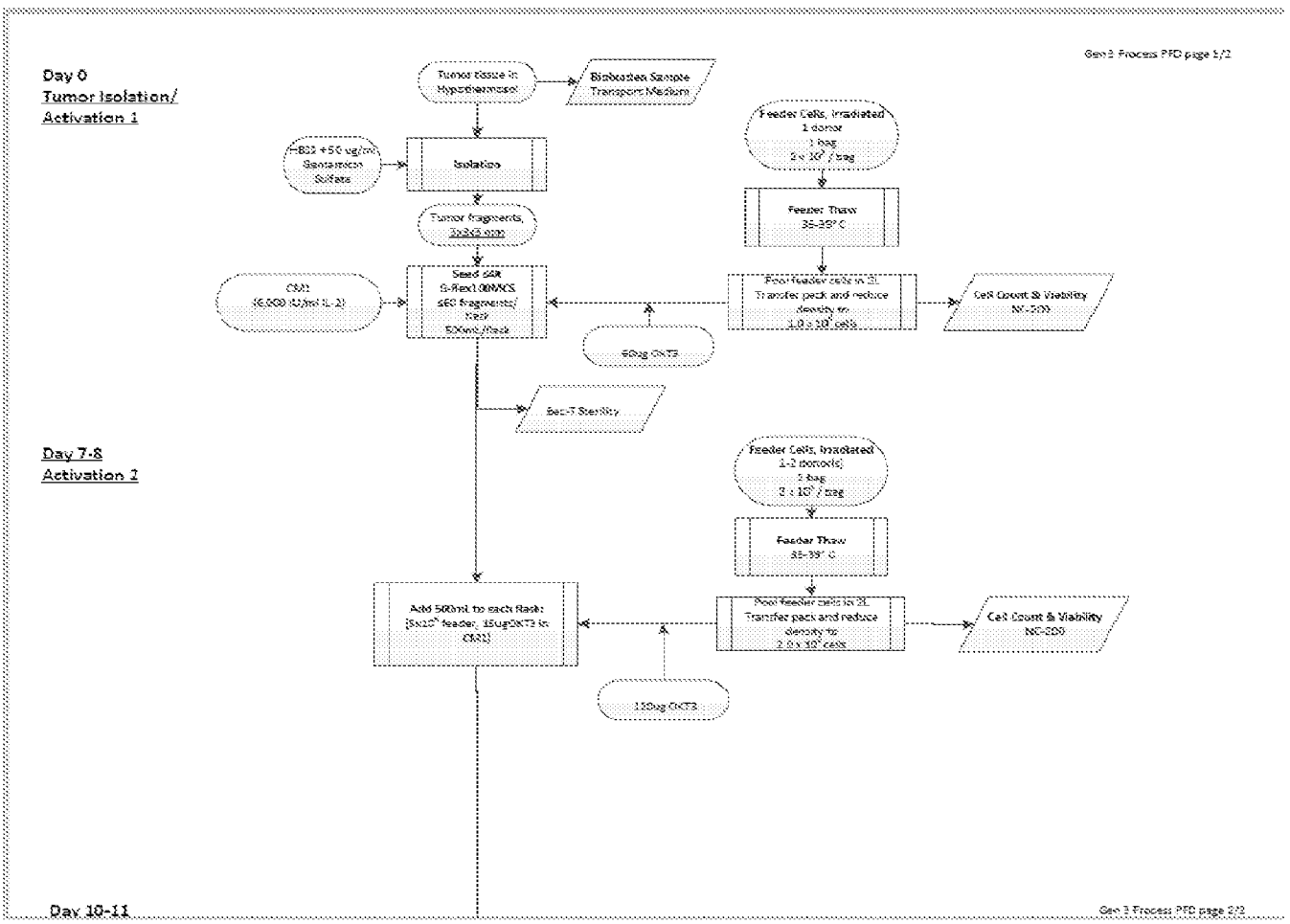

Gen 3 Process PFD page 1/2
Day 0
Tumor Isolation/
Activation 1
Tumor tissue in Hypothermosol
Biobanken Sample Transport Medium
Feeder Cells, Irradiated
1 donor
1 bag
2 x 10^8 / bag
RPMI +50 ug/ml Gentamicin Sulfate
Isolation
Feeder Thaw
35-39° C
Tumor fragments, 2x2x2 mm
CM1
(6,000 IU/ml IL-2)
Seed GRP
G-Rex100MCS
≤60 fragments/
Rack
500ml /Rack
Pool Feeder cells in 2L
Transfer pack and reduce density to:
1.0 x 10^6 cells
Cell Count & Viability
NC-200
45kg OKT3
Day 1 Sterility
Day 7-8
Activation 2
Feeder Cells, Irradiated
1-2 donor(s)
2 bag
2 x 10^8 / bag
Feeder Thaw
35-39° C
Add 500ml to each Rack:
[5x10^8 feeder, 15ug OKT3 in CM1]
Pool Feeder cells in 2L
Transfer pack and reduce density to:
2.0 x 10^6 cells
Cell Count & Viability
NC-200
150ug OKT3
Day 10-11
Gen 3 Process PFD page 2/2

Figure 26B

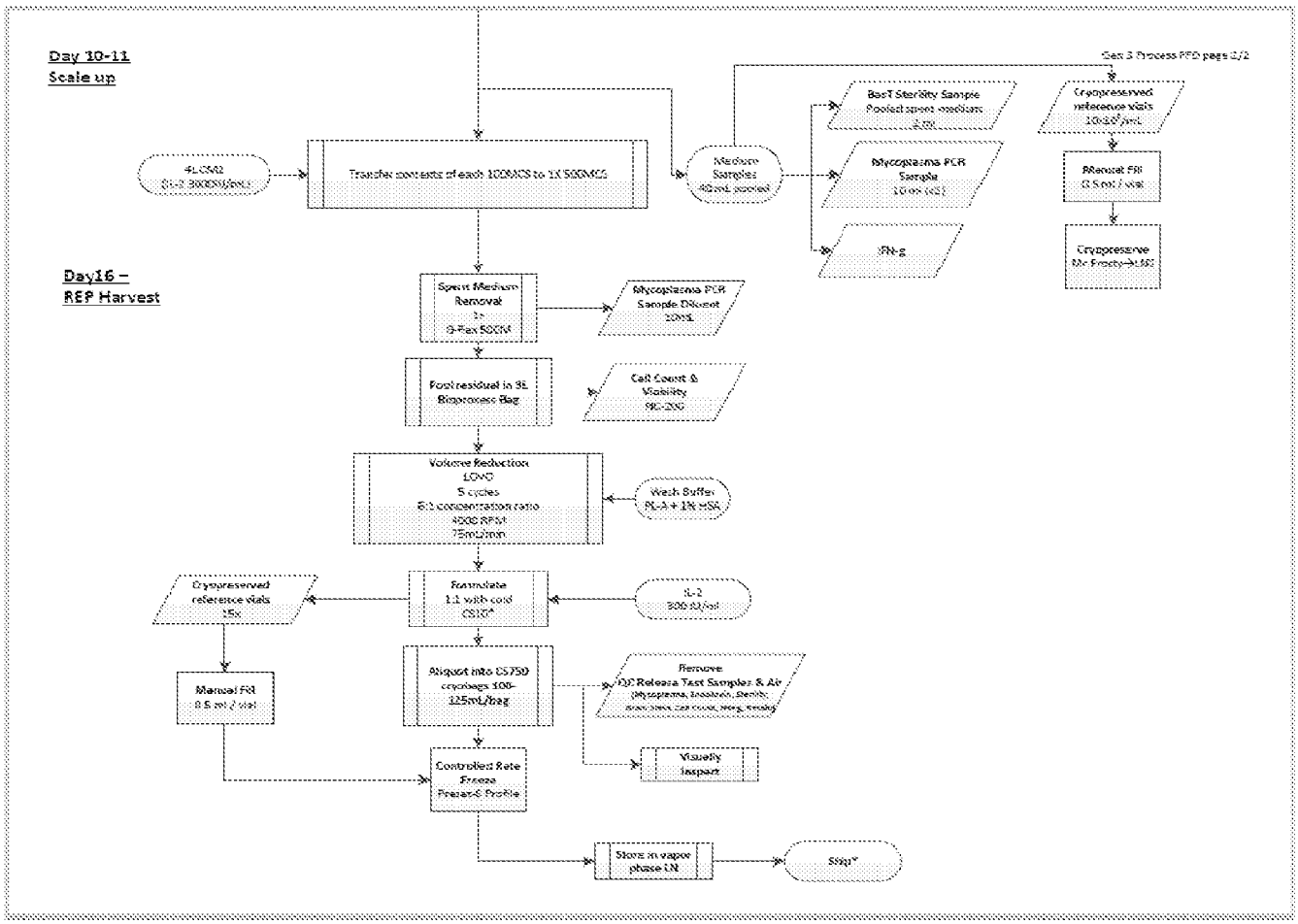

Day 10-11
Scale up
Day16 –
REP Harvest
Transfer contents of each 100MCS to 1X 500MCS
Medium Samples
Mycoplasma PCR Sample
IFN-g
Spent Medium Removal
Post residual in 3L Bioprocess Bag
Cell Count & Viability
Volume Reduction
LOVO
5 cycles
Wash Buffer
Formulate
1:1 with cold CS10
IL-2
Aliquot into CS750 cryobags 100-125mL/bag
Manual FR
Controlled Rate Freeze
Visually Inspect
Store in vapor phase LN

Figure 27

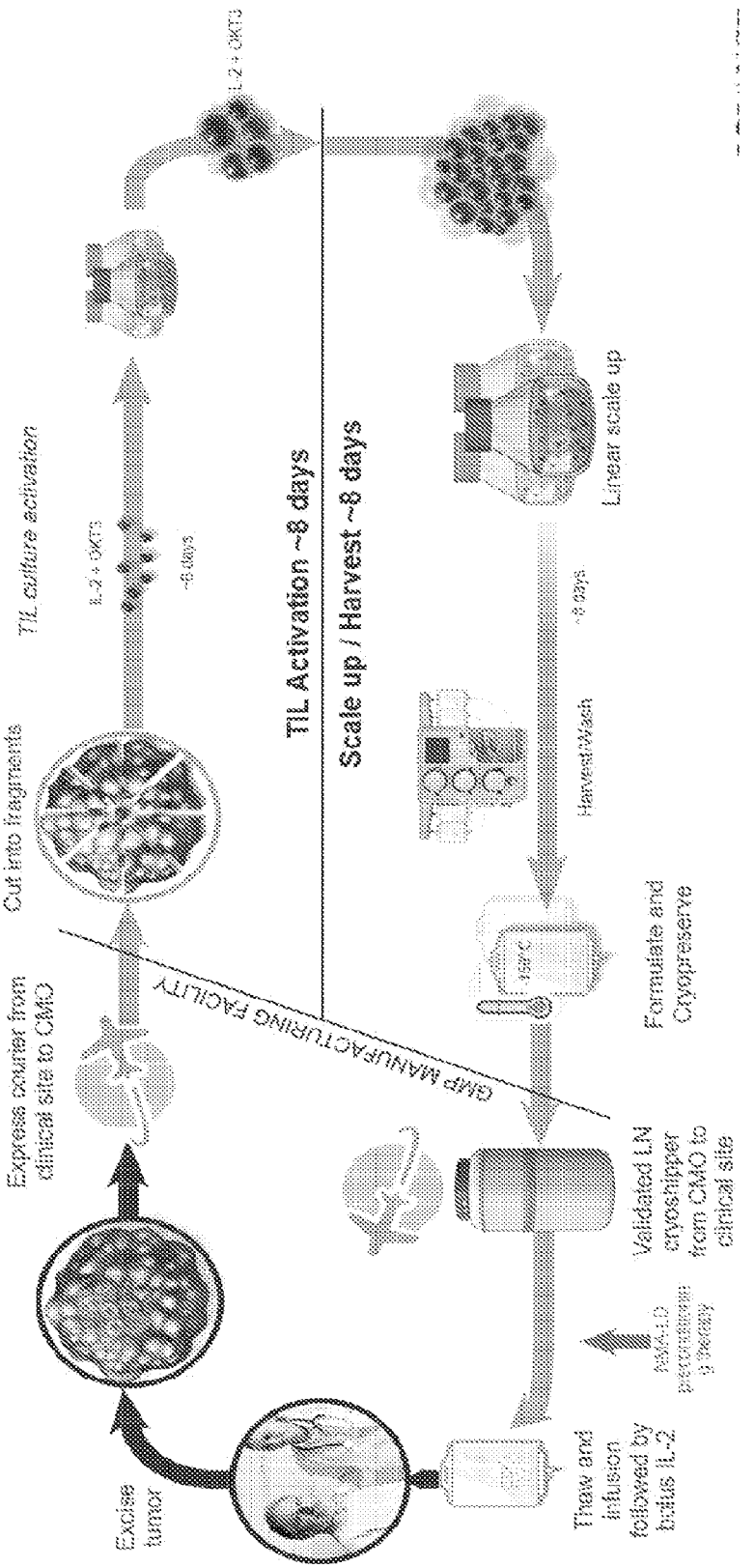

Excise tumor
Express courier from clinical site to CMO
GMP MANUFACTURING FACILITY
Cut into fragments
TIL culture activation
IL-2 + OKT3
~8 days
TIL Activation ~8 days
Scale up / Harvest ~8 days
Harvest/Wash
~8 days
Linear scale up
Formulate and Cryopreserve
Validated LN cryoshipper from CMO to clinical site
Thaw and infusion followed by bolus IL-2

Figure 28

STEP	Gen 2	Gen 2.1	Gen 3.0
Pre REP- day 0	≤ 50 fragments/ 1 G-Rex 100MCS - **11 days**	≤ 180 fragments/ 3 G-Rex Pre-formulated CM1 warmed media 100MCS - **11 days**	*Fresh or Frozen Tumor* Whole tumor with ≤ 30 fragments up to 60 fragments per 1 G-Rex 100MCS (up to 4 G-Rex), preformulated warmed media - **7 days. Pre REP Feeders 250e6 cells + OKT-3 (15ug)**
REP Initiation	Direct to REP- Day 11- **<200e^6 TIL** 1 G-Rex 500MCS	Direct to REP- Day 11- **<200 e^6 TIL** Pre-formulated CM2 warmed media in one G-Rex 500MCS	Direct to REP- Day 7-**all cells TIL- same G-Rex 100MCS (100MCS up to 4 GREX), Standard media or Defined Media (Serum free). Addition Feeders 500e6 cells +OKT-3 (30ug)**
TIL propagation or Scale up	1 to 5 G-REX 500MCS **Split day 16**	2 to 5 G-REX 500MCS Pre-formulated CM4 warmed media **Split day 16**	From G-REX 100MCS transfer TIL suspension to G-REX 500MCS , up to 4 GREX 500 MCS- Standard media or Defined Media (Serum Free) **Scale up on day 10 or 11**
Harvest	**Harvest day 22,** LOVO-automated cell washer	**Harvest day 22,** LOVO-automated cell washer (5 wash cycle)	**Harvest day 14 or 16** LOVO- automated cell washer (5 wash cycle)
Final formulation	Cryopreserved Product 300IU/ml IL2- CS10 in LN_2 multiple aliquots	Cryopreserved Product 300IU/ml IL2- CS10 in LN_2 multiple aliquots	Cryopreserved product 300IU/ml IL-2-CS10 in LN_2 multiple aliquots
Process time	22 days	22 days	16 days

Figure 29

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.

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Figure 30

Table 2: Description of Gen 3 optimization conditions.

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 31

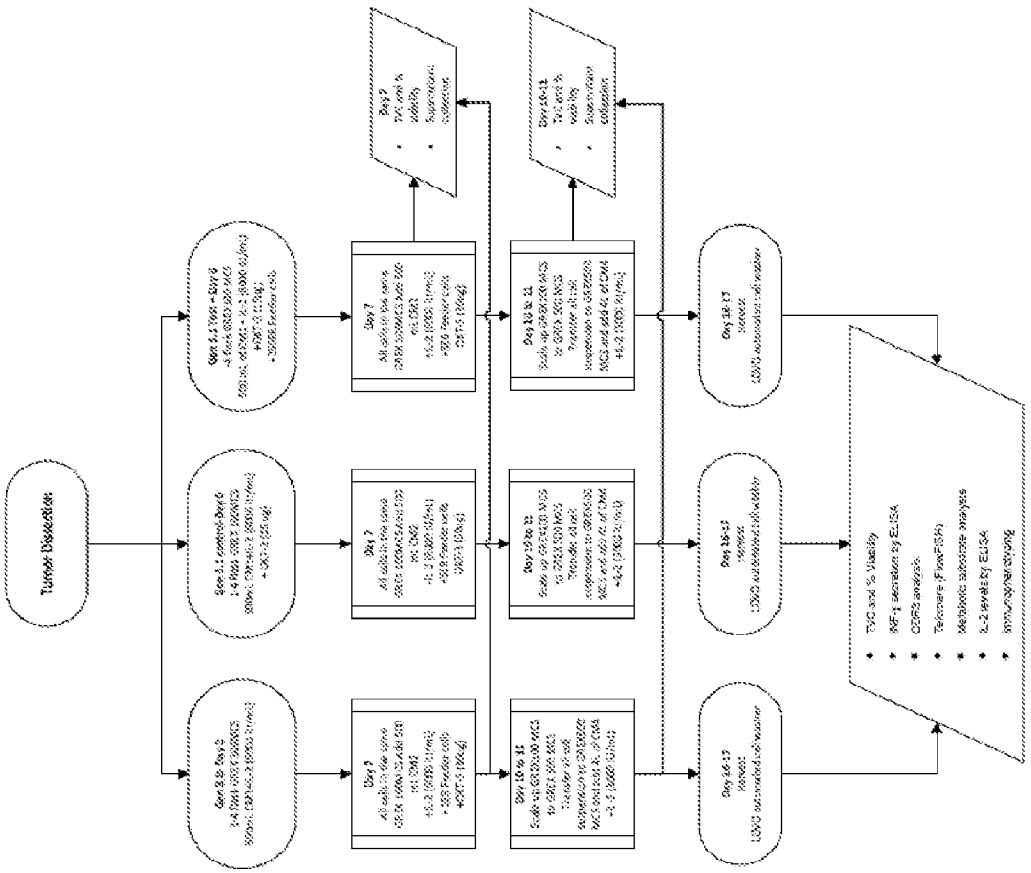

Tumor Dissection
Day 16-17
Harvest
Day 16-17
Harvest
Day 16-17
Harvest
TNC and % Viability
IFN-γ secretion by ELISA
CSF5 analysis
Telomere (FlowFISH)
Metabolic substrate analysis
IL-2 levels by ELISA
Immunophenotyping

Figure 32

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 33

Test	Acceptance Criteria	Gen 3.1Test 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 34

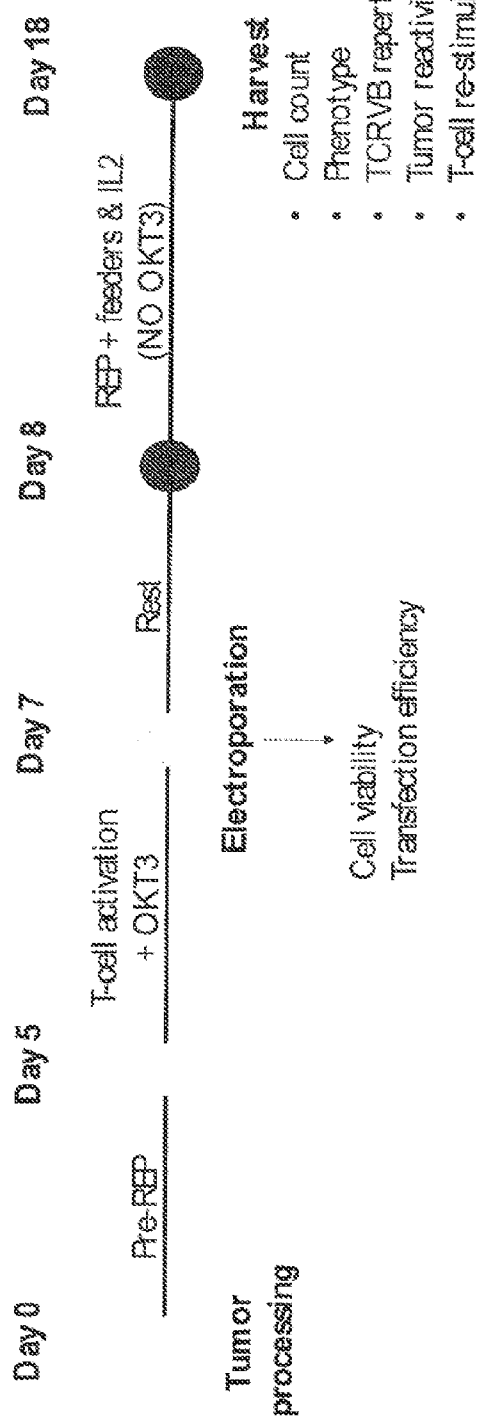

Day 0
Day 5
Day 7
Day 8
Day 18
Pre-REP
T-cell activation
+ OKT3
Rest
REP + feeders & IL2
(NO OKT3)
Tumor processing
Electroporation
Cell viability
Transfection efficiency
Harvest
Cell count
Phenotype
TCRVB repertoire
Tumor reactivity
T-cell re-stimulation

Figure 35

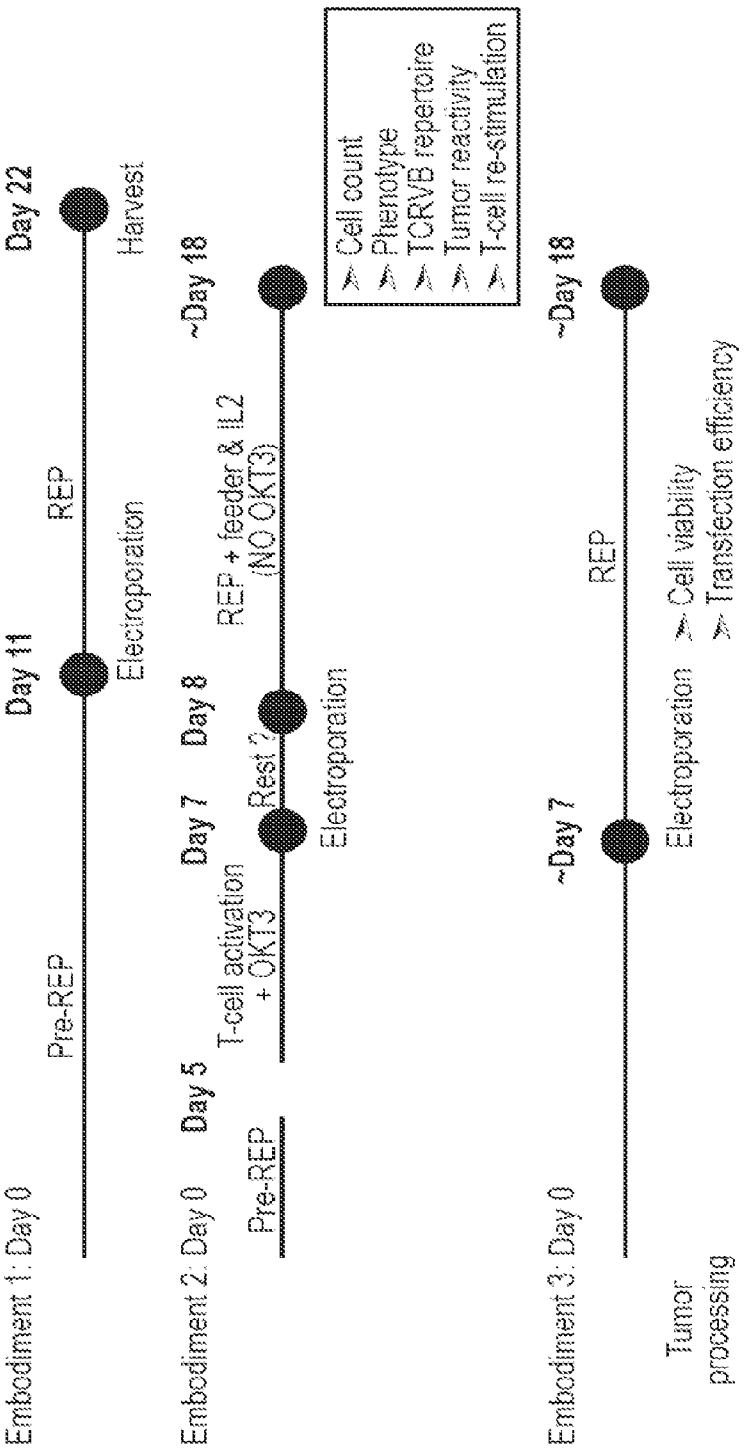

Embodiment 1: Day 0
Pre-REP
Day 11
Electroporation
REP
Day 22
Harvest
Embodiment 2: Day 0
Pre-REP
Day 5
T-cell activation + OKT3
Day 7
Rest ?
Electroporation
Day 8
REP + feeder & IL2 (NO OKT3)
~Day 18
Embodiment 3: Day 0
Tumor processing
~Day 7
Electroporation
REP
~Day 18
Cell count
Phenotype
TCRVB repertoire
Tumor reactivity
T-cell re-stimulation
Cell viability
Transfection efficiency

FIGURE 36A

Signal Sequence: MDWTWILFLVAAATRVHS (SEQ ID NO:252)
Linker (Lr1): SGGGGSGGGGSGGGGSGGGGSLQ (SEQ ID NO:246)
Anchor (Ar2): (human B7.1 transmembrane-intracellular amino acid sequence)
LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO:239)
FurinA-P2A sequence: RAKRSGSGATNFSLLKQAGDVEENPGP (SEQ ID NO:251)

Tethered IL-12-Lr1-Ar2

MDWTWILFLVAAATRVHSIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKT
LTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTT
ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYE
NYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTS
ATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGGGSRNLPVATPDPGMFPCLHHSQNLLRAVSNML
QKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYE
DLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAF
RIRAVTIDRVMSYLNASSGGGGSGGGGSGGGGSGGGGSGGGSLQLLPSWAITLISVNGIFVICCLTYCFAPRC
RERRRNERLRRESVRPV (SEQ ID NO:303)

Tethered IL-12-Lr1-Ar2

ATGGATTGGACCTGGATTCTGTTCCTCGTGGCCGCCGCTACCCGCGTGCACTCCATCTGGGAGCTGAAGAAAGAC
GTGTACGTGGTGGAATTGGATTGGTACCCCGACGCCCCAGGCGAGATGGTGGTTCTGACTTGCGACACGCCGGA
GGAGGACGGCATCACCTGGACGCTGGACCAGAGCAGCGAAGTGCTGGGCTCTGGTAAGACCTTAACCATCCAGG
TCAAGGAGTTCGGTGATGCAGGACAGTACACGTGTCACAAGGGCGGGGAGGTCTTGTCCCATAGCCTGCTGCTCC
TGCACAAGAAGGAAGACGGCATCTGGTCTACGGATATCCTTAAAGACCAGAAGGAGCCCAAGAACAAAACCTTCC
TGCGATGCGAGGCTAAGAATTACTCCGGGCGCTTTACCTGTTGGTGGCTCACAACCATCTCGACCGACCTGACCTT
CTCCGTGAAGTCTTCCAGGGGATCTTCTGATCCGCAAGGCGTTACCTGCGGGGCCGCTACCCTGTCGGCTGAGCGC
GTCCGTGGTGACAACAAGGAGTACGAGTACTCGGTGGAATGCCAGGAGGACAGCGCCTGCCCAGCGGCCGAAGA
GTCCTTGCCCATCGAGGTGATGGTGGACGCTGTACACAAGCTCAAGTACGAGAACTACACCTCCTCCTTCTTTATAC
GCGACATCATCAAGCCTGACCCCCCTAAAAACCTGCAGCTGAAGCCCCTTAAAAACTCTCGACAGGTCGAGGTATC
CTGGGAGTACCCCGACACCTGGTCTACTCCTCATTCGTATTTCAGCCTGACCTTCTGCGTGCAGGTGCAGGGCAAG
TCCAAGCGCGAGAAGAAGGACCGCGTGTTCACCGACAAAACCAGCGCGACTGTCATTTGTCGGAAGAACGCGAG
CATCTCCGTACGGGCACAGGATCGCTATTACTCCAGCAGTTGGTCAGAGTGGGCTTCGGTTCCGTGTTCTGGAGGA
GGTGGCGGCGGCTCACGCAACCTGCCCGTGGCGACGCCGGACCCGGGTATGTTTCCTTGTCTGCACCACTCTCAG
AACTTGCTCCGTGCCGTCTCGAATATGCTGCAGAAGGCCCGCCAGACTCTGGAGTTCTATCCATGCACTAGTGAGG
AGATCGATCATGAGGACATCACCAAGGACAAGACATCCACCGTGGAGGCTTGTCTGCCGCTGGAGTTGACAAAGA
ACGAGAGCTGCCTCAATAGCCGCGAGACTTCCTTCATCACCAACGGCTCCTGCCTCGCGAGCCGTAAGACCTCCTT
TATGATGGCGCTGTGCCTGTCAAGTATCTACGAAGATCTGAAAATGTATCAGGTGGAGTTCAAAACTATGAACGC
AAAACTCCTGATGGATCCCAAGCGCCAAATTTTCTTGGACCAGAACATGCTTGCCGTGATCGACGAGCTGATGCAG
GCCCTGAACTTCAACTCCGAGACCGTCCCTCAGAAGTCGTCTCTGGAGGAACCCGACTTTTACAAGACAAAGATCA
AGCTGTGCATCCTGCTACACGCGTTCCGCATTCGGGCCGTGACCATTGACCGCGTGATGTCCTACCTGAATGCCTC
GTCCGGCGGGGGGGGCTCCGGTGGGGGCGGCTCTGGCGGAGGCGGCTCAGGTGGCGGCGGCTCCGGCGGAGG
TTCGCTTCAGCTTCTGCCCAGTTGGGCAATCACTCTGATTTCCGTCAACGGAATCTTCGTCATCTGCTGCCTGACCTA
CTGCTTTGCTCCCAGATGTAGAGAGCGCCGCCGCAACGAGCGCCTGCGTCGTGAGAGCGTGAGGCCTGTG (SEQ
ID NO:304)

FIGURE 36B

Tethered IL-18-Lr1-Ar2

MDWTWILFLVAAATRVHSYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDS
QPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEGYFLACEKE
RDLFKLILKKEDELGDRSIMFTVQNEDSGGGGSGGGGSGGGGSGGGGSGGGSLQLLPSWAITLISVNGIFVIC
CLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO:305)

Tethered IL-18-Lr1-Ar2

ATGGATTGGACCTGGATTTTGTTCCTGGTGGCTGCAGCCACTCGGGTCCACAGCTACTTCGGCAAGCTG
GAGAGTAAACTTTCCGTGATCCGCAACCTCAACGACCAAGTGCTGTTCATTGACCAGGGCAACCGCCCC
CTCTTTGAAGACATGACCGACAGCGATTGTCGTGACAATGCGCCCCGCACCATCTTTATAATTTCCATGT
ACAAGGATAGCCAGCCGCGGGGAATGGCGGTAACGATCTCGGTGAAGTGTGAAAAAATCTCGACCCTG
TCATGCGAGAACAAGATCATTTCCTTCAAAGAGATGAATCCACCCGACAACATCAAGGACACCAAGTCC
GACATCATCTTCTTCCAGCGCAGCGTGCCTGGGCATGACAACAAGATGCAGTTCGAGAGCTCGTCTTAC
GAGGGCTATTTCCTGGCCTGCGAGAAGGAGCGTGACCTGTTCAAGCTTATCCTGAAGAAGGAGGACGA
GCTGGGGGACCGCAGCATCATGTTTACAGTGCAGAACGAGGATTCTGGTGGCGGCGGTAGCGGGGGC
GGGGGTTCTGGCGGCGGAGGCTCCGGTGGAGGAGGCTCCGGCGGCGGTTCGTTGCAGCTGCTGCCCA
GTTGGGCCATCACTCTAATCTCCGTCAACGGCATCTTCGTCATCTGTTGCCTGACCTACTGCTTTGCTCCG
AGGTGTAGAGAGCGACGCCGCAACGAGCGCCTCCGCCGTGAGTCCGTGCGCCCTGTT (SEQ ID
NO:306)

Tethered DR-IL-18 (6-27 variant)-Lr1-Ar2 (yellow highlight: mutated residues)

MDWTWILFLVAAATRVHSYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISKYSDS
RARGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFARVPGHGRKTQFESSSYEGYFLACEKERDL
FKLILKKEDELGDRSIMFTVQNEDSGGGGSGGGGSGGGGSGGGGSGGGSLQLLPSWAITLISVNGIFVICCLT
YCFAPRCRERRRNERLRRESVRPV (SEQ ID NO:307)

Tethered DR-IL-18 (6-27 variant)-Lr1-Ar2

ATGGATTGGACCTGGATCCTGTTCCTGGTGGCGGCGGCAACTCGCGTACATAGCTATTTCGGCAAGCTG
GAGAGCAAACTATCAGTGATCCGCAACCTCAACGACCAGGTCTTGTTTATTGACCAGGGCAACCGCCCC
CTGTTCGAAGACATGACAGACAGCGATTGTCGCGACAATGCTCCTCGCACCATCTTTATCATCTCCAAGT
ACTCCGATTCTCGAGCCAGAGGTCTCGCCGTGACCATCTCCGTGAAGTGCGAGAAGATATCCACTCTTTC
ATGCGAGAACAAGATCATTTCCTTCAAGGAGATGAATCCACCCGACAACATCAAGGACACCAAGTCCGA
CATCATCTTCTTCGCTCGTGTGCCTGGCCACGGCCGCAAGACCCAGTTCGAGAGTTCCAGCTACGAGGG
CTACTTCCTGGCCTGTGAGAAAGAGCGGGACCTTTTCAAATTGATTCTGAAGAAGGAGGACGAGCTGG
GCGACCGTAGCATCATGTTTACGGTGCAGAACGAGGATTCTGGCGGGGGCGGTTCTGGAGGGGGTGG
GTCGGGCGGTGGTGGCTCCGGGGGAGGCGGCTCCGGAGGAGGCTCGCTGCAGCTGCTGCCCAGTTGG
GCCATCACGCTCATTTCTGTCAACGGCATCTTCGTCATCTGTTGCCTGACCTACTGCTTTGCTCCGAGGTG
TAGGGAACGTCGTCGCAACGAGCGCCTGCGCCGCGAGTCTGTTCGGCCGGTG (SEQ ID NO:308)

FIGURE 36C

Tethered IL-12/IL-15

MDWTWILFLVAAATRVHSIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKT
LTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTT
ISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYE
NYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTS
ATVICRKNASISVRAQDRYYSSSWSEWASVPCS*GGGGGGS*RNLPVATPDPGMFPCLHHSQNLLRAVSNML
QKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYE
DLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAF
RIRAVTIDRVMSYLNAS*GGGGSGGGGSGGGGSGGGGSGGGGSLQLLPSWAITLISVNGIFVICCLTYCFAPRC*
RERRRNERLRRESVRPVRAKRSGSGATNFSLLKQAGDVEENPGP*MDWTWILFLVAAATRVHS*NWVNVISD
LKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESG
CKECEELEEKNIKEFLQSFVHIVQMFINTS*GGGGSGGGGSGGGGSGGGGSGGGGSLQLLPSWAITLISVNGIF*
VICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO:309)

Tethered IL-12/IL-15

ATGGATTGGACCTGGATTCTGTTTTTGGTGGCGGCCGCCACCCGCGTCCATTCCATATGGGAATTAAAGAAGGACGTGTACGT
GGTGGAGCTTGACTGGTACCCTGACGCTCCCGGCGAGATGGTGGTCCTGACCTGCGATACCCCCGAGGAGGACGGCATCACC
TGGACCCTGGACCAGAGCTCCGAGGTGCTGGGAAGCGGTAAAACCCTCACCATACAGGTGAAGGAGTTCGGTGATGCTGGCC
AGTACACCTGTCACAAAGGCGGGGAGGTACTTTCCCATTCGCTGCTCCTGCTTCACAAGAAGGAAGACGGCATTTGGTCGACC
GATATTCTGAAGGACCAGAAGGAACCAAAAAACAAGACCTTTTTGCGTTGCGAGGCCAAGAACTATTCCGGCCGCTTCACCTG
CTGGTGGCTAACGACCATCTCAACCGACCTGACCTTCTCCGTTAAGTCTAGCCGTGGTAGCTCCGACCCTCAGGGGGTAACCTG
CGGGGCTGCGACGCTGAGTGCGGAGCGGGTACGCGGCGACAACAAGGAGTACGAGTACTCCGTGGAATGTCAGGAGGATA
GTGCCTGCCCAGCGGCCGAGGAGTCCCTGCCCATCGAGGTAATGGTGGACGCGGTCCACAAGCTGAAGTACGAGAACTACAC
TAGCTCCTTCTTTATTAGAGACATCATCAAGCCCGACCCGCCTAAGAACCTGCAGCTGAAACCTCTCAAAAACTCCCGCCAGGT
TGAAGTGTCGTGGGAGTACCCTGACACCTGGTCTACACCGCACAGCTATTTCTCGCTTACATTCTGCGTGCAGGTGCAGGGCA
AATCCAAGCGCGAGAAGAAAGACCGCGTGTTCACCGACAAAACCTCTGCTACAGTGATCTGCCGAAAGAATGCTTCTATCTCC
GTGCGTGCTCAGGACCGCTACTACTCATCAAGCTGGAGTGAGTGGGCCTCCGTGCCATGTTCCGGGGGGGGCGGCGGTGGCT
CCCGCAACCTGCCCGTTGCAACGCCCGACCCGGGTATGTTCCCATGCCTGCACCACTCCCAGAATCTGTTACGTGCTGTAAGCA
ATATGCTTCAGAAGGCTCGCCAGACTCTGGAGTTCTACCCCTGCACTTCGGAGGAGATCGACCACGAAGACATCACCAAGGAC
AAGACATCCACCGTGGAGGCGTGCCTCCCTTTGGAGCTCACAAAGAACGAGTCCTGTCTAAACTCAAGGGAGACTAGCTTCAT
CACTAATGGATCGTGTCTGGCGTCGCGCAAGACCTCTTTCATGATGGCCCTGTGCCTGTCCAGCATCTATGAGGACCTTAAAAT
GTATCAGGTCGAGTTCAAGACTATGAACGCAAAGCTGCTGATGGATCCCAAGCGGCAAATTTTCCTGGACCAGAACATGCTGG
CGGTGATCGACGAGCTGATGCAGGCCCTCAACTTCAACAGCGAAACTGTTCCTCAGAAGTCCTCCCTGGAGGAGCCGGACTTT
TACAAGACCAAGATCAAGCTATGTATTTTGTTACACGCCTTCCGCATCCGCGCTGTCACTATTGACCGCGTGATGTCTTACCTGA
ACGCATCTAGTGGGGGCGGAGGTTCCGGGGGAGGCGGCTCTGGCGGTGGCGGCTCTGGCGGTGGTGGTTCAGGAGGCGGG
TCCCTTCAGCTGCTCCCGTCGTGGGCCATCACACTGATCTCTGTGAACGGCATCTTCGTCATCTGTTGTCTTACTTACTGCTTTGC
CCCCAGGTGTAGGGAGAGACGCCGCAATGAACGGCTGAGGCGTGAGAGCGTGCGCCCGGTGAGGGCTAAGCGCAGCGGCT
CCGGCGCGACCAACTTTTCGCTGCTGAAGCAGGCCGGGGACGTGGAGGAGAATCCAGGCCCGATGGATTGGACCTGGATCCT
GTTCTTGGTGGCCGCGGCTACTCGGGTGCACTCTAATTGGGTCAACGTGATCTCCGACTTGAAAAAGATCGAGGATCTGATCC
AGAGCATGCATATCGACGCCACGCTGTACACCGAGAGCGATGTGCACCCCTCTTGCAAGGTGACCGCCATGAAGTGCTTTCTG
CTGGAGCTGCAGGTCATCTCTCTTGAGTCGGGCGATGCATCTATTCATGACACCGTCGAGAACCTAATCATCCTGGCCAACAAC
TCACTGTCTTCAAACGGCAACGTGACGGAGTCTGGGTGCAAGGAATGTGAGGAATTGGAGGAGAAAAACATCAAGGAGTTCC
TGCAGTCCTTCGTCCACATCGTGCAGATGTTTATTAACACGAGTAGTGGCGGAGGTGGCAGCGGAGGCGGCGGCTCCGGCGG
TGGAGGAAGTGGAGGCGGCGGCTCTGGTGGCGGTTCCCTACAATTGCTTCCCTCTTGGGCTATCACCCTCATCAGCGTCAACG
GTATCTTCGTCATCTGTTGTCTGACCTACTGCTTTGCCCCTCGATGCCGAGAGCGCCGTCGCAACGAGCGCCTGCGCCGGGAGT
CCGTGCGCCCCGTT (SEQ ID NO:310)

FIGURE 36D

Tethered IL-18/IL-15

MDWTWILFLVAAATRVHSYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDS
QPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEGYFLACEKE
RDLFKLILKKEDELGDRSIMFTVQNEDSGGGGSGGGGSGGGGSGGGGSGGGSLQLLPSWAITLISVNGIFVIC
CLTYCFAPRCRERRRNERLRRESVRPVRAKRSGSGATNFSLLKQAGDVEENPGPMDWTWILFLVAAATRVH
SNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLS
SNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGGSGGGSLQLLPSW
AITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO:311)

Tethered IL-18/IL-15

ATGGATTGGACCTGGATCCTTTTCTTGGTGGCGGCTGCAACCCGGGTTCATAGCTATTTCGGCAAGCTG
GAGTCCAAGCTCTCCGTGATCCGCAACCTCAACGACCAGGTGCTGTTCATCGACCAGGGCAACCGCCCC
CTGTTTGAGGACATGACAGATTCTGATTGCCGCGACAACGCGCCGCGCACCATCTTCATTATCTCTATGT
ACAAGGACTCCCAGCCACGTGGTATGGCCGTCACGATCAGCGTGAAGTGCGAGAAGATTTCTACCCTGT
CCTGCGAGAACAAGATCATCTCGTTCAAGGAGATGAACCCTCCCGACAATATCAAGGACACCAAGTCCG
ACATCATCTTCTTCCAGCGATCAGTGCCAGGCCACGACAACAAGATGCAGTTCGAGAGCAGCAGCTACG
AGGGCTACTTCCTTGCGTGCGAGAAAGAGCGCGATCTGTTCAAACTGATCCTCAAGAAGGAAGACGAG
CTCGGGGACCGCAGCATCATGTTTACCGTACAGAACGAGGACAGCGGCGGCGGCGGCTCCGGTGGAG
GCGGTAGTGGCGGAGGCGGCTCAGGCGGCGGCGGCTCCGGAGGTGGGTCGTTGCAGCTCTTGCCGAG
TTGGGCTATCACTCTTATCTCCGTAAACGGCATCTTCGTGATATGCTGCCTGACGTACTGTTTTGCTCCTA
GGTGTCGCGAGCGCCGCCGGAACGAGAGACTGCGACGGGAATCCGTGCGCCCGGTCCGCGCGAAGCG
TAGTGGCTCCGGTGCTACCAACTTTTCTCTGCTGAAGCAGGCCGGCGACGTGGAGGAGAATCCCGGCCC
TATGGACTGGACATGGATCCTGTTCCTGGTGGCTGCGGCCACCCGCGTCCACTCAAATTGGGTCAATGT
GATTTCTGACCTGAAGAAAATTGAGGATCTCATCCAGTCCATGCACATTGACGCCACTCTGTACACCGAG
AGCGATGTTCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTCCTGTTGGAGCTGCAGGTCATTTCGC
TCGAGTCTGGCGACGCATCGATCCATGACACCGTGGAGAACCTGATTATCCTGGCCAACAACTCCCTTTC
TTCAAATGGCAACGTGACTGAAAGCGGGTGCAAAGAGTGTGAGGAGCTGGAAGAAAAGAACATCAAA
GAGTTCCTGCAATCTTTCGTCCACATTGTGCAGATGTTTATTAACACGTCCTCGGGTGGCGGGGGGTCG
GGCGGGGGTGGTTCCGGCGGAGGCGGGTCCGGAGGAGGGGGCTCTGGTGGGGGATCCCTACAGCTA
CTGCCCTCTTGGGCCATCACCCTTATCTCCGTCAACGGTATCTTCGTGATCTGTTGCCTGACTTACTGCTTT
GCCCCGAGATGTCGTGAGCGTAGGAGGAACGAGCGCCTGCGCCGCGAGAGCGTGCGTCCCGTG (SEQ
ID NO:312)

FIGURE 36E

Tethered anti-CD40scFV (APX005M)

MDWTWILFLVAAATRVHSQVQLVESGGGVVQPGRSLRLSCAASGFSFSSTYVCWVRQAPGKGLEWIACIYT
GDGTNYSASWAKGRFTISKDSSKNTVYLQMNSLRAEDTAVYFCARPDITYGFAINFWGPGTLVTVSSGGGGS
GGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTIKCQASQSISSRLAWYQQKPGKPPKLLIYRASTLASG
VPSRFSGSGSGTDFTLTISSLQPEDVATYYCQCTGYGISWPIGGGTKVEIKSGGGGSGGGGSGGGGSGGGGS
GGGSLQLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO:313)

Tethered anti-CD40scFV (APX005M)

ATGGATTGGACCTGGATCCTGTTCCTGGTGGCGGCCGCTACACGAGTCCACAGCCAGGTCCAGCTCGTG
GAGAGCGGAGGCGGCGTGGTGCAGCCAGGCCGCTCGCTGCGCCTGTCATGCGCAGCGTCCGGTTTCTC
CTTCTCGTCGACCTACGTGTGCTGGGTCCGCCAGGCCCCTGGCAAAGGCCTGGAGTGGATTGCTTGTAT
TTATACCGGCGACGGCACCAACTACAGCGCAAGTTGGGCCAAGGGCCGTTTCACCATCTCCAAGGATAG
TAGCAAGAACACGGTATACCTGCAGATGAACTCTCTTCGCGCCGAGGACACCGCCGTGTATTTCTGTGCC
CGGCCCGACATCACTTATGGCTTCGCGATTAACTTTTGGGGCCCTGGAACCCTGGTGACCGTGTCCTCCG
GCGGCGGTGGCTCTGGGGGGGGCGGGTCCGGCGGAGGCGGCAGCGGAGGCGGCGGCTCCGACATCC
AGATGACCCAGTCGCCCAGTTCTTTGTCCGCTTCCGTGGGGGACAGGGTCACCATAAAGTGCCAGGCTT
CTCAGAGCATCTCGTCCCGCCTGGCGTGGTACCAGCAGAAGCCCGGGAAGCCCCCAAAGCTGCTCATCT
ACCGGGCTTCCACTCTGGCCAGCGGTGTTCCTTCCCGCTTCTCTGGCTCCGGGTCGGGCACTGACTTTAC
ATTGACGATCTCTTCGCTTCAGCCGGAAGATGTAGCTACTTACTACTGTCAATGCACTGGCTACGGCATC
AGTTGGCCCATCGGTGGAGGCACCAAGGTGGAGATCAAAAGCGGGGGAGGCGGTTCTGGCGGTGGAG
GGTCTGGCGGGGGTGGCTCCGGGGGCGGTGGTAGCGGTGGTGGATCCCTACAGCTGCTGCCCTCTTGG
GCCATCACGCTCATTTCAGTTAATGGCATCTTCGTCATCTGCTGCCTGACCTACTGCTTTGCGCCGAGGTG
TAGAGAGCGGCGTCGCAACGAGCGCCTGCGTCGCGAGAGCGTGCGCCCGGTG (SEQ ID NO:314)

FIGURE 36F

Tethered anti-CD40scFV (Dacetuzumab)

MDWTWILFLVAAATRVHSEVQLVESGGGLVQPGGSLRLSCAASGYSFTGYYIHWVRQAPGKGLEWVARVI
PNAGGTSYNQKFKGRFTLSVDNSKNTAYLQMNSLRAEDTAVYYCAREGIYWWGQGTLVTVSSGGGGSGG
GGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRSSQSLVHSNGNTFLHWYQQKPGKAPKLLIYTVSN
RFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCSQTTHVPWTFGQGTKVEIKSGGGGSGGGGSGGGGSGG
GGSGGGSLQLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO:315)

Tethered anti-CD40scFV (Dacetuzumab)

ATGGATTGGACCTGGATCCTGTTCCTGGTGGCGGCGGCTACACGTGTGCACTCCGAGGTGCAGTTGGTG
GAGAGCGGCGGTGGTCTGGTGCAGCCGGGCGGGAGCCTGCGCCTGTCGTGCGCCGCTTCCGGCTACTC
CTTCACCGGTTATTACATCCACTGGGTGCGACAGGCCCCGGGCAAAGGCCTGGAGTGGGTCGCTCGCGT
AATTCCTAACGCGGGTGGCACCAGCTACAACCAGAAATTTAAGGGCAGATTCACCCTCAGCGTTGACAA
CTCCAAGAACACGGCCTACCTGCAGATGAACTCCCTTCGCGCCGAGGACACCGCCGTGTACTACTGTGCT
CGCGAGGGCATCTATTGGTGGGGCCAGGGAACTCTAGTTACCGTGTCCTCCGGCGGCGGCGGCTCGGG
CGGCGGGGGTTCTGGTGGGGGCGGTTCCGGAGGGGGAGGGTCCGACATCCAGATGACCCAGAGCCCC
TCTAGTCTGAGCGCATCCGTGGGGGACAGGGTCACCATCACCTGCCGCTCGTCACAATCGCTTGTCCACT
CAAATGGCAACACTTTCCTGCATTGGTACCAGCAGAAGCCCGGCAAGGCGCCCAAGCTGCTCATCTACA
CCGTGAGCAACCGCTTTTCAGGCGTGCCATCCCGCTTCTCTGGCTCCGGCTCCGGTACAGATTTCACCCTT
ACCATTAGCTCGTTGCAGCCAGAAGACTTTGCCACCTATTTCTGTTCTCAGACGACTCACGTCCCGTGGA
CCTTCGGACAGGGCACCAAGGTGGAGATCAAGAGCGGCGGAGGTGGGAGTGGAGGCGGCGGGTCCG
GAGGGGGCGGTTCTGGCGGCGGTGGTTCTGGCGGAGGGTCTCTGCAGCTGCTACCCAGTTGGGCCATC
ACTCTCATTTCTGTCAACGGCATCTTCGTCATATGTTGCCTGACCTACTGCTTTGCTCCTAGGTGTCGTGA
GCGCCGCCGCAACGAGCGGTTGCGGCGTGAATCCGTGCGCCCTGTA (SEQ ID NO:316)

FIGURE 36F

Tethered anti-CD40scFV (Lucatutuzumab)

MDWTWILFLVAAATRVHSQVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVI
SYEESNRYHADSVKGRFTISRDNSKITLYLQMNSLRTEDTAVYYCARDGGIAAPGPDYWGQGTLVTVSSGGG
GSGGGGSGGGGSGGGGSDIVMTQSPLSLTVTPGEPASISCRSSQSLLYSNGYNYLDWYLQKPGQSPQVLISL
GSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQARQTPFTFGPGTKVDIRGGGGSGGGGSGGG
GSGGGGSGGGSLQLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO:317)

Tethered anti-CD40scFV (Lucatutuzumab)

ATGGACTGGACCTGGATCCTGTTCCTGGTCGCAGCGGCCACCCGCGTACATTCTCAGGTGCAGCTGGTG
GAATCCGGGGGTGGCGTGGTGCAACCAGGCCGTAGCCTGCGCCTGAGCTGTGCTGCGTCCGGCTTTAC
TTTTTCGTCTTACGGCATGCACTGGGTGCGCCAGGCCCCGGGTAAAGGCTTGGAGTGGGTCGCGGTTAT
CAGCTATGAGGAGAGCAACCGCTACCACGCGGACTCCGTGAAGGGCCGATTCACCATCTCGCGGGACA
ACTCCAAGATAACCCTGTACCTGCAGATGAACTCTCTGCGGACTGAAGACACCGCCGTGTACTACTGTGC
CCGCGATGGCGGAATCGCCGCTCCCGGGCCCGACTATTGGGGCCAGGGCACACTCGTTACCGTCTCCTC
GGGAGGCGGCGGCAGTGGTGGTGGAGGTTCCGGCGGCGGGGGCTCCGGGGGTGGCGGCTCTGACAT
CGTGATGACGCAGAGCCCTTTGAGCCTAACAGTTACCCCCGGGGAGCCCGCTTCGATTTCATGCCGCTCC
TCCCAGAGCCTGCTGTACTCAAATGGCTACAACTACCTGGACTGGTACCTCCAGAAGCCGGGTCAGTCCC
CTCAGGTGCTCATCTCGTTGGGCAGCAACAGGGCCTCTGGCGTGCCAGACCGCTTCTCCGGATCTGGCA
GCGGAACCGATTTCACCCTGAAGATCTCTAGAGTGGAGGCTGAGGACGTGGGCGTGTATTACTGTATGC
AGGCCCGACAGACTCCCTTCACCTTCGGCCCCGGGACCAAGGTGGATATCCGCTCGGGAGGCGGCGGC
AGCGGTGGGGGAGGCAGCGGTGGTGGCGGATCCGGGGGGGGCGGCTCTGGTGGCGGATCCCTCCAG
CTGCTGCCGAGTTGGGCTATCACTCTTATTTCCGTAAATGGGATTTTCGTCATCTGCTGCCTTACCTACTG
CTTTGCACCTAGATGTCGCGAGCGTAGGCGCAACGAGCGGCTTCGTCGCGAGTCCGTCCGCCCTGTC
(SEQ ID NO:318)

FIGURE 36G

Tethered anti-CD40scFV (Selicrelumab)

MDWTWILFLVAAATRVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMG
WINPDSGGTNYAQKFQGRVTMTRDTSISTAYMELNRLRSDDTAVYYCARDQPLGYCTNGVCSYFDYWGQ
GTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCRASQGIYSWLAWYQQKPG
KAPNLLIYTASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANIFPLTFGGGTKVEIKSGGGGSGGG
GSGGGGSGGGGSGGGSLQLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO:319)

Tethered anti-CD40scFV (Selicrelumab)

ATGGATTGGACCTGGATCCTGTTCCTGGTGGCTGCTGCCACCCGTGTGCACAGCCAGGTGCAGCTGGTG
CAATCCGGCGCCGAGGTGAAGAAGCCCGGCGCCAGCGTGAAGGTATCGTGTAAGGCTTCGGGCTACAC
GTTCACCGGCTACTACATGCATTGGGTCCGCCAGGCCCCCGGACAGGGCCTGGAGTGGATGGGCTGGA
TTAACCCCGACTCCGGCGGCACCAACTACGCGCAGAAATTTCAGGGGCGAGTGACTATGACAAGGGAC
ACTTCCATCTCCACAGCCTACATGGAGCTCAACCGCCTGCGCAGCGATGACACCGCAGTGTACTATTGTG
CTCGCGACCAGCCTCTAGGTTACTGCACTAATGGCGTATGTAGCTATTTCGACTATTGGGGCCAGGGCAC
CCTGGTCACCGTGTCCAGCGGCGGAGGGGGCTCAGGCGGAGGTGGCTCAGGAGGTGGCGGCTCGGGG
GGCGGTGGGTCCGACATTCAGATGACCCAGTCCCCGAGCTCGGTCTCTGCTTCTGTGGGAGACCGCGTG
ACGATAACCTGCCGTGCCTCCCAGGGCATCTACAGTTGGCTTGCGTGGTACCAGCAGAAGCCAGGCAAG
GCGCCAAACCTGCTGATTTACACCGCATCCACTCTTCAGTCAGGCGTTCCTTCCCGCTTCAGTGGCTCTGG
TTCTGGCACCGATTTCACGCTGACCATCTCCTCCTTGCAGCCGGAAGACTTTGCCACTTACTACTGCCAAC
AGGCCAACATCTTCCCTCTGACCTTCGGCGGTGGCACCAAGGTGGAGATCAAATCTGGCGGAGGGGGC
TCGGGCGGGGGTGGTTCTGGCGGCGGGGGAAGTGGTGGGGGCGGATCTGGTGGTGGTAGCCTGCAG
CTCTTGCCCTCCTGGGCGATCACCCTCATCAGCGTCAATGGCATCTTCGTCATCTGTTGCCTGACCTACTG
CTTTGCGCCGAGATGTCGAGAGCGCCGGCGCAACGAGCGCCTGCGTCGGGAGTCCGTGCGCCCCGTG
(SEQ ID NO:320)

FIGURE 36H

CD40L

MIETYNQTSPRSAATGLPISMKIFMYLLTVFLITQMIGSALFAVYLHRRLDKIEDERNLHEDFVFMKTIQRCNT
GERSLSLLNCEEIKSQFEGFVKDIMLNKEETKKENSFEMQKGDQNPQIAAHVISEASSKTTSVLQWAEKGYYT
MSNNLVTLENGKQLTVKRQGLYYIYAQVTFCSNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQ
QSIHLGGVFELQPGASVFVNVTDPSQVSHGTGFTSFGLLKL (SEQ ID NO:273)

CD40L

ATGATCGAGACCTACAACCAGACTTCCCCACGTAGCGCCGCCACTGGTCTACCCATCTCGATGAAAATCT
TCATGTATCTGCTGACCGTGTTCCTGATCACCCAGATGATTGGCTCCGCTCTGTTTGCCGTGTACTTGCAC
CGCCGCCTGGACAAAATTGAGGACGAGCGGAATCTGCACGAAGATTTCGTATTTATGAAGACCATCCAG
AGGTGTAACACTGGCGAGCGTTCTCTTTCGTTGCTTAACTGCGAAGAGATCAAGAGCCAGTTCGAGGGC
TTCGTCAAGGACATCATGCTCAACAAGGAGGAGACTAAGAAGGAGAACTCCTTTGAGATGCAGAAGGG
TGATCAGAACCCCCAAATTGCAGCGCATGTGATAAGCGAGGCTTCCTCCAAGACAACCAGTGTGCTGCA
GTGGGCGGAGAAGGGCTACTACACCATGTCAAATAACCTGGTGACCCTGGAGAACGGCAAGCAGCTGA
CGGTGAAGCGCCAGGGGCTCTATTACATCTACGCGCAGGTCACGTTCTGTTCGAACCGCGAAGCCTCCT
CTCAGGCTCCCTTCATCGCCTCACTGTGCCTGAAATCTCCGGGACGCTTCGAGCGAATCCTGCTCCGCGC
CGCCAACACGCACTCCTCCGCCAAGCCTTGCGGGCAGCAGAGCATCCACCTTGGTGGGGTTTTTGAGCT
GCAGCCTGGCGCGTCCGTGTTCGTCAACGTGACCGACCCGTCCCAGGTCTCTCATGGAACCGGCTTCACC
TCCTTCGGCCTGCTCAAGTTG (SEQ ID NO:321)

FIGURE 36I

Tethered CD40L/IL-15

MIETYNQTSPRSAATGLPISMKIFMYLLTVFLITQMIGSALFAVYLHRRLDKIEDERNLHEDFVFMKTIQRCNT
GERSLSLLNCEEIKSQFEGFVKDIMLNKEETKKENSFEMQKGDQNPQIAAHVISEASSKTTSVLQWAEKGYYT
MSNNLVTLENGKQLTVKRQGLYYIYAQVTFCSNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQ
QSIHLGGVFELQPGASVFVNVTDPSQVSHGTGFTSFGLLKLGGGGGSGGGGSGGGGSGGGGSGGGSLQLLP
SWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPVRAKRSGSGATNFSLLKQAGDVEENPGPMDW
TWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIH
DTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGG
GGGGSLQLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO:322)

Tethered CD40L/IL-15

ATGATCGAAACTTACAACCAGACGAGCCCGCGCTCCGCCGCCACCGGGCTGCCCATCTCTATGAAAATCT
TCATGTATCTGCTGACCGTGTTTCTGATCACCCAGATGATCGGTTCCGCGCTGTTTGCCGTGTACCTGCAC
CGCCGCCTGGACAAGATAGAAGACGAGCGCAACCTGCACGAAGATTTCGTCTTCATGAAGACCATCCAG
CGGTGTAATACCGGCGAGAGGAGCCTGAGCTTGCTCAACTGCGAGGAGATCAAAAGTCAGTTCGAGGG
CTTCGTTAAGGACATCATGCTCAACAAGGAGGAGACCAAGAAGGAGAACTCCTTCGAGATGCAAAAGG
GGGACCAGAACCCTCAGATCGCCGCGCATGTAATTTCCGAGGCCTCATCCAAGACGACTTCTGTCCTACA
GTGGGCAGAGAAGGGCTACTACACCATGTCTAATAACCTGGTCACCCTTGAGAACGGCAAGCAACTGAC
AGTGAAGCGCCAGGGCCTCTACTACATCTACGCGCAGGTGACCTTCTGCAGCAACCGCGAGGCTTCCTC
CCAAGCTCCCTTCATCGCATCTCTGTGCCTGAAAAGCCCTGGCCGCTTCGAGCGGATTCTCCTTCGTGCA
GCAAACACTCACTCGTCCGCCAAGCCCTGTGGACAGCAGAGCATCCACCTGGGCGGCGTGTTCGAGCTG
CAGCCAGGCGCCTCCGTGTTCGTCAATGTGACCGACCCGTCCCAGGTGTCCCATGGCACTGGCTTCACCT
CCTTCGGCCTCCTCAAACTGTCCGGCGGCGGTGGCAGTGGTGGCGGGGGATCAGGTGGCGGCGGCTCC
GGAGGTGGCGGGTCCGGGGGAGGTTCTCTTCAGCTTCTGCCTAGCTGGGCGATCACCCTGATCTCCGTT
AATGGCATCTTCGTCATTTGTTGTTTGACCTATTGCTTTGCCCCGAGATGTCGAGAGCGTAGACGCAACG
AGCGCCTGCGCCGTGAAAGCGTGCGACCTGTGCGCGCTAAGCGCAGCGGCTCCGGCGCTACCAACTTCT
CATTACTCAAGCAGGCTGGAGATGTAGAGGAGAATCCAGGGCCAATGGATTGGACCTGGATCCTGTTCC
TGGTGGCCGCCGCGACTCGCGTCCACAGTAATTGGGTCAACGTGATCTCTGACCTGAAAAAGATTGAGG
ACTTGATCCAGAGCATGCACATTGACGCTACCCTGTACACGGAGAGTGACGTGCACCCCTCGTGCAAGG
TGACAGCCATGAAGTGCTTTTTGCTGGAGTTGCAGGTCATCTCTCTAGAGAGCGGTGATGCCTCCATCCA
TGACACCGTGGAGAACCTTATCATCCTGGCCAACAACAGCCTGTCGAGCAACGGCAACGTGACCGAGTC
CGGGTGCAAGGAATGTGAGGAGCTGGAGGAGAAAAACATCAAGGAGTTCCTGCAGTCGTTTGTGCACA
TCGTGCAGATGTTTATTAACACCTCTTCTGGCGGTGGGGGATCGGGGGGGGGCGGCTCCGGCGGTGGC
GGTTCTGGAGGCGGTGGTTCTGGCGGGGGATCTTTACAGCTGCTGCCCAGTTGGGCGATCACTCTCATT
TCCGTGAACGGCATTTTCGTCATTTGCTGTTTGACCTACTGCTTTGCTCCGAGGTGTCGCGAGCGTCGCC
GCAACGAGCGCCTACGGCGGGAATCGGTGAGGCCCGTG (SEQ ID NO:323)

FIGURE 37A

Signal Sequence: MDWTWILFLVAAATRVHS (SEQ ID NO:252)
Signal Sequence: MYRMQLLSCIALSLALVTNS (SEQ ID NO:254)
Linker (Lr1): SGGGGSGGGGSGGGGSGGGGSGGGSLQ (SEQ ID NO:246)
Anchor (Ar2): (human B7.1 transmembrane-intracellular amino acid sequence)
LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO:239)
FurinA-P2A sequence: RAKRSGSGATNFSLLKQAGDVEENPGP (SEQ ID NO:251)
)

Tethered IL-2
MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEE
ELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
SGGGGSGGGGSGGGGSGGGGSGGGSLQLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO:324)

Tethered IL-2
ATGTATCGGATGCAGCTGCTGTCTTGTATTGCTCTATCACTGGCGCTGGTGACCAACTCCGCTCCTACTTCCAGCTC
GACCAAGAAGACCCAGCTCCAACTTGAGCACCTGCTGCTTGATCTTCAGATGATCCTGAACGGCATCAACAACTAC
AAAAACCCCAAGCTGACCCGCATGCTCACTTTCAAGTTCTACATGCCTAAAAAGGCCACCGAGCTCAAGCACTTGC
AGTGCCTGGAGGAGGAGTTGAAGCCGTTGGAAGAGGTGCTCAACCTGGCCCAGTCCAAGAATTTCCATCTAAGAC
CCCGCGACCTGATCTCTAACATCAACGTGATTGTACTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCGAGTA
CGCGGACGAGACTGCAACCATCGTGGAGTTCCTGAACCGCTGGATCACGTTCTGTCAGAGCATCATCTCGACCCTT
ACATCTGGCGGCGGGGGGTCGGGCGGTGGGGGCTCTGGAGGAGGCGGTAGTGGGGGTGGCGGCTCCGGTGGA
GGCAGCCTGCAGCTGCTCCCAAGTTGGGCCATCACCCTGATTAGCGTCAATGGCATCTTCGTCATATGTTGTCTGA
CCTACTGCTTTGCCCCCCGCTGCCGAGAACGTCGCCGCAACGAGCGGCTGCGCAGGGAGTCCGTGCGCCCGGTGT
GA (SEQ ID NO:325)

FIGURE 37B

Tethered IL-12

MDWTWILFLVAAATRVHSIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQVKEF
GDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGS
SDPQGVTCGAATLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQL
KPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEW
ASVPCS*GGGGGGS*RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPL
ELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELM
QALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS*GGGGSGGSGGGSGGGSGGSGGGSGGGSLQ*
LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO:326)

Tethered IL-12

ATGTATCGGATGCAGCTGCTGTCTTGTATTGCTCTATCACTGGCGCTGGTGACCAACTCCGCTCCTACTTCCAGCTC
GACCAAGAAGACCCAGCTCCAACTTGAGCACCTGCTGCTTGATCTTCAGATGATCCTGAACGGCATCAACAACTAC
AAAAACCCCAAGCTGACCCGCATGCTCACTTTCAAGTTCTACATGCCTAAAAAGGCCACCGAGCTCAAGCACTTGC
AGTGCCTGGAGGAGGAGTTGAAGCCGTTGGAAGAGGTGCTCAACCTGGCCCAGTCCAAGAATTTCCATCTAAGAC
CCCGCGACCTGATCTCTAACATCAACGTGATTGTACTGGAGCTGAAGGGCTCCGAGACCACCTTCATGTGCGAGTA
CGCGGACGAGACTGCAACCATCGTGGAGTTCCTGAACCGCTGGATCACGTTCTGTCAGAGCATCATCTCGACCCTT
ACATCTGGCGGCGGGGGGTCGGGCGGTGGGGGCTCTGGAGGAGGCGGTAGTGGGGGTGGCGGCTCCGGTGGA
GGCAGCCTGCAGCTGCTCCCAAGTTGGGCCATCACCCTGATTAGCGTCAATGGCATCTTCGTCATATGTTGTCTGA
CCTACTGCTTTGCCCCCCGCTGCCGAGAACGTCGCCGCAACGAGCGGCTGCGCAGGGAGTCCGTGCGCCCGGTGT
GA (SEQ ID NO:327)

FIGURE 37C

Tethered IL-15

MDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDT
VENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGGSGGGGSLQ
LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO:328)

Tethered IL-15

ATGGATTGGACCTGGATCCTGTTCCTGGTGGCTGCGGCCACCCGTGTACACAGCAACTGGGTCAATGTGATCTCCG
ACCTGAAGAAGATTGAGGACCTCATCCAGTCTATGCACATTGACGCAACACTTTACACCGAGAGCGATGTGCACCC
CTCTTGCAAGGTGACTGCCATGAAATGTTTCTTGCTCGAATTGCAGGTCATCTCTCTGGAGAGTGGGGACGCTTCC
ATACATGACACCGTGGAGAACCTGATCATCCTGGCCAACAACAGCCTCTCGTCAAATGGCAACGTGACTGAGAGC
GGGTGCAAAGAGTGTGAGGAGCTGGAGGAGAAGAACATCAAGGAGTTCCTGCAGAGCTTCGTCCACATCGTGCA
GATGTTTATTAACACGTCGTCCGGAGGCGGGGGCTCTGGTGGCGGCGGCTCCGGGGGAGGCGGCTCCGGTGGC
GGTGGTTCCGGCGGAGGCTCGCTACAGCTGCTGCCCAGTTGGGCCATCACCCTTATTTCTGTCAACGGCATCTTCG
TTATCTGTTGCCTGACCTACTGCTTTGCGCCGAGGTGTCGCGAGCGGCGTCGCAACGAGCGCCTGCGCCGAGAAT
CCGTGCGCCCTGTGTGA (SEQ ID NO:329)

CD40L

MIETYNQTSPRSAATGLPISMKIFMYLLTVFLITQMIGSALFAVYLHRRLDKIEDERNLHEDFVFMKTIQRCNTGERSLSLL
NCEEIKSQFEGFVKDIMLNKEETKKENSFEMQKGDQNPQIAAHVISEASSKTTSVLQWAEKGYYTMSNNLVTLENGKQ
LTVKRQGLYYIYAQVTFCSNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQQSIHLGGVFELQPGASVFVNV
TDPSQVSHGTGFTSFGLLKL (SEQ ID NO:273)

CD40L

ATGATCGAGACCTACAACCAGACTTCCCCACGTAGCGCCGCCACTGGTCTACCCATCTCGATGAAAATCTTCATGTA
TCTGCTGACCGTGTTCCTGATCACCCAGATGATTGGCTCCGCTCTGTTTGCCGTGTACTTGCACCGCCGCCTGGACA
AAATTGAGGACGAGCGGAATCTGCACGAAGATTTCGTATTTATGAAGACCATCCAGAGGTGTAACACTGGCGAGC
GTTCTCTTTCGTTGCTTAACTGCGAAGAGATCAAGAGCCAGTTCGAGGGCTTCGTCAAGGACATCATGCTCAACAA
GGAGGAGACTAAGAAGGAGAACTCCTTTGAGATGCAGAAGGGTGATCAGAACCCCCAAATTGCAGCGCATGTGA
TAAGCGAGGCTTCCTCCAAGACAACCAGTGTGCTGCAGTGGGCGGAGAAGGGCTACTACACCATGTCAAATAACC
TGGTGACCCTGGAGAACGGCAAGCAGCTGACGGTGAAGCGCCAGGGGCTCTATTACATCTACGCGCAGGTCACG
TTCTGTTCGAACCGCGAAGCCTCCTCTCAGGCTCCCTTCATCGCCTCACTGTGCCTGAAATCTCCGGGACGCTTCGA
GCGAATCCTGCTCCGCGCCGCCAACACGCACTCCTCCGCCAAGCCTTGCGGGCAGCAGAGCATCCACCTTGGTGG
GGTTTTTGAGCTGCAGCCTGGCGCGTCCGTGTTCGTCAACGTGACCGACCCGTCCCAGGTCTCTCATGGAACCGGC
TTCACCTCCTTCGGCCTGCTCAAGTTG (SEQ ID NO:321)

FIGURE 37D

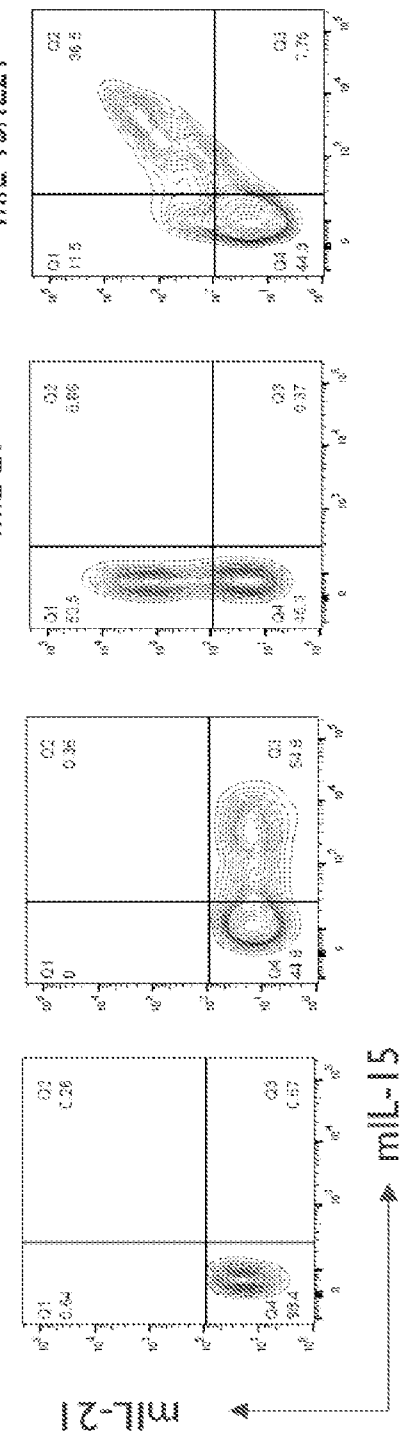
GFP

ATGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCA
CAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCAC
CGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCC
GACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCA
AGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTG
AAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGT
CTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCA
GCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACT
ACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGA
CCGCCGCCGGGATCACTCACGGCATGGACGAGCTGTACAAGTGA (SEQ ID NO:330)

Tethered IL-21

MDWTWILFLVAAATRVHSQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQK
AQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRT
HGSEDSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSLQLLPSWAITLISVNGIFVICC
LTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO:331)

FIGURE 38A



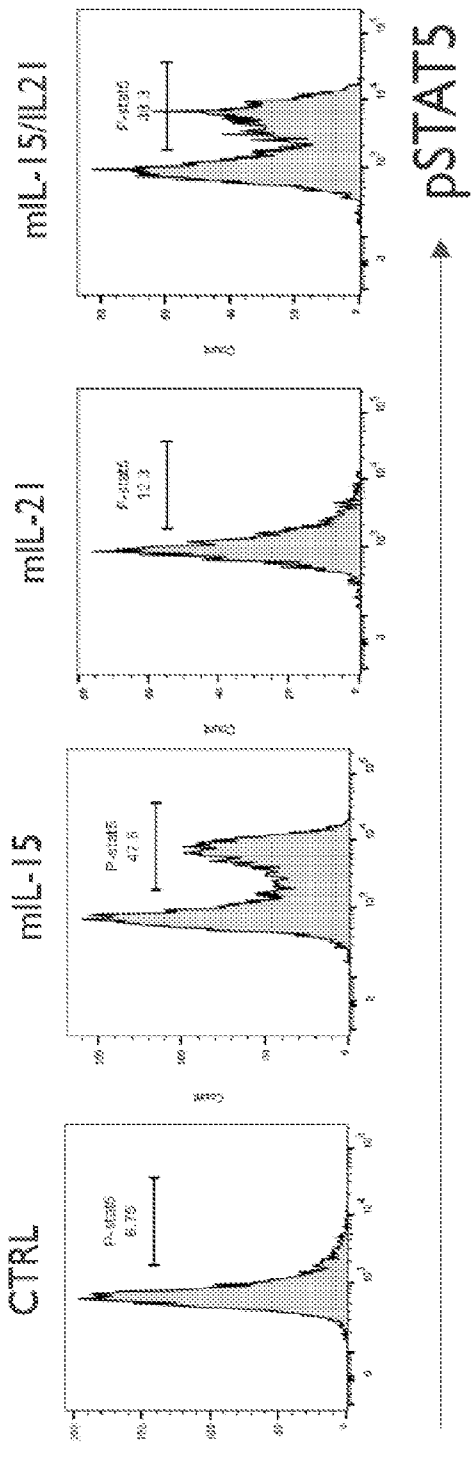


FIGURE 38B

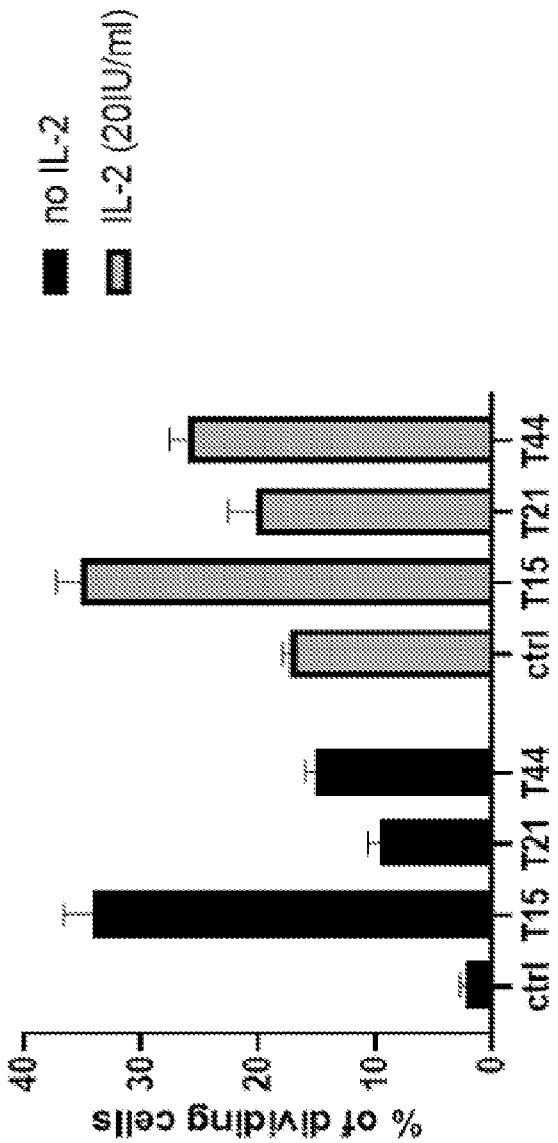

no IL-2
IL-2 (20IU/ml)
% of dividing cells
0
10
20
30
40
ctrl
T15
T21
T44
ctrl
T15
T21
T44

FIGURE 38C

Figure 39

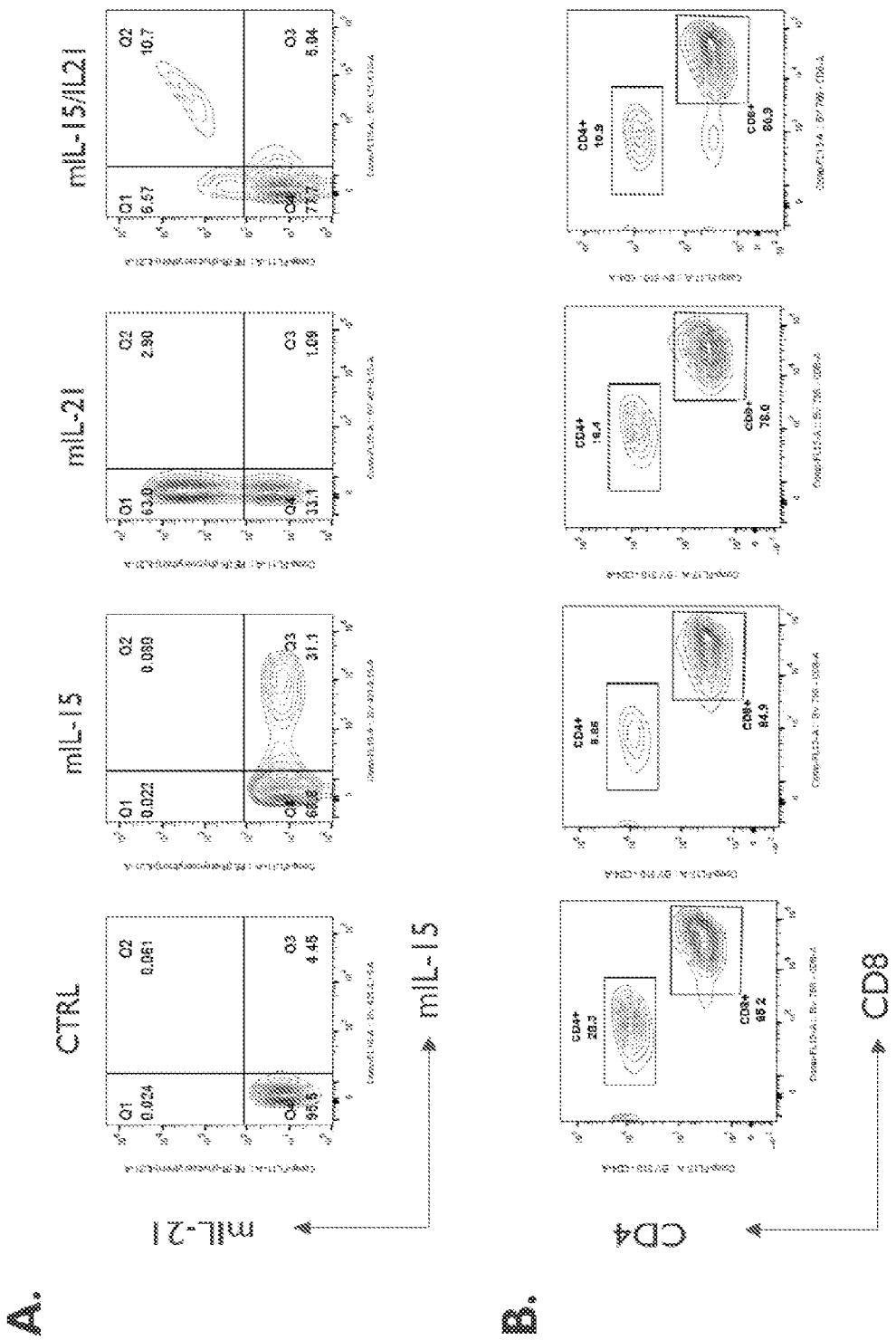


FIGURE 40A

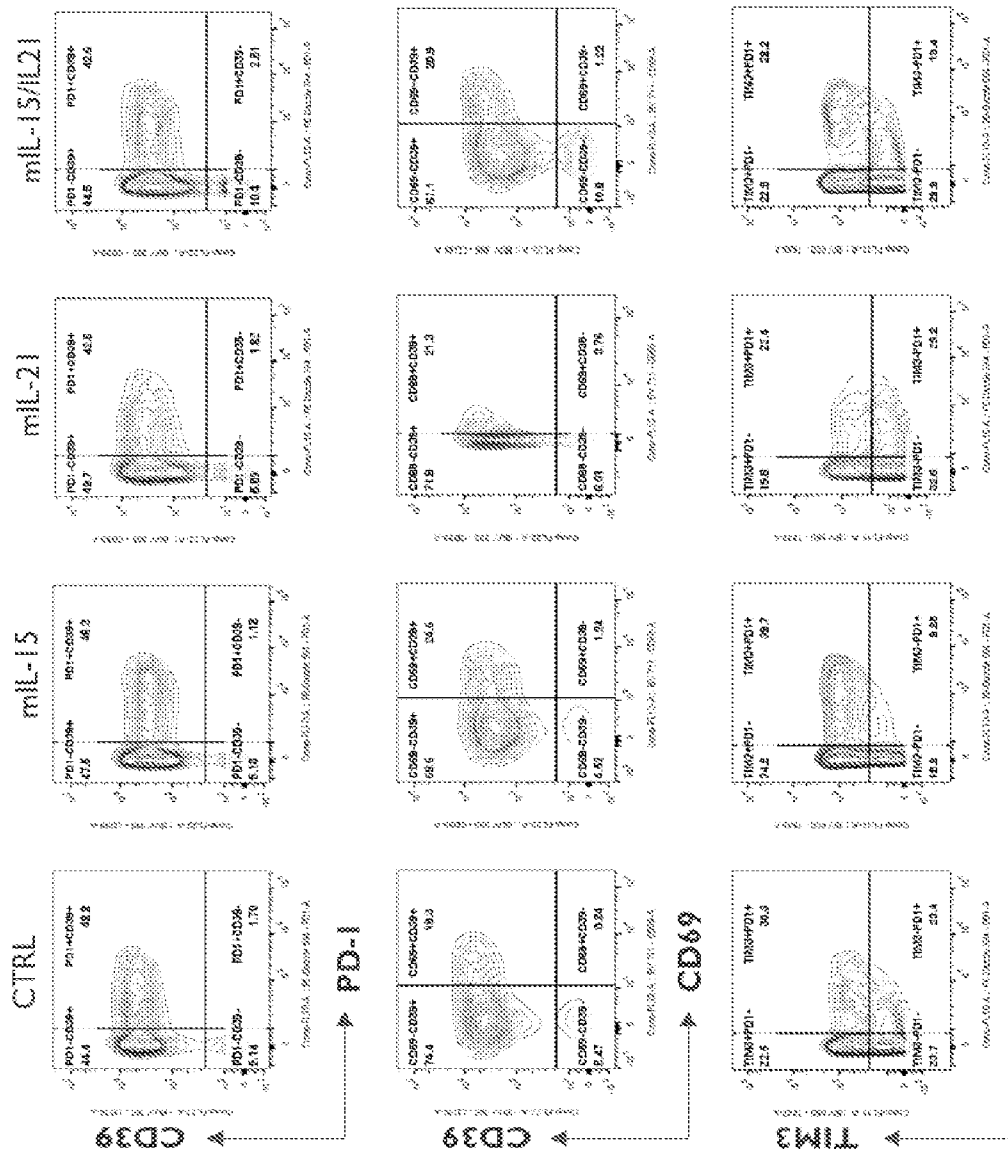


FIGURE 40B

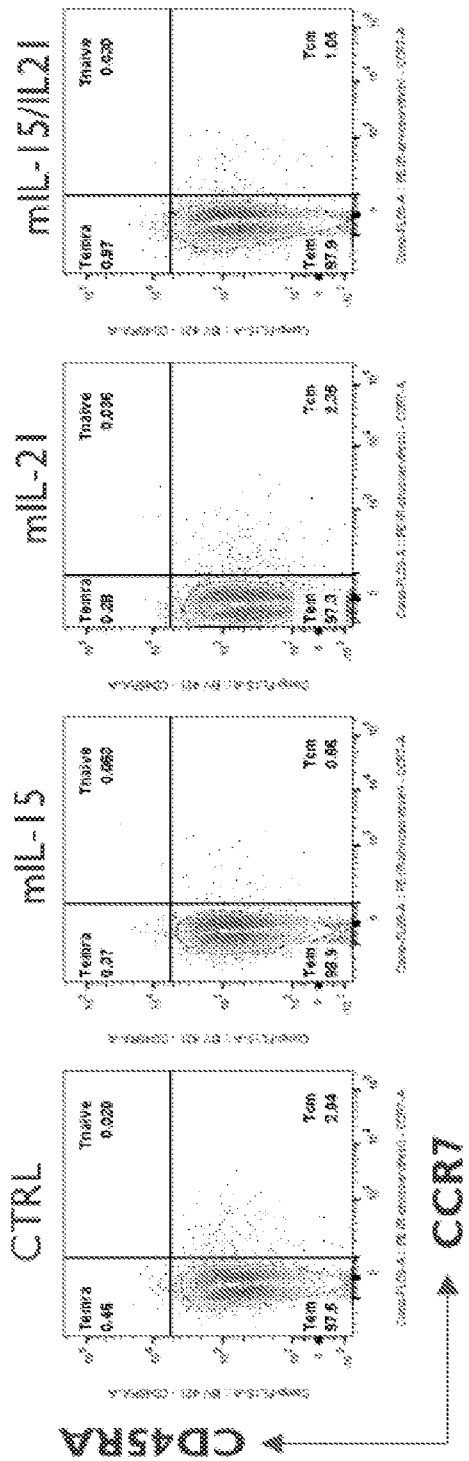

CTRL
mIL-15
mIL-21
mIL-15/IL21
Tnaive
Tcm
Tem
Temra
CD45RA
CCR7

FIGURE 40C

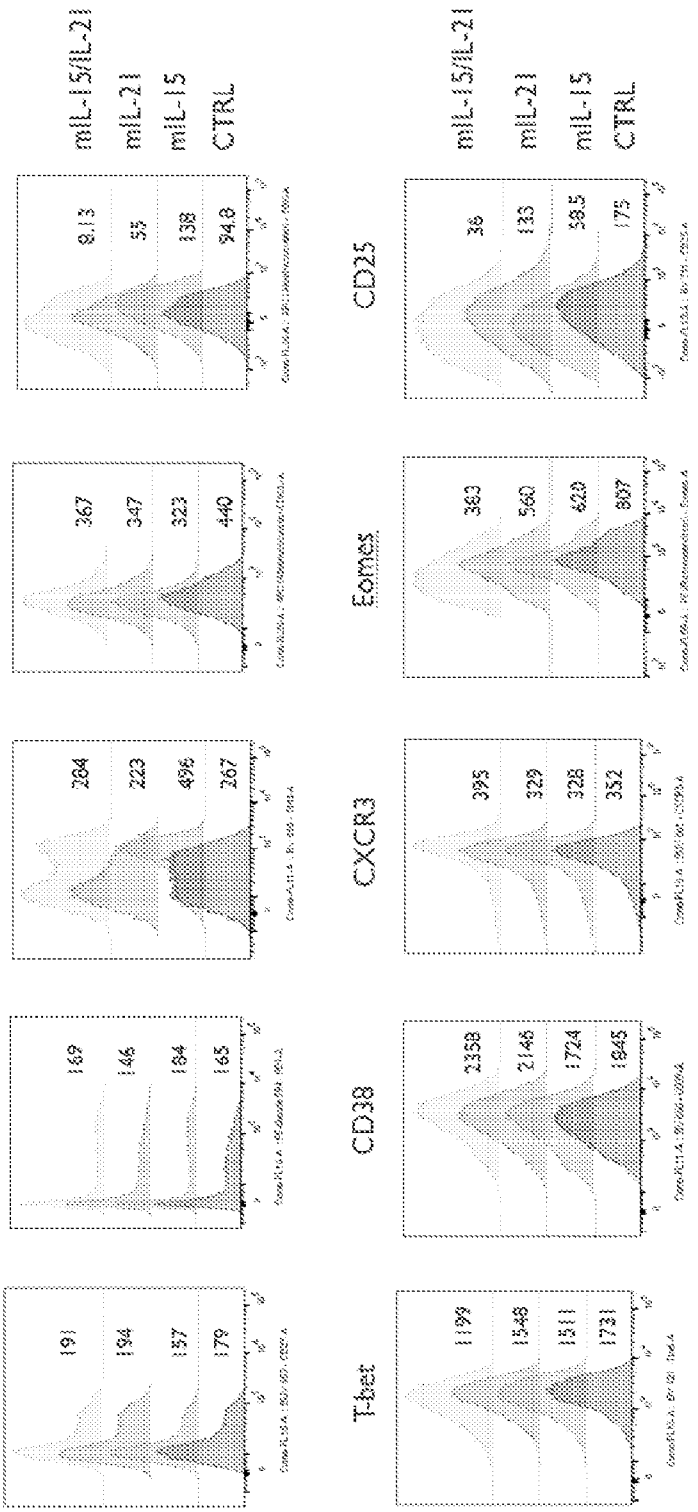
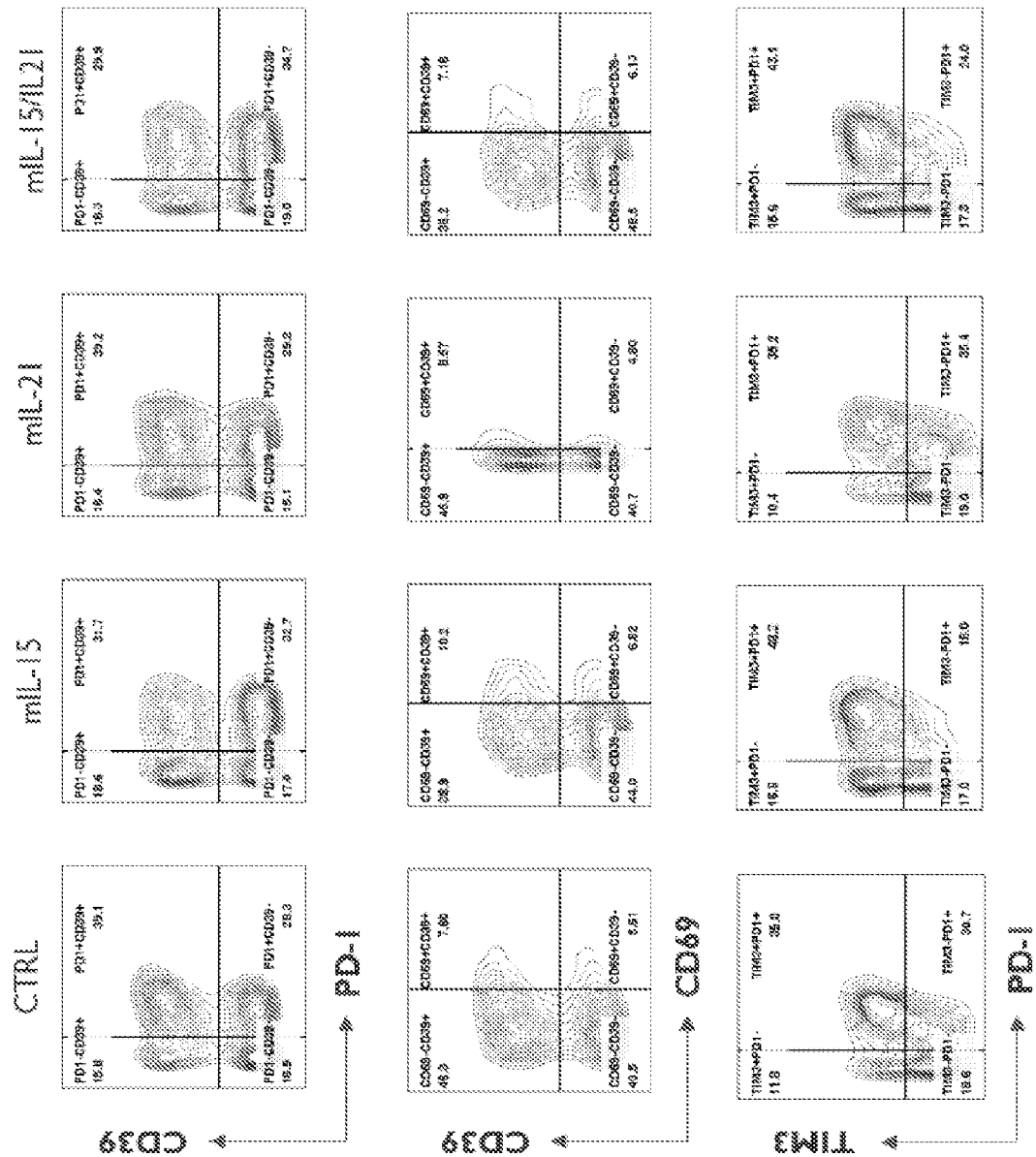

CD27
191
194
157
179
PD1
169
146
184
165
TIM3
284
223
496
267
CD62L
367
347
323
440
TOX
8.13
55
138
94.8
mIL-15/IL-21
mIL-21
mIL-15
CTRL
T-bet
1199
1548
1311
1731
CD38
2358
2146
1724
1845
CXCR3
395
329
328
352
Eomes
383
560
420
807
CD25
36
133
58.5
175
mIL-15/IL-21
mIL-21
mIL-15
CTRL

FIGURE 41A



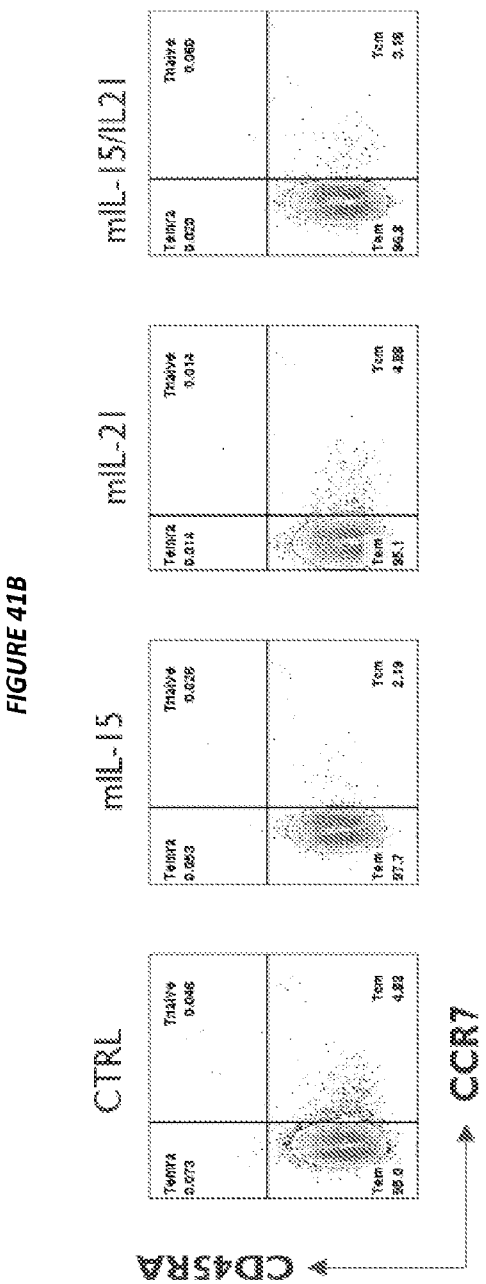


FIGURE 41B

FIGURE 41C

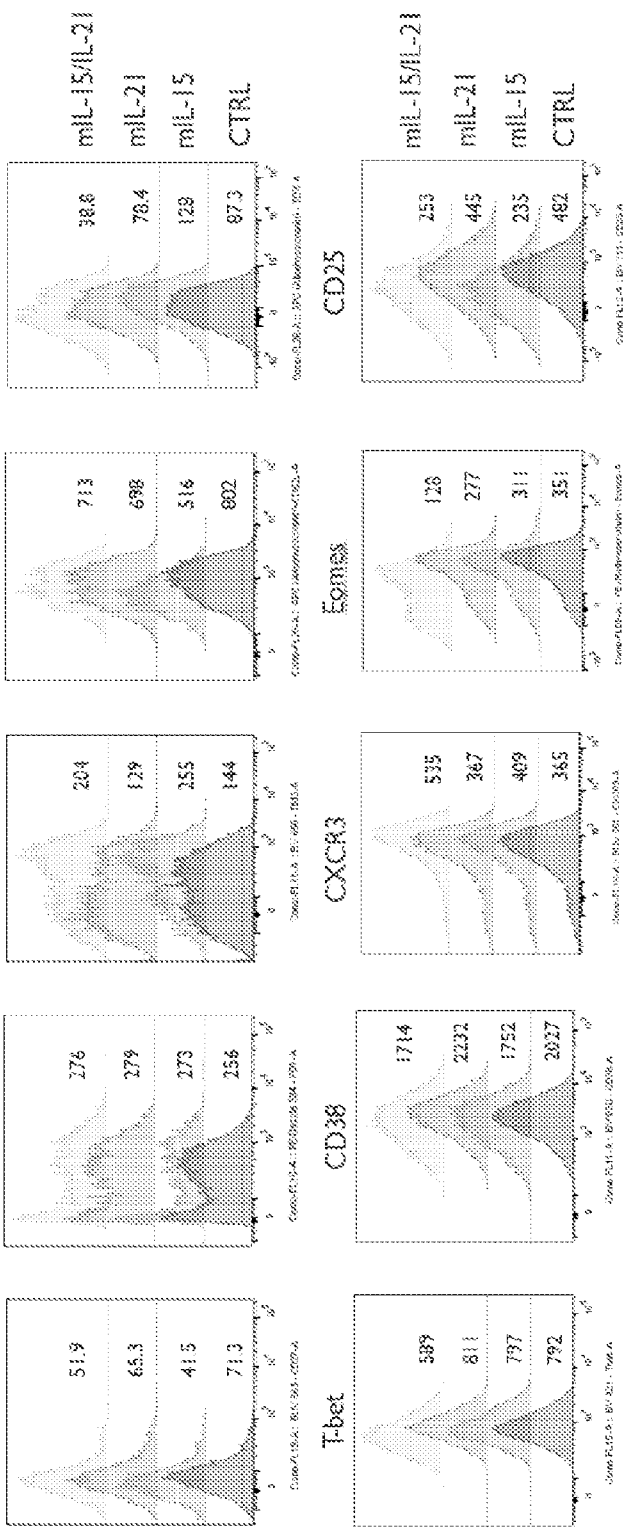

CD27
51.9
65.3
41.5
71.3
PD1
276
279
273
256
TIM3
204
129
255
144
CD62L
713
698
516
802
TOX
38.8
78.4
129
87.3
mIL-15/IL-21
mIL-21
mIL-15
CTRL
T-bet
589
811
737
792
CD38
1714
2232
1752
2027
CXCR3
535
367
409
365
Eomes
126
277
311
351
CD25
253
445
235
482
mIL-15/IL-21
mIL-21
mIL-15
CTRL

Figure 1

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 F**

Final Formulation and/or Transfer to Infusion Bag

(optionally cryopreserve)