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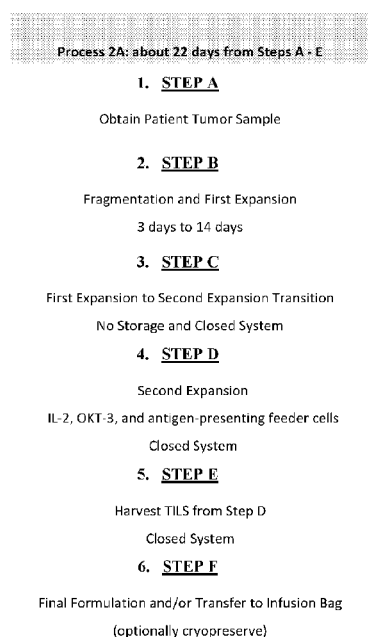
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(54) Title: USE OF TUMOR INFLTRATING LYMPHOCYTES FOR TREATING NSCLC PATIENTS REFRACTORY FOR AN-
TI-PD-1 ANTIBODY

Figure 1



(57) Abstract: The present invention provides improved and/or shortened processes and methods for preparing TILs in order to prepare therapeutic populations of TILs with increased therapeutic efficacy for the treatment of non-small cell lung carcinoma (NSCLC), wherein the NSCLC is refractory to treatment with an anti -PD- 1 antibody.

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TREATMENT OF NSCLC PATIENTS REFRACTORY FOR ANTI-PD-1 ANTIBODY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/725,976, filed on August 31, 2018, and U.S. Provisional Patent Application No. 62/726,919, filed on September 4, 2018, which are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on August 23, 2019, is named 116983-5043-WO_ST25.txt and is 168 kilobytes in size.

BACKGROUND OF THE INVENTION

[0003] Treatment of bulky, refractory cancers using adoptive transfer of tumor infiltrating lymphocytes (TILs) represents a powerful approach to therapy for patients with poor prognoses. Gattinoni, *et al.*, *Nat. Rev. Immunol.* **2006**, 6, 383-393. A large number of TILs are required for successful immunotherapy, and a robust and reliable process is needed for commercialization. This has been a challenge to achieve because of technical, logistical, and regulatory issues with cell expansion. IL-2-based TIL expansion followed by a “rapid expansion process” (REP) has become a preferred method for TIL expansion because of its speed and efficiency. Dudley, *et al.*, *Science* **2002**, 298, 850-54; Dudley, *et al.*, *J. Clin. Oncol.* **2005**, 23, 2346-57; Dudley, *et al.*, *J. Clin. Oncol.* **2008**, 26, 5233-39; Riddell, *et al.*, *Science* **1992**, 257, 238-41; Dudley, *et al.*, *J. Immunother.* **2003**, 26, 332-42. REP can result in a 1,000-fold expansion of TILs over a 14-day period, although it requires a large excess (*e.g.*, 200-fold) of irradiated allogeneic peripheral blood mononuclear cells (PBMCs, also known as mononuclear cells (MNCs)), often from multiple donors, as feeder cells, as well as anti-CD3 antibody (OKT3) and high doses of IL-2. Dudley, *et al.*, *J. Immunother.* **2003**, 26, 332-42. TILs that have undergone an REP procedure have produced successful adoptive cell therapy following host immunosuppression in patients with melanoma. Current infusion

acceptance parameters rely on readouts of the composition of TILs (*e.g.*, CD28, CD8, or CD4 positivity) and on fold expansion and viability of the REP product.

[0004] Current TIL manufacturing and treatment processes are limited by length, cost, sterility concerns, and other factors described herein such that the potential to treat patients which are refractory to anti-PD1 and as such have been severely limited. There is an urgent need to provide TIL manufacturing processes and therapies based on such processes that are appropriate for use in treating patients for whom very few or no viable treatment options remain. The present invention meets this need by providing a shortened manufacturing process for use in generating TILs which can then be employed in the treatment of non-small cell lung carcinoma (NSCLC) patients whom are refractory to anti-PD-1 treatment.

BRIEF SUMMARY OF THE INVENTION

[0005] The present invention provides improved and/or shortened methods for expanding TILs and producing therapeutic populations of TILs for use in treatment of non-small cell lung carcinoma (NSCLC) patients whom are refractory to anti-PD-1 treatment.

[0006] The present invention provides a method of treating non-small cell lung carcinoma (NSCLC) with a population of tumor infiltrating lymphocytes (TILs) 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 NSCLC tumor in a patient, including from multiple tumor fragments or biopsies;
- (c) contacting the tumor fragments with a first cell culture medium;
- (d) performing an initial expansion of the first population of TILs in the first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, wherein the first cell culture medium comprises IL-2;
- (e) performing a rapid expansion of the second population of TILs in a second cell culture medium to obtain a third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; wherein the second cell culture medium comprises IL-2, OKT-3 (anti-CD3 antibody), and optionally irradiated allogeneic peripheral blood mononuclear cells (PBMCs); and wherein the rapid expansion is

performed over a period of 14 days or less;

(f) harvesting the third population of TILs; and

(g) administering a therapeutically effective portion of the third population of TILs to a patient with the NSCLC;

wherein the NSCLC is refractory to treatment with an anti-PD-1 antibody.

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

[0008] In some embodiments, obtaining the first population of TILs comprises a multilesional sampling method.

[0009] In some embodiments, the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 and/or anti-PD-L2 antibody.

[0010] In some embodiments, the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody.

[0011] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent.

[0012] In some embodiments, the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has been previously treated with a chemotherapeutic agent.

[0013] In some embodiments, the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has been previously treated with a chemotherapeutic agent.

[0014] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent.

- [0015] In some embodiments, the refractory NSCLC has low expression of PD-L1.
- [0016] In some embodiments, the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has low expression of PD-L1.
- [0017] In some embodiments, the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has low expression of PD-L1.
- [0018] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent and has low expression of PD-L1.
- [0019] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent and has low expression of PD-L1.
- [0020] In some embodiments, the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has bulky disease at baseline.
- [0021] In some embodiments, the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has bulky disease at baseline.
- [0022] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent and has bulky disease at baseline.
- [0023] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent and has bulky disease at baseline.
- [0024] In some embodiments, bulky disease is indicated where the maximal tumor diameter is greater than 7 cm measured in either the transverse or coronal plane or swollen lymph nodes with a short-axis diameter of 20 mm or greater.
- [0025] In some embodiments, the refractory NSCLC is refractory to at least two prior systemic treatment courses, not including neo-adjuvant or adjuvant therapies.
- [0026] In some embodiments, the refractory NSCLC is refractory to an anti-PD-1 antibody selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, JS001, TSR-042, pidilizumab, (BGB-A317, SHR-1210, REGN2810, MDX-1106, PDR001, anti-PD-1 from clone: RMP1-14; and an anti-PD-1 antibodies disclosed in U.S. Patent No. 8,008,449, durvalumab, atezolizumab, avelumab, and fragments, derivatives, variants, as well as biosimilars thereof.

[0027] In some embodiments, the refractory NSCLC is refractory to pembrolizumab or a biosimilar thereof.

[0028] In some embodiments, the refractory NSCLC is refractory to nivolumab or a biosimilar thereof.

[0029] In some embodiments, the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof.

[0030] In some embodiments, the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof and pembrolizumab or a biosimilar thereof.

[0031] In some embodiments, the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof and nivolumab or a biosimilar thereof.

[0032] In some embodiments, the refractory NSCLC is refractory to durvalumab or a biosimilar thereof.

[0033] In some embodiments, the refractory NSCLC is refractory to atezolizumab or a biosimilar thereof.

[0034] In some embodiments, the refractory NSCLC is refractory to avelumab or a biosimilar thereof.

[0035] In some embodiments, the initial expansion is performed over a period of 21 days or less.

[0036] In some embodiments, the initial expansion is performed over a period of 14 days or less.

[0037] In some embodiments, the initial expansion is performed over a period of about 11 days and the rapid expansion is performed over a period of about 11 days.

[0038] In some embodiments, the IL-2 is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL in the first cell culture medium.

[0039] In some embodiments, 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 in the second cell culture medium.

[0040] In some embodiments, the initial expansion is performed using a gas permeable container.

[0041] In some embodiments, the rapid expansion is performed using a gas permeable container.

[0042] In some embodiments, the first cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

[0043] In some embodiments, the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

[0044] In some embodiments, the method further comprises the step of treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the third population of TILs to the patient.

[0045] In some embodiments, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m²/day for two days followed by administration of fludarabine at a dose of 25 mg/m²/day for five days.

[0046] In some embodiments, the method further comprises the step of treating the patient with an IL-2 regimen starting on the day after administration of the third population of TILs to the patient.

[0047] 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.

[0048] In some embodiments, the invention provides a method of treating non-small cell lung carcinoma (NSCLC) with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

- (a) resecting one or more tumors from a patient, the one or more tumors comprising a first population of TILs;
- (b) fragmenting the one or more tumors into tumor fragments;
- (c) contacting the tumor fragments with a first cell culture medium;
- (d) performing an initial expansion of the first population of TILs in the first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, wherein the first cell culture medium comprises IL-2;
- (e) performing a rapid expansion of the second population of TILs in a second cell

culture medium to obtain a third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; wherein the second cell culture medium comprises IL-2, OKT-3 (anti-CD3 antibody), and optionally irradiated allogeneic peripheral blood mononuclear cells (PBMCs); and wherein the rapid expansion is performed over a period of 14 days or less;

(f) harvesting the third population of TILs; and

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

wherein the cancer is refractory to treatment with an anti-PD-1 antibody.

[0049] In some embodiments, the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody.

[0050] In some embodiments, the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody.

[0051] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent.

[0052] In some embodiments, the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has been previously treated a chemotherapeutic agent.

[0053] In some embodiments, the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has been previously treated a chemotherapeutic agent.

[0054] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent.

[0055] In some embodiments, the refractory NSCLC has low expression of PD-L1.

[0056] In some embodiments, the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has low expression of PD-L1.

[0057] In some embodiments, the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has low expression of PD-L1.

[0058] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent and has low expression of PD-L1.

[0059] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent and has low expression of PD-L1

[0060] In some embodiments, the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has bulky disease at baseline.

[0061] In some embodiments, the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has bulky disease at baseline.

[0062] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent and has bulky disease at baseline.

[0063] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent and has bulky disease at baseline.

[0064] In some embodiments, bulky disease is indicated where the maximal tumor diameter is greater than 7 cm measured in either the transverse or coronal plane or swollen lymph nodes with a short-axis diameter of 20 mm or greater.

[0065] In some embodiments, the refractory NSCLC is refractory to at least two prior systemic treatment courses, not including neo-adjuvant or adjuvant therapies.

[0066] In some embodiments, the refractory NSCLC is refractory to an anti-PD-1 antibody selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, JS001, TSR-042, pidilizumab, (BGB-A317, SHR-1210, REGN2810, MDX-1106, PDR001, anti-PD-1 from clone: RMP1-14; and an anti-PD-1 antibodies disclosed in U.S. Patent No. 8,008,449, durvalumab, atezolizumab, avelumab, and fragments, derivatives, variants, as well as biosimilars thereof.

[0067] In some embodiments, the refractory NSCLC is refractory to pembrolizumab or a biosimilar thereof.

[0068] In some embodiments, the refractory NSCLC is refractory to nivolumab or a biosimilar thereof.

[0069] In some embodiments, the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof.

[0070] In some embodiments, the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof and pembrolizumab or a biosimilar thereof.

[0071] In some embodiments, the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof and nivolumab or a biosimilar thereof.

[0072] In some embodiments, the refractory NSCLC is refractory to durvalumab or a biosimilar thereof.

[0073] In some embodiments, the refractory NSCLC is refractory to atezolizumab or a biosimilar thereof.

[0074] In some embodiments, the refractory NSCLC is refractory to avelumab or a biosimilar thereof.

[0075] In some embodiments, the initial expansion is performed over a period of 21 days or less.

[0076] In some embodiments, the initial expansion is performed over a period of 14 days or less.

[0077] In some embodiments, the initial expansion is performed over a period of about 11 days and the rapid expansion is performed over a period of about 11 days.

[0078] In some embodiments, the IL-2 is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL in the first cell culture medium.

[0079] In some embodiments, 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 in the second cell culture medium.

[0080] In some embodiments, the initial expansion is performed using a gas permeable container.

[0081] In some embodiments, the rapid expansion is performed using a gas permeable container.

[0082] In some embodiments, the first cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

[0083] In some embodiments, the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

[0084] In some embodiments, the method further comprises the step of treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the third population of TILs to the patient.

[0085] In some embodiments, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m²/day for two days followed by administration of fludarabine at a dose of 25 mg/m²/day for five days.

[0086] In some embodiments, the method further comprises the step of treating the patient with an IL-2 regimen starting on the day after administration of the third population of TILs to the patient.

[0087] 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.

[0088] In some embodiments, the invention provides a method for treating a subject with non-small cell lung carcinoma (NSCLC), wherein the cancer is refractory to treatment with an anti-PD-1 antibody, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

- (a) obtaining and/or receiving a first population of TILs from one or more tumors resected from a subject by processing the one or more tumors 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 ~~3-113~~ 44 days to obtain the second population of TILs, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs, 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~~ 7-14 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second 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; and
- (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; and
- (h) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (g) to the subject.

[0089] In some embodiments, “obtaining” indicates the TILs employed in the method and/or process can be derived directly from the sample (including from a surgical resection, needle biopsy, core biopsy, small biopsy, or other sample) as part of the method and/or process steps. In some embodiments, “receiving” indicates the TILs employed in the method and/or process can be derived indirectly from the sample (including from a surgical resection, needle biopsy, core biopsy, small biopsy, or other sample) and then employed in the method and/or process, (for example, where step (a) begins with TILs that have already been derived from the sample by a separate process not included in part (a), such TILs could be referred to as “received”).

[0090] In some embodiments, the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody.

[0091] In some embodiments, the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody.

[0092] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent.

[0093] In some embodiments, the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has been previously treated a chemotherapeutic agent.

[0094] In some embodiments, the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has been previously treated a chemotherapeutic agent.

[0095] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent.

[0096] In some embodiments, the refractory NSCLC has low expression of PD-L1.

[0097] In some embodiments, the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has low expression of PD-L1.

[0098] In some embodiments, the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has low expression of PD-L1.

[0099] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent and has low expression of PD-L1.

[00100] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent and has low expression of PD-L1.

[00101] In some embodiments, the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has bulky disease at baseline.

[00102] In some embodiments, the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has bulky disease at baseline.

[00103] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent and has bulky disease at baseline.

[00104] In some embodiments, the refractory NSCLC has been treated with a chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent and has bulky disease at baseline.

[00105] In some embodiments, bulky disease is indicated where the maximal tumor diameter is greater than 7 cm measured in either the transverse or coronal plane or swollen lymph nodes with a short-axis diameter of 20 mm or greater.

[00106] In some embodiments, the refractory NSCLC is refractory to at least two prior systemic treatment courses, not including neo-adjuvant or adjuvant therapies.

[00107] In some embodiments, the refractory NSCLC is refractory to an anti-PD-1 antibody selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, JS001, TSR-042, pidilizumab, (BGB-A317, SHR-1210, REGN2810, MDX-1106, PDR001, anti-PD-1 from clone: RMP1-14; and an anti-PD-1 antibodies disclosed in U.S. Patent No. 8,008,449, durvalumab, atezolizumab, avelumab, and fragments, derivatives, variants, as well as biosimilars thereof.

[00108] In some embodiments, the refractory NSCLC is refractory to pembrolizumab or a biosimilar thereof.

[00109] In some embodiments, the refractory NSCLC is refractory to nivolumab or a biosimilar thereof.

[00110] In some embodiments, the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof.

[00111] In some embodiments, the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof and pembrolizumab or a biosimilar thereof.

[00112] In some embodiments, the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof and nivolumab or a biosimilar thereof.

[00113] In some embodiments, the refractory NSCLC is refractory to durvalumab or a biosimilar thereof.

[00114] In some embodiments, the refractory NSCLC is refractory to atezolizumab or a biosimilar thereof.

[00115] In some embodiments, the refractory NSCLC is refractory to avelumab or a biosimilar thereof.

[00116] In some embodiments, the initial expansion is performed over a period of 21 days or less.

[00117] In some embodiments, the initial expansion is performed over a period of 14 days or less.

[00118] In some embodiments, the initial expansion is performed over a period of about 3-11 days and the second expansion is performed over a period of about 7-11 days.

[00119] In some embodiments, the initial expansion is performed over a period of about 11 days and the rapid expansion is performed over a period of about 11 days.

[00120] In some embodiments, the IL-2 is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL in the first cell culture medium.

[00121] In some embodiments, 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 in the second cell culture medium.

[00122] In some embodiments, the initial expansion is performed using a gas permeable container.

[00123] In some embodiments, the rapid expansion is performed using a gas permeable container.

[00124] In some embodiments, the first cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

[00125] In some embodiments, the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

[00126] In some embodiments, the method further comprises the step of treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the third population of TILs to the patient.

[00127] In some embodiments, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m²/day for two days followed by administration of fludarabine at a dose of 25 mg/m²/day for five days.

[00128] In some embodiments, the method further comprises the step of treating the patient with an IL-2 regimen starting on the day after administration of the third population of TILs to the patient.

[00129] 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[00130] **Figure 1:** Exemplary Process 2A chart providing an overview of Steps A through F.

[00131] **Figure 2:** Process Flow Chart of Process 2A.

[00132] **Figure 3:** Shows a diagram of an embodiment of a cryopreserved TIL exemplary manufacturing process (~22 days).

[00133] **Figure 4:** Shows a diagram of an embodiment of process 2A, a 22-day process for TIL manufacturing.

[00134] **Figure 5:** Comparison table of Steps A through F from exemplary embodiments of process 1C and process 2A.

[00135] **Figure 6:** Detailed comparison of an embodiment of process 1C and an embodiment of process 2A.

[00136] **Figure 7:** Study Flowcharts for Combination Cohorts: Cohort 1A (MM), Cohort 2A (HNSCC), and Cohort 3A (NSCLC). Abbreviations: Cy=cyclophosphamide; EOA=end of assessment; EOS=end of study; EOT=end of treatment; Flu=fludarabine; IL-2=interleukin-2; NMA-LD=nonmyeloablative lymphodepletion; Q3W=every 3 weeks; TIL=tumor infiltrating lymphocytes. Patients in Cohorts 1A, 2A, and 3A will receive a single infusion of pembrolizumab after the completion of their tumor resection for TIL production and baseline scans before the initiation of the NMA-LD regimen. For this particular study, the next dose of pembrolizumab was not administered earlier than following the completion of IL-2 and continue Q3W $\pm$ 3 days thereafter for $\leq$ 2 years (24 months) or until disease progression or unacceptable toxicity, whichever occurred first.

[00137] **Figure 8:** Study Flowchart for Single-agent Cohort: Cohort 3B (NSCLC). Abbreviations: Cy=cyclophosphamide; EOA=end of assessment; EOS=end of study; EOT=end of treatment; Flu=fludarabine; IL-2=interleukin-2; NMA-LD=nonmyeloablative lymphodepletion; TIL=tumor infiltrating lymphocytes.

[00138] **Figure 9:** Shows a diagram of an embodiment of process 2A, a 22-day process for TIL manufacturing.

[00139] **Figure 10:** Provides the structures I-A and I-B, the cylinders refer to individual polypeptide binding domains. Structures I-A and I-B comprise three linearly-linked TNFRSF binding domains derived from e.g., 4-1BBL or an antibody that binds 4-1BB, which fold to form a trivalent protein, which is then linked to a second trivalent protein through IgG1-Fc

(including CH3 and CH2 domains) is then used to link two of the trivalent proteins together through disulfide bonds (small elongated ovals), stabilizing the structure and providing an agonists capable of bringing together the intracellular signaling domains of the six receptors and signaling proteins to form a signaling complex. The TNFRSF binding domains denoted as cylinders may be scFv domains comprising, e.g., a VH and a VL chain connected by a linker that may comprise hydrophilic residues and Gly and Ser sequences for flexibility, as well as Glu and Lys for solubility.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

- [00140]** SEQ ID NO:1 is the amino acid sequence of the heavy chain of muromonab.
- [00141]** SEQ ID NO:2 is the amino acid sequence of the light chain of muromonab.
- [00142]** SEQ ID NO:3 is the amino acid sequence of a recombinant human IL-2 protein.
- [00143]** SEQ ID NO:4 is the amino acid sequence of aldesleukin.
- [00144]** SEQ ID NO:5 is the amino acid sequence of a recombinant human IL-4 protein.
- [00145]** SEQ ID NO:6 is the amino acid sequence of a recombinant human IL-7 protein.
- [00146]** SEQ ID NO:7 is the amino acid sequence of a recombinant human IL-15 protein.
- [00147]** SEQ ID NO:8 is the amino acid sequence of a recombinant human IL-21 protein.
- [00148]** SEQ ID NO:9 is the amino acid sequence of human 4-1BB.
- [00149]** SEQ ID NO:10 is the amino acid sequence of murine 4-1BB.
- [00150]** SEQ ID NO:11 is the heavy chain for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).
- [00151]** SEQ ID NO:12 is the light chain for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).
- [00152]** SEQ ID NO:13 is the heavy chain variable region (VH) for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).
- [00153]** SEQ ID NO:14 is the light chain variable region (VL) for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).
- [00154]** SEQ ID NO:15 is the heavy chain CDRI for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00155] SEQ ID NO:16 is the heavy chain CDR2 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00156] SEQ ID NO:17 is the heavy chain CDR3 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00157] SEQ ID NO:18 is the light chain CDR1 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00158] SEQ ID NO:19 is the light chain CDR2 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00159] SEQ ID NO:20 is the light chain CDR3 for the 4-1BB agonist monoclonal antibody utomilumab (PF-05082566).

[00160] SEQ ID NO:21 is the heavy chain for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00161] SEQ ID NO:22 is the light chain for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00162] SEQ ID NO:23 is the heavy chain variable region (VH) for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00163] SEQ ID NO:24 is the light chain variable region (VL) for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00164] SEQ ID NO:25 is the heavy chain CDR1 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00165] SEQ ID NO:26 is the heavy chain CDR2 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00166] SEQ ID NO:27 is the heavy chain CDR3 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00167] SEQ ID NO:28 is the light chain CDR1 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00168] SEQ ID NO:29 is the light chain CDR2 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00169] SEQ ID NO:30 is the light chain CDR3 for the 4-1BB agonist monoclonal antibody urelumab (BMS-663513).

[00170] SEQ ID NO:31 is an Fc domain for a TNFRSF agonist fusion protein.

[00171] SEQ ID NO:32 is a linker for a TNFRSF agonist fusion protein.

[00172] SEQ ID NO:33 is a linker for a TNFRSF agonist fusion protein.

[00173] SEQ ID NO:34 is a linker for a TNFRSF agonist fusion protein.

[00174] SEQ ID NO:35 is a linker for a TNFRSF agonist fusion protein.

[00175] SEQ ID NO:36 is a linker for a TNFRSF agonist fusion protein.

[00176] SEQ ID NO:37 is a linker for a TNFRSF agonist fusion protein.

[00177] SEQ ID NO:38 is a linker for a TNFRSF agonist fusion protein.

[00178] SEQ ID NO:39 is a linker for a TNFRSF agonist fusion protein.

[00179] SEQ ID NO:40 is a linker for a TNFRSF agonist fusion protein.

[00180] SEQ ID NO:41 is a linker for a TNFRSF agonist fusion protein.

[00181] SEQ ID NO:42 is an Fc domain for a TNFRSF agonist fusion protein.

[00182] SEQ ID NO:43 is a linker for a TNFRSF agonist fusion protein.

[00183] SEQ ID NO:44 is a linker for a TNFRSF agonist fusion protein.

[00184] SEQ ID NO:45 is a linker for a TNFRSF agonist fusion protein.

[00185] SEQ ID NO:46 is a 4-1BB ligand (4-1BBL) amino acid sequence.

[00186] SEQ ID NO:47 is a soluble portion of 4-1BBL polypeptide.

[00187] SEQ ID NO:48 is a heavy chain variable region (VH) for the 4-1BB agonist antibody 4B4-1-1 version 1.

[00188] SEQ ID NO:49 is a light chain variable region (VL) for the 4-1BB agonist antibody 4B4-1-1 version 1.

[00189] SEQ ID NO:50 is a heavy chain variable region (VH) for the 4-1BB agonist antibody 4B4-1-1 version 2.

[00190] SEQ ID NO:51 is a light chain variable region (VL) for the 4-1BB agonist antibody 4B4-1-1 version 2.

[00191] SEQ ID NO:52 is a heavy chain variable region (VH) for the 4-1BB agonist antibody H39E3-2.

[00192] SEQ ID NO:53 is a light chain variable region (VL) for the 4-1BB agonist antibody H39E3-2.

[00193] SEQ ID NO:54 is the amino acid sequence of human OX40.

[00194] SEQ ID NO:55 is the amino acid sequence of murine OX40.

[00195] SEQ ID NO:56 is the heavy chain for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00196] SEQ ID NO:57 is the light chain for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00197] SEQ ID NO:58 is the heavy chain variable region (VH) for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00198] SEQ ID NO:59 is the light chain variable region (VL) for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00199] SEQ ID NO:60 is the heavy chain CDR1 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00200] SEQ ID NO:61 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00201] SEQ ID NO:62 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00202] SEQ ID NO:63 is the light chain CDR1 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00203] SEQ ID NO:64 is the light chain CDR2 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00204] SEQ ID NO:65 is the light chain CDR3 for the OX40 agonist monoclonal antibody tavolixizumab (MEDI-0562).

[00205] SEQ ID NO:66 is the heavy chain for the OX40 agonist monoclonal antibody 11D4.

[00206] SEQ ID NO:67 is the light chain for the OX40 agonist monoclonal antibody 11D4.

[00207] SEQ ID NO:68 is the heavy chain variable region (VH) for the OX40 agonist monoclonal antibody 11D4.

[00208] SEQ ID NO:69 is the light chain variable region (VL) for the OX40 agonist monoclonal antibody 11D4.

[00209] SEQ ID NO:70 is the heavy chain CDR1 for the OX40 agonist monoclonal antibody 11D4.

[00210] SEQ ID NO:71 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody 11D4.

[00211] SEQ ID NO:72 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody 11D4.

[00212] SEQ ID NO:73 is the light chain CDR1 for the OX40 agonist monoclonal antibody 11D4.

[00213] SEQ ID NO:74 is the light chain CDR2 for the OX40 agonist monoclonal antibody 11D4.

[00214] SEQ ID NO:75 is the light chain CDR3 for the OX40 agonist monoclonal antibody 11D4.

[00215] SEQ ID NO:76 is the heavy chain for the OX40 agonist monoclonal antibody 18D8.

[00216] SEQ ID NO:77 is the light chain for the OX40 agonist monoclonal antibody 18D8.

[00217] SEQ ID NO:78 is the heavy chain variable region (VH) for the OX40 agonist monoclonal antibody 18D8.

[00218] SEQ ID NO:79 is the light chain variable region (VL) for the OX40 agonist monoclonal antibody 18D8.

[00219] SEQ ID NO:80 is the heavy chain CDR1 for the OX40 agonist monoclonal antibody 18D8.

[00220] SEQ ID NO:81 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody 18D8.

[00221] SEQ ID NO:82 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody 18D8.

[00222] SEQ ID NO:83 is the light chain CDR1 for the OX40 agonist monoclonal antibody 18D8.

[00223] SEQ ID NO:84 is the light chain CDR2 for the OX40 agonist monoclonal antibody 18D8.

[00224] SEQ ID NO:85 is the light chain CDR3 for the OX40 agonist monoclonal antibody 18D8.

[00225] SEQ ID NO:86 is the heavy chain variable region (VH) for the OX40 agonist monoclonal antibody Hu119-122.

[00226] SEQ ID NO:87 is the light chain variable region (VL) for the OX40 agonist monoclonal antibody Hu119-122.

[00227] SEQ ID NO:88 is the heavy chain CDR1 for the OX40 agonist monoclonal antibody Hu119-122.

[00228] SEQ ID NO:89 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody Hu119-122.

[00229] SEQ ID NO:90 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody Hu119-122.

[00230] SEQ ID NO:91 is the light chain CDR1 for the OX40 agonist monoclonal antibody Hu119-122.

[00231] SEQ ID NO:92 is the light chain CDR2 for the OX40 agonist monoclonal antibody Hu119-122.

[00232] SEQ ID NO:93 is the light chain CDR3 for the OX40 agonist monoclonal antibody Hu119-122.

[00233] SEQ ID NO:94 is the heavy chain variable region (VH) for the OX40 agonist monoclonal antibody Hu106-222.

[00234] SEQ ID NO:95 is the light chain variable region (VL) for the OX40 agonist monoclonal antibody Hu106-222.

[00235] SEQ ID NO:96 is the heavy chain CDR1 for the OX40 agonist monoclonal antibody Hu106-222.

[00236] SEQ ID NO:97 is the heavy chain CDR2 for the OX40 agonist monoclonal antibody Hu106-222.

[00237] SEQ ID NO:98 is the heavy chain CDR3 for the OX40 agonist monoclonal antibody Hu106-222.

[00238] SEQ ID NO:99 is the light chain CDR1 for the OX40 agonist monoclonal antibody Hu106-222.

[00239] SEQ ID NO:100 is the light chain CDR2 for the OX40 agonist monoclonal antibody Hu106-222.

[00240] SEQ ID NO:101 is the light chain CDR3 for the OX40 agonist monoclonal antibody Hu106-222.

[00241] SEQ ID NO:102 is an OX40 ligand (OX40L) amino acid sequence.

[00242] SEQ ID NO:103 is a soluble portion of OX40L polypeptide.

[00243] SEQ ID NO:104 is an alternative soluble portion of OX40L polypeptide.

[00244] SEQ ID NO:105 is the heavy chain variable region (VH) for the OX40 agonist monoclonal antibody 008.

[00245] SEQ ID NO:106 is the light chain variable region (VL) for the OX40 agonist monoclonal antibody 008.

[00246] SEQ ID NO:107 is the heavy chain variable region (VH) for the OX40 agonist monoclonal antibody 011.

[00247] SEQ ID NO:108 is the light chain variable region (VL) for the OX40 agonist monoclonal antibody 011.

[00248] SEQ ID NO:109 is the heavy chain variable region (VH) for the OX40 agonist monoclonal antibody 021.

[00249] SEQ ID NO:110 is the light chain variable region (VL) for the OX40 agonist monoclonal antibody 021.

[00250] SEQ ID NO:111 is the heavy chain variable region (VH) for the OX40 agonist monoclonal antibody 023.

[00251] SEQ ID NO:112 is the light chain variable region (VL) for the OX40 agonist monoclonal antibody 023.

[00252] SEQ ID NO:113 is the heavy chain variable region (VH) for an OX40 agonist monoclonal antibody.

[00253] SEQ ID NO:114 is the light chain variable region (VL) for an OX40 agonist monoclonal antibody.

[00254] SEQ ID NO:115 is the heavy chain variable region (VH) for an OX40 agonist monoclonal antibody.

[00255] SEQ ID NO:116 is the light chain variable region (VL) for an OX40 agonist monoclonal antibody.

[00256] SEQ ID NO:117 is the heavy chain variable region (VH) for a humanized OX40 agonist monoclonal antibody.

[00257] SEQ ID NO:118 is the heavy chain variable region (VH) for a humanized OX40 agonist monoclonal antibody.

[00258] SEQ ID NO:119 is the light chain variable region (VL) for a humanized OX40 agonist monoclonal antibody.

[00259] SEQ ID NO:120 is the light chain variable region (VL) for a humanized OX40 agonist monoclonal antibody.

[00260] SEQ ID NO:121 is the heavy chain variable region (VH) for a humanized OX40 agonist monoclonal antibody.

[00261] SEQ ID NO:122 is the heavy chain variable region (VH) for a humanized OX40 agonist monoclonal antibody.

[00262] SEQ ID NO:123 is the light chain variable region (VL) for a humanized OX40 agonist monoclonal antibody.

[00263] SEQ ID NO:124 is the light chain variable region (VL) for a humanized OX40 agonist monoclonal antibody.

[00264] SEQ ID NO:125 is the heavy chain variable region (VH) for an OX40 agonist monoclonal antibody.

[00265] SEQ ID NO:126 is the light chain variable region (VL) for an OX40 agonist monoclonal antibody.

- [00266]** SEQ ID NO:127 is the heavy chain amino acid sequence of the PD-1 inhibitor nivolumab.
- [00267]** SEQ ID NO:128 is the light chain amino acid sequence of the PD-1 inhibitor nivolumab.
- [00268]** SEQ ID NO:129 is the heavy chain variable region (V_H) amino acid sequence of the PD-1 inhibitor nivolumab.
- [00269]** SEQ ID NO:130 is the light chain variable region (V_L) amino acid sequence of the PD-1 inhibitor nivolumab.
- [00270]** SEQ ID NO:131 is the heavy chain CDR1 amino acid sequence of the PD-1 inhibitor nivolumab.
- [00271]** SEQ ID NO:132 is the heavy chain CDR2 amino acid sequence of the PD-1 inhibitor nivolumab.
- [00272]** SEQ ID NO:133 is the heavy chain CDR3 amino acid sequence of the PD-1 inhibitor nivolumab.
- [00273]** SEQ ID NO:134 is the light chain CDR1 amino acid sequence of the PD-1 inhibitor nivolumab.
- [00274]** SEQ ID NO:135 is the light chain CDR2 amino acid sequence of the PD-1 inhibitor nivolumab.
- [00275]** SEQ ID NO:136 is the light chain CDR3 amino acid sequence of the PD-1 inhibitor nivolumab.
- [00276]** SEQ ID NO:137 is the heavy chain amino acid sequence of the PD-1 inhibitor pembrolizumab.
- [00277]** SEQ ID NO:138 is the light chain amino acid sequence of the PD-1 inhibitor pembrolizumab.
- [00278]** SEQ ID NO:139 is the heavy chain variable region (V_H) amino acid sequence of the PD-1 inhibitor pembrolizumab.
- [00279]** SEQ ID NO:140 is the light chain variable region (V_L) amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00280] SEQ ID NO:141 is the heavy chain CDR1 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00281] SEQ ID NO:142 is the heavy chain CDR2 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00282] SEQ ID NO:143 is the heavy chain CDR3 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00283] SEQ ID NO:144 is the light chain CDR1 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00284] SEQ ID NO:145 is the light chain CDR2 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00285] SEQ ID NO:146 is the light chain CDR3 amino acid sequence of the PD-1 inhibitor pembrolizumab.

[00286] SEQ ID NO:147 is the heavy chain amino acid sequence of the PD-L1 inhibitor durvalumab.

[00287] SEQ ID NO:148 is the light chain amino acid sequence of the PD-L1 inhibitor durvalumab.

[00288] SEQ ID NO:149 is the heavy chain variable region (V_H) amino acid sequence of the PD-L1 inhibitor durvalumab.

[00289] SEQ ID NO:150 is the light chain variable region (V_L) amino acid sequence of the PD-L1 inhibitor durvalumab.

[00290] SEQ ID NO:151 is the heavy chain CDR1 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00291] SEQ ID NO:152 is the heavy chain CDR2 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00292] SEQ ID NO:153 is the heavy chain CDR3 amino acid sequence of the PD-L1 inhibitor durvalumab.

[00293] SEQ ID NO:154 is the light chain CDR1 amino acid sequence of the PD-L1 inhibitor durvalumab.

- [00294]** SEQ ID NO:155 is the light chain CDR2 amino acid sequence of the PD-L1 inhibitor durvalumab.
- [00295]** SEQ ID NO:156 is the light chain CDR3 amino acid sequence of the PD-L1 inhibitor durvalumab.
- [00296]** SEQ ID NO:157 is the heavy chain amino acid sequence of the PD-L1 inhibitor avelumab.
- [00297]** SEQ ID NO:158 is the light chain amino acid sequence of the PD-L1 inhibitor avelumab.
- [00298]** SEQ ID NO:159 is the heavy chain variable region (V_H) amino acid sequence of the PD-L1 inhibitor avelumab.
- [00299]** SEQ ID NO:160 is the light chain variable region (V_L) amino acid sequence of the PD-L1 inhibitor avelumab.
- [00300]** SEQ ID NO:161 is the heavy chain CDR1 amino acid sequence of the PD-L1 inhibitor avelumab.
- [00301]** SEQ ID NO:162 is the heavy chain CDR2 amino acid sequence of the PD-L1 inhibitor avelumab.
- [00302]** SEQ ID NO:163 is the heavy chain CDR3 amino acid sequence of the PD-L1 inhibitor avelumab.
- [00303]** SEQ ID NO:164 is the light chain CDR1 amino acid sequence of the PD-L1 inhibitor avelumab.
- [00304]** SEQ ID NO:165 is the light chain CDR2 amino acid sequence of the PD-L1 inhibitor avelumab.
- [00305]** SEQ ID NO:166 is the light chain CDR3 amino acid sequence of the PD-L1 inhibitor avelumab.
- [00306]** SEQ ID NO:167 is the heavy chain amino acid sequence of the PD-L1 inhibitor atezolizumab.
- [00307]** SEQ ID NO:168 is the light chain amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00308] SEQ ID NO:169 is the heavy chain variable region (V_H) amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00309] SEQ ID NO:170 is the light chain variable region (V_L) amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00310] SEQ ID NO:171 is the heavy chain CDR1 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00311] SEQ ID NO:172 is the heavy chain CDR2 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00312] SEQ ID NO:173 is the heavy chain CDR3 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00313] SEQ ID NO:174 is the light chain CDR1 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00314] SEQ ID NO:175 is the light chain CDR2 amino acid sequence of the PD-L1 inhibitor atezolizumab.

[00315] SEQ ID NO:176 is the light chain CDR3 amino acid sequence of the PD-L1 inhibitor atezolizumab.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

[00316] Adoptive cell therapy utilizing TILs cultured *ex vivo* by the Rapid Expansion Protocol (REP) has produced successful adoptive cell therapy following host immunosuppression in patients with cancer such as melanoma. Current infusion acceptance parameters rely on readouts of the composition of TILs (*e.g.*, CD28, CD8, or CD4 positivity) and on the numerical folds of expansion and viability of the REP product.

[00317] Current REP protocols give little insight into the health of the TIL that will be infused into the patient. T cells undergo a profound metabolic shift during the course of their maturation from naïve to effector T cells (see Chang, *et al.*, *Nat. Immunol.* **2016**, *17*, 364, hereby expressly incorporated in its entirety, and in particular for the discussion and markers of anaerobic and aerobic metabolism). For example, naïve T cells rely on mitochondrial respiration to produce ATP, while mature, healthy effector T cells such as TIL are highly

glycolytic, relying on aerobic glycolysis to provide the bioenergetics substrates they require for proliferation, migration, activation, and anti-tumor efficacy.

[00318] Current TIL manufacturing and treatment processes are limited by length, cost, sterility concerns, and other factors described herein such that the potential to treat patients which are refractory to anti-PD1 and as such have been severely limited. There is an urgent need to provide TIL manufacturing processes and therapies based on such processes that are appropriate for use in treating patients for whom very few or no viable treatment options remain. The present invention meets this need by providing a shortened manufacturing process for use in generating TILs which can then be employed in the treatment of non-small cell lung carcinoma (NSCLC) patients whom are refractory to anti-PD-1 treatment.

II. Definitions

[00319] 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.

[00320] 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.

[00321] The term “*in vivo*” refers to an event that takes place in a subject's body.

[00322] 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.

[00323] 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.

[00324] 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.

[00325]

[00326] 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.

[00327] 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.

[00328] 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.

[00329] 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.

[00330] 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 $\alpha\beta$, 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.

[00331] 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.

[00332] 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 secrete 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.

[00333] 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 secrete 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.

[00334] 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.

[00335] 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.

[00336] 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. Preferably, the peripheral blood mononuclear cells are irradiated allogeneic peripheral blood mononuclear cells. PBMCs are a type of antigen-presenting cell.

[00337] 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, oteelixizumab, teplizumab, and visilizumab.

[00338] 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.

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

[00339] 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 NKTR-214, available from Nektar Therapeutics, South San Francisco, CA, USA. NKTR-214 and pegylated IL-2 suitable for use in the invention is 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 4902,502, 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.

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	PTSSSTKKTK LQLEHLLLDL QMILNGINNY KNPKLTRMLT FKFYMPKKAT ELKHLQCLEE ELKPLEEVLN LAQSKNFHLR PRDLISNINV IVLELKGSSET TFMCEYADET ATIVEFLNRW ITFSQSIIST LT	60 120 132
SEQ ID NO:5 recombinant human IL-4 (rhIL-4)	MHKCDITLQE IIKTLNSLTE QKTLCTELTV TDIFAASKNT TEKETFCAA TVLRQFYSHH EKDTRCLGAT AQQFHRHKQL IRFLKRLDRN LWGLAGLNSC PVKEANQSTL ENFLERLTKI MREKYSKCSS	60 120 130
SEQ ID NO:6 recombinant human IL-7 (rhIL-7)	MDCDIEGKDG KQYESVLMVS IDQLDLSMKE IGSNCLNNEF NFFKRHICDA NKEGMFLFRA ARKLRQFLKM NSTGDFDLHL LKVSEGTTIL LNCTGQVKGR KPAALGEAQP TKSLEENKSL KEQKKLNDLC FLKRLQEI KTCWNKILMGT KEH	60 120 153
SEQ ID NO:7 recombinant human IL-15 (rhIL-15)	MNVVNVISDL KKIEDLIQSM HIDATLYTES DVHPSCKVTA MKCFLLLELQV ISLESGDASI HDTVENLIIL ANNSLSSNGN VTESGCKECE ELEEKNIKEF LQSFVHIVQM FINTS	60 115
SEQ ID NO:8 recombinant human IL-21 (rhIL-21)	MQDRHMIRMRL QLIDIVDQLK NYVNDLVPEF LPAPEDVETN CEWSAFSCFQ KAQLKSANTG NNERIINVTI KKLKRKPPST NAGRRQKHRL TCPSCDSYEK KPPKEFLERF KSLQKMIHQ HLSSRTHGSE DS	60 120 132

[00340] 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 IgG₁ expression from B cells. Recombinant human IL-4 suitable for use in the invention is commercially available from multiple suppliers, including ProSpec-Tany TechnoGene Ltd., East Brunswick, NJ, USA (Cat. No. CYT-211) and ThermoFisher Scientific, Inc., Waltham, MA, USA (human IL-15 recombinant protein, Cat. No. Gibco CTP0043). The amino acid sequence of recombinant human IL-4 suitable for use in the invention is given in Table 2 (SEQ ID NO:5).

[00341] The term “IL-7” (also referred to herein as “IL7”) refers to a glycosylated tissue-derived cytokine known as interleukin 7, which may be obtained from stromal and epithelial cells, as well as from dendritic cells. Fry and Mackall, *Blood* **2002**, 99, 3892-904. IL-7 can stimulate the development of T cells. IL-7 binds to the IL-7 receptor, a heterodimer consisting of IL-7 receptor alpha and common gamma chain receptor, which in a series of signals important for T cell development within the thymus and survival within the periphery. Recombinant human IL-7 suitable for use in the invention is commercially available from

multiple suppliers, including ProSpec-Tany TechnoGene Ltd., East Brunswick, NJ, USA (Cat. No. CYT-254) and ThermoFisher Scientific, Inc., Waltham, MA, USA (human IL-15 recombinant protein, Cat. No. Gibco PHC0071). The amino acid sequence of recombinant human IL-7 suitable for use in the invention is given in Table 2 (SEQ ID NO:6).

[00342] 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:7).

[00343] The term “IL-21” (also referred to herein as “IL21”) refers to the pleiotropic cytokine protein known as interleukin-21, and includes all forms of IL-21 including human and mammalian forms, conservative amino acid substitutions, glycoforms, biosimilars, and variants thereof. IL-21 is described, *e.g.*, in Spolski and Leonard, *Nat. Rev. Drug. Disc.* **2014**, 13, 379-95, the disclosure of which is incorporated by reference herein. IL-21 is primarily produced by natural killer T cells and activated human CD4⁺ T cells. Recombinant human IL-21 is a single, non-glycosylated polypeptide chain containing 132 amino acids with a molecular mass of 15.4 kDa. Recombinant human IL-21 is commercially available from multiple suppliers, including ProSpec-Tany TechnoGene Ltd., East Brunswick, NJ, USA (Cat. No. CYT-408-b) and ThermoFisher Scientific, Inc., Waltham, MA, USA (human IL-21 recombinant protein, Cat. No. 14-8219-80). The amino acid sequence of recombinant human IL-21 suitable for use in the invention is given in Table 2 (SEQ ID NO:8).

[00344] When “an anti-tumor effective amount”, “an tumor-inhibiting effective amount”, or “therapeutic amount” is indicated, the precise amount of the compositions of the present invention to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject). It can generally be stated that a pharmaceutical composition

comprising the tumor infiltrating lymphocytes (e.g. secondary TILs or genetically modified cytotoxic lymphocytes) described herein may be administered at a dosage of 10^4 to 10^{11} cells/kg body weight (e.g., 10^5 to 10^6 , 10^5 to 10^{10} , 10^5 to 10^{11} , 10^6 to 10^{10} , 10^6 to 10^{11} , 10^7 to 10^{11} , 10^7 to 10^{10} , 10^8 to 10^{11} , 10^8 to 10^{10} , 10^9 to 10^{11} , or 10^9 to 10^{10} cells/kg body weight), including all integer values within those ranges. Tumor infiltrating lymphocytes (including in some cases, genetically modified cytotoxic lymphocytes) compositions may also be administered multiple times at these dosages. The tumor infiltrating lymphocytes (including in some cases, genetically) can be administered by using infusion techniques that are commonly known in immunotherapy (*see*, e.g., Rosenberg et al., *New Eng. J. of Med.* 319: 1676, 1988). The optimal dosage and treatment regime for a particular patient can readily be determined by one skilled in the art of medicine by monitoring the patient for signs of disease and adjusting the treatment accordingly.

[00345] 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.

[00346] In an embodiment, 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 an embodiment, 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 an embodiment, 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.

[00347] Experimental findings indicate that lymphodepletion prior to adoptive transfer of tumor-specific T lymphocytes plays a key role in enhancing treatment efficacy by eliminating

regulatory T cells and competing elements of the immune system (“cytokine sinks”). Accordingly, some embodiments of the invention utilize a lymphodepletion step (sometimes also referred to as “immunosuppressive conditioning”) on the patient prior to the introduction of the rTILs of the invention.

[00348] 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.

[00349] 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.

[00350] 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).

[00351] 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.

[00352] 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.

[00353] 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).

[00354] 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 $\alpha\beta$, 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 γ) 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.

[00355] 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.

[00356] 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.

[00357] 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.

[00358] 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.

[00359] 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.”

[00360] 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 (C1q) of the classical complement system.

[00361] 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.

[00362] 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.

[00363] 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 F_d fragment consisting of the V_H and $CH1$ domains; (iv) a F_v 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 F_v 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 F_v (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.

[00364] 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.

[00365] 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 an embodiment, 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.

[00366] 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*.

[00367] As used herein, “isotype” refers to the antibody class (e.g., IgM or IgG1) that is encoded by the heavy chain constant region genes.

[00368] 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.”

[00369] 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.

[00370] 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.

[00371] 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.

[00372] 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.

[00373] 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 α -L-fucosidase removes fucosyl residues from antibodies as described in Tarentino, *et al.*, *Biochem.* **1975**, *14*, 5516-5523.

[00374] “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 (C₁-C₁₀)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.

[00375] 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. TIL Manufacturing Processes

[00376] An exemplary TIL process known as process 2A containing some of these features is depicted in Figure 2, and some of the advantages of this embodiment of the present invention over process 1C are described in Figures F and G. An embodiment of process 2A is shown Figure 1.

[00377] As discussed herein, the present invention can include a step relating to the restimulation of cryopreserved TILs to increase their metabolic activity and thus relative health prior to transplant into a patient, and methods of testing said metabolic health. As generally outlined herein, TILs are generally taken from a patient sample and manipulated to expand their number prior to transplant into a patient. In some embodiments, the TILs may be optionally genetically manipulated as discussed below.

[00378] In some embodiments, the TILs may be cryopreserved. Once thawed, they may also be restimulated to increase their metabolism prior to infusion into a patient.

[00379] In some embodiments, the first expansion (including processes referred to as the preREP as well as processes shown in Figure 1 as Step A) is shortened to 3 to 14 days and the

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

[00380] The “Step” Designations A, B, C, *etc.*, below are in reference to Figure 1 and in reference to certain embodiments described herein. The ordering of the Steps below and in Figure 1 is exemplary and any combination or order of steps, as well as additional steps, repetition of steps, and/or omission of steps is contemplated by the present application and the methods disclosed herein.

A. STEP A: Obtain Patient tumor sample

[00381] In general, TILs are initially obtained from a patient tumor sample (“primary TILs”) and then expanded into a larger population for further manipulation as described herein, optionally cryopreserved, restimulated as outlined herein and optionally evaluated for phenotype and metabolic parameters as an indication of TIL health.

[00382] A patient tumor sample may be obtained using methods known in the art, generally via surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells. In some embodiments, multilesional sampling is used. In some embodiments, surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells includes multilesional sampling (*i.e.*, obtaining samples from one or more tumor sites and/or locations in the patient, as well as one or more tumors in the same location or in close proximity). In general, the tumor sample may be from any solid tumor, including primary tumors, invasive tumors or metastatic tumors. The tumor sample may also be a liquid tumor, such as a tumor obtained from a hematological malignancy. The solid tumor may be of lung tissue. In some embodiments, useful TILs are obtained from non-small cell lung carcinoma (NSCLC).

[00383] Once obtained, the tumor sample is generally fragmented using sharp dissection into small pieces of between 1 to about 8 mm³, with from about 2-3 mm³ being particularly

useful. The TILs are cultured from these fragments using enzymatic tumor digests. Such tumor digests may be produced by incubation in enzymatic media (*e.g.*, Roswell Park Memorial Institute (RPMI) 1640 buffer, 2 mM glutamate, 10 mcg/mL gentamicine, 30 units/mL of DNase and 1.0 mg/mL of collagenase) followed by mechanical dissociation (*e.g.*, using a tissue dissociator). Tumor digests may be produced by placing the tumor in enzymatic media and mechanically dissociating the tumor for approximately 1 minute, followed by incubation for 30 minutes at 37 °C in 5% CO₂, followed by repeated cycles of mechanical dissociation and incubation under the foregoing conditions until only small tissue pieces are present. At the end of this process, if the cell suspension contains a large number of red blood cells or dead cells, a density gradient separation using FICOLL branched hydrophilic polysaccharide may be performed to remove these cells. Alternative methods known in the art may be used, such as those described in U.S. Patent Application Publication No. 2012/0244133 A1, the disclosure of which is incorporated by reference herein. Any of the foregoing methods may be used in any of the embodiments described herein for methods of expanding TILs or methods treating a cancer.

[00384] In general, the harvested cell suspension is called a “primary cell population” or a “freshly harvested” cell population.

[00385] In some embodiments, fragmentation includes physical fragmentation, including for example, dissection as well as digestion. In some embodiments, the fragmentation is physical fragmentation. In some embodiments, the fragmentation is dissection. In some embodiments, the fragmentation is by digestion. In some embodiments, TILs can be initially cultured from enzymatic tumor digests and tumor fragments obtained from patients. In an embodiment, TILs can be initially cultured from enzymatic tumor digests and tumor fragments obtained from patients.

[00386] In some embodiments, where the tumor is a solid tumor, the tumor undergoes physical fragmentation after the tumor sample is obtained in, for example, Step A (as provided in Figure 1). In some embodiments, the fragmentation occurs before cryopreservation. In some embodiments, the fragmentation occurs after cryopreservation. In some embodiments, the fragmentation occurs after obtaining the tumor and in the absence of any cryopreservation. In some embodiments, the tumor is fragmented and 10, 20, 30, 40 or more fragments or pieces are placed in each container for the first expansion. In some embodiments, the tumor is fragmented and 30 or 40 fragments or pieces are placed in each container for the first expansion. In some embodiments, the tumor is fragmented and 40

fragments or pieces are placed in each container for the first expansion. In some embodiments, the multiple fragments comprise about 4 to about 50 fragments, wherein each fragment has a volume of about 27 mm^3 . In some embodiments, the multiple fragments comprise about 30 to about 60 fragments with a total volume of about 1300 mm^3 to about 1500 mm^3 . In some embodiments, the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3 . In some embodiments, the multiple fragments comprise about 50 fragments with a total mass of about 1 gram to about 1.5 grams. In some embodiments, the multiple fragments comprise about 4 fragments.

[00387] In some embodiments, the TILs are obtained from tumor fragments. In some embodiments, the tumor fragment is obtained by sharp dissection. In some embodiments, the tumor fragment is between about 1 mm^3 and 10 mm^3 . In some embodiments, the tumor fragment is between about 1 mm^3 and 8 mm^3 . In some embodiments, the tumor fragment is about 1 mm^3 . In some embodiments, the tumor fragment is about 2 mm^3 . In some embodiments, the tumor fragment is about 3 mm^3 . In some embodiments, the tumor fragment is about 4 mm^3 . In some embodiments, the tumor fragment is about 5 mm^3 . In some embodiments, the tumor fragment is about 6 mm^3 . In some embodiments, the tumor fragment is about 7 mm^3 . In some embodiments, the tumor fragment is about 8 mm^3 . In some embodiments, the tumor fragment is about 9 mm^3 . In some embodiments, the tumor fragment is about 10 mm^3 . In some embodiments, the tumors are $1\text{-}4 \text{ mm} \times 1\text{-}4 \text{ mm} \times 1\text{-}4 \text{ mm}$. In some embodiments, the tumors are $1 \text{ mm} \times 1 \text{ mm} \times 1 \text{ mm}$. In some embodiments, the tumors are $2 \text{ mm} \times 2 \text{ mm} \times 2 \text{ mm}$. In some embodiments, the tumors are $3 \text{ mm} \times 3 \text{ mm} \times 3 \text{ mm}$. In some embodiments, the tumors are $4 \text{ mm} \times 4 \text{ mm} \times 4 \text{ mm}$.

[00388] In some embodiments, the tumors are resected in order to minimize the amount of hemorrhagic, necrotic, and/or fatty tissues on each piece. In some embodiments, the tumors are resected in order to minimize the amount of hemorrhagic tissue on each piece. In some embodiments, the tumors are resected in order to minimize the amount of necrotic tissue on each piece. In some embodiments, the tumors are resected in order to minimize the amount of fatty tissue on each piece.

[00389] In some embodiments, the tumor fragmentation is performed in order to maintain the tumor internal structure. In some embodiments, the tumor fragmentation is performed without preforming a sawing motion with a scalpel. In some embodiments, the TILs are obtained from tumor digests. In some embodiments, tumor digests were generated by incubation in enzyme media, for example but not limited to RPMI 1640, 2 mM GlutaMAX,

10 mg/mL gentamicin, 30 U/mL DNase, and 1.0 mg/mL collagenase, followed by mechanical dissociation (GentleMACS, Miltenyi Biotec, Auburn, CA). After placing the tumor in enzyme media, the tumor can be mechanically dissociated for approximately 1 minute. The solution can then be incubated for 30 minutes at 37 °C in 5% CO₂ and it then mechanically disrupted again for approximately 1 minute. After being incubated again for 30 minutes at 37 °C in 5% CO₂, the tumor can be mechanically disrupted a third time for approximately 1 minute. In some embodiments, after the third mechanical disruption if large pieces of tissue were present, 1 or 2 additional mechanical dissociations were applied to the sample, with or without 30 additional minutes of incubation at 37 °C in 5% CO₂. In some embodiments, at the end of the final incubation if the cell suspension contained a large number of red blood cells or dead cells, a density gradient separation using Ficoll can be performed to remove these cells.

[00390] In some embodiments, the harvested cell suspension prior to the first expansion step is called a “primary cell population” or a “freshly harvested” cell population.

[00391] In some embodiments, cells can be optionally frozen after sample harvest and stored frozen prior to entry into the expansion described in Step B, which is described in further detail below, as well as exemplified in Figure 1.

B. STEP B: First Expansion

[00392] In some embodiments, the present methods provide for obtaining young TILs, which are capable of increased replication cycles upon administration to a subject/patient and as such may provide additional therapeutic benefits over older TILs (*i.e.*, TILs which have further undergone more rounds of replication prior to administration to a subject/patient). Features of young TILs have been described in the literature, for example Donia, et al., *Scandinavian Journal of Immunology*, 75:157–167 (2012); Dudley et al., *Clin Cancer Res*, 16:6122–6131 (2010); Huang et al., *J Immunother*, 28(3):258–267 (2005); Besser et al., *Clin Cancer Res*, 19(17):OF1–OF9 (2013); Besser et al., *J Immunother* 32:415–423 (2009); Robbins, et al., *J Immunol* 2004; 173:7125–7130; Shen et al., *J Immunother*, 30:123–129 (2007); Zhou, et al., *J Immunother*, 28:53–62 (2005); and Tran, et al., *J Immunother*, 31:742–751 (2008), all of which are incorporated herein by reference in their entireties.

[00393] The diverse antigen receptors of T and B lymphocytes are produced by somatic recombination of a limited, but large number of gene segments. These gene segments: V (variable), D (diversity), J (joining), and C (constant), determine the binding

specificity and downstream applications of immunoglobulins and T-cell receptors (TCRs). The present invention provides a method for generating TILs which exhibit and increase the T-cell repertoire diversity. In some embodiments, the TILs obtained by the present method exhibit an increase in the T-cell repertoire diversity. In some embodiments, the TILs obtained by the present method exhibit an increase in the T-cell repertoire diversity as compared to freshly harvested TILs and/or TILs prepared using other methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, the TILs obtained by the present method exhibit an increase in the T-cell repertoire diversity as compared to freshly harvested TILs and/or TILs prepared using methods referred to as process 1C, as exemplified in Figure 5 and/or Figure 6. In some embodiments, the TILs obtained in the first expansion exhibit an increase in the T-cell repertoire diversity. In some embodiments, the increase in diversity is an increase in the immunoglobulin diversity and/or the T-cell receptor diversity. In some embodiments, the diversity is in the immunoglobulin is in the immunoglobulin heavy chain. In some embodiments, the diversity is in the immunoglobulin is in the immunoglobulin light chain. In some embodiments, the diversity is in the T-cell receptor. In some embodiments, the diversity is in one of the T-cell receptors selected from the group consisting of alpha, beta, gamma, and delta receptors. In some embodiments, there is an increase in the expression of T-cell receptor (TCR) alpha and/or beta. In some embodiments, there is an increase in the expression of T-cell receptor (TCR) alpha. In some embodiments, there is an increase in the expression of T-cell receptor (TCR) beta. In some embodiments, there is an increase in the expression of TCRab (*i.e.*, TCR α/β).

[00394] After dissection or digestion of tumor fragments, for example such as described in Step A of Figure 1, the resulting cells are cultured in serum containing IL-2 under conditions that favor the growth of TILs over tumor and other cells. In some embodiments, the tumor digests are incubated in 2 mL wells in media comprising inactivated human AB serum with 6000 IU/mL of IL-2. This primary cell population is cultured for a period of days, generally from 3 to 14 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this primary cell population is cultured for a period of 7 to 14 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this primary cell population is cultured for a period of 10 to 14 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, this

primary cell population is cultured for a period of about 11 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells.

[00395] In a preferred embodiment, expansion of TILs may be performed using an initial bulk TIL expansion step (for example such as those described in Step B of Figure 1, which can include processes referred to as pre-REP) as described below and herein, followed by a second expansion (Step D, including processes referred to as rapid expansion protocol (REP) steps) as described below under Step D and herein, followed by optional cryopreservation, and followed by a second Step D (including processes referred to as restimulation REP steps) as described below and herein. The TILs obtained from this process may be optionally characterized for phenotypic characteristics and metabolic parameters as described herein.

[00396] In embodiments where TIL cultures are initiated in 24-well plates, for example, using Costar 24-well cell culture cluster, flat bottom (Corning Incorporated, Corning, NY, each well can be seeded with 1×10^6 tumor digest cells or one tumor fragment in 2 mL of complete medium (CM) with IL-2 (6000 IU/mL; Chiron Corp., Emeryville, CA). In some embodiments, the tumor fragment is between about 1 mm^3 and 10 mm^3 .

[00397] In some embodiments, the first expansion culture medium is referred to as “CM”, an abbreviation for culture media. In some embodiments, CM for Step B consists of RPMI 1640 with GlutaMAX, supplemented with 10% human AB serum, 25 mM Hepes, and 10 mg/mL gentamicin. In embodiments where cultures are initiated in gas-permeable flasks with a 40 mL capacity and a 10 cm^2 gas-permeable silicon bottom (for example, G-Rex10; Wilson Wolf Manufacturing, New Brighton, MN) (Fig. 1), each flask was loaded with $10\text{--}40 \times 10^6$ viable tumor digest cells or 5–30 tumor fragments in 10–40 mL of CM with IL-2. Both the G-Rex10 and 24-well plates were incubated in a humidified incubator at 37°C in 5% CO_2 and 5 days after culture initiation, half the media was removed and replaced with fresh CM and IL-2 and after day 5, half the media was changed every 2–3 days.

[00398] After preparation of the tumor fragments, the resulting cells (*i.e.*, fragments) are cultured in serum containing IL-2 under conditions that favor the growth of TILs over tumor and other cells. In some embodiments, the tumor digests are incubated in 2 mL wells in media comprising inactivated human AB serum (or, in some cases, as outlined herein, in the presence of aAPC cell population) with 6000 IU/mL of IL-2. This primary cell population is cultured for a period of days, generally from 10 to 14 days, resulting in a bulk TIL population, generally about 1×10^8 bulk TIL cells. In some embodiments, the growth media

during the first expansion comprises IL-2 or a variant thereof. In some embodiments, the IL is recombinant human IL-2 (rhIL-2). In some embodiments the IL-2 stock solution has a specific activity of $20\text{-}30 \times 10^6$ IU/mg for a 1 mg vial. In some embodiments the IL-2 stock solution has a specific activity of 20×10^6 IU/mg for a 1 mg vial. In some embodiments the IL-2 stock solution has a specific activity of 25×10^6 IU/mg for a 1 mg vial. In some embodiments the IL-2 stock solution has a specific activity of 30×10^6 IU/mg for a 1 mg vial. In some embodiments, the IL-2 stock solution has a final concentration of $4\text{-}8 \times 10^6$ IU/mg of IL-2. In some embodiments, the IL-2 stock solution has a final concentration of $5\text{-}7 \times 10^6$ IU/mg of IL-2. In some embodiments, the IL-2 stock solution has a final concentration of 6×10^6 IU/mg of IL-2. In some embodiments, the IL-2 stock solution is prepared as described in Example 5. In some embodiments, the first expansion culture media comprises about 10,000 IU/mL of IL-2, about 9,000 IU/mL of IL-2, about 8,000 IU/mL of IL-2, about 7,000 IU/mL of IL-2, about 6,000 IU/mL of IL-2 or about 5,000 IU/mL of IL-2. In some embodiments, the first expansion culture media comprises about 9,000 IU/mL of IL-2 to about 5,000 IU/mL of IL-2. In some embodiments, the first expansion culture media comprises about 8,000 IU/mL of IL-2 to about 6,000 IU/mL of IL-2. In some embodiments, the first expansion culture media comprises about 7,000 IU/mL of IL-2 to about 6,000 IU/mL of IL-2. In some embodiments, the first expansion culture media comprises about 6,000 IU/mL of IL-2. In an embodiment, the cell culture medium further comprises IL-2. In some embodiments, the cell culture medium comprises about 3,000 IU/mL of IL-2. In an embodiment, the cell culture medium further comprises IL-2. In a preferred embodiment, the cell culture medium comprises about 3,000 IU/mL of IL-2. In an embodiment, the cell culture medium comprises about 1,000 IU/mL, about 1,500 IU/mL, about 2,000 IU/mL, about 2,500 IU/mL, about 3,000 IU/mL, about 3,500 IU/mL, about 4,000 IU/mL, about 4,500 IU/mL, about 5,000 IU/mL, about 5,500 IU/mL, about 6,000 IU/mL, about 6,500 IU/mL, about 7,000 IU/mL, about 7,500 IU/mL, or about 8,000 IU/mL of IL-2. In an embodiment, the cell culture medium comprises between 1,000 and 2,000 IU/mL, between 2,000 and 3,000 IU/mL, between 3,000 and 4,000 IU/mL, between 4,000 and 5,000 IU/mL, between 5,000 and 6,000 IU/mL, between 6,000 and 7,000 IU/mL, between 7,000 and 8,000 IU/mL, or about 8,000 IU/mL of IL-2.

[00399] In some embodiments, first expansion culture media comprises about 500 IU/mL of IL-15, about 400 IU/mL of IL-15, about 300 IU/mL of IL-15, about 200 IU/mL of IL-15, about 180 IU/mL of IL-15, about 160 IU/mL of IL-15, about 140 IU/mL of IL-15, about 120 IU/mL of IL-15, or about 100 IU/mL of IL-15. In some embodiments, the first expansion

culture media comprises about 500 IU/mL of IL-15 to about 100 IU/mL of IL-15. In some embodiments, the first expansion culture media comprises about 400 IU/mL of IL-15 to about 100 IU/mL of IL-15. In some embodiments, the first expansion culture media comprises about 300 IU/mL of IL-15 to about 100 IU/mL of IL-15. In some embodiments, the first expansion culture media comprises about 200 IU/mL of IL-15. In some embodiments, the cell culture medium comprises about 180 IU/mL of IL-15. In an embodiment, the cell culture medium further comprises IL-15. In a preferred embodiment, the cell culture medium comprises about 180 IU/mL of IL-15.

[00400] In some embodiments, first expansion culture media comprises about 20 IU/mL of IL-21, about 15 IU/mL of IL-21, about 12 IU/mL of IL-21, about 10 IU/mL of IL-21, about 5 IU/mL of IL-21, about 4 IU/mL of IL-21, about 3 IU/mL of IL-21, about 2 IU/mL of IL-21, about 1 IU/mL of IL-21, or about 0.5 IU/mL of IL-21. In some embodiments, the first expansion culture media comprises about 20 IU/mL of IL-21 to about 0.5 IU/mL of IL-21. In some embodiments, the first expansion culture media comprises about 15 IU/mL of IL-21 to about 0.5 IU/mL of IL-21. In some embodiments, the first expansion culture media comprises about 12 IU/mL of IL-21 to about 0.5 IU/mL of IL-21. In some embodiments, the first expansion culture media comprises about 10 IU/mL of IL-21 to about 0.5 IU/mL of IL-21. In some embodiments, the first expansion culture media comprises about 5 IU/mL of IL-21 to about 1 IU/mL of IL-21. In some embodiments, the first expansion culture media comprises about 2 IU/mL of IL-21. In some embodiments, the cell culture medium comprises about 1 IU/mL of IL-21. In some embodiments, the cell culture medium comprises about 0.5 IU/mL of IL-21. In an embodiment, the cell culture medium further comprises IL-21. In a preferred embodiment, the cell culture medium comprises about 1 IU/mL of IL-21.

[00401] In an embodiment, the cell culture medium comprises OKT-3 antibody. In some embodiments, the cell culture medium comprises about 30 ng/mL of OKT-3 antibody. In an embodiment, the cell culture medium comprises about 0.1 ng/mL, about 0.5 ng/mL, about 1 ng/mL, about 2.5 ng/mL, about 5 ng/mL, about 7.5 ng/mL, about 10 ng/mL, about 15 ng/mL, about 20 ng/mL, about 25 ng/mL, about 30 ng/mL, about 35 ng/mL, about 40 ng/mL, about 50 ng/mL, about 60 ng/mL, about 70 ng/mL, about 80 ng/mL, about 90 ng/mL, about 100 ng/mL, about 200 ng/mL, about 500 ng/mL, and about 1 µg/mL of OKT-3 antibody. In an embodiment, the cell culture medium comprises between 0.1 ng/mL and 1 ng/mL, between 1 ng/mL and 5 ng/mL, between 5 ng/mL and 10 ng/mL, between 10 ng/mL and 20 ng/mL, between 20 ng/mL and 30 ng/mL, between 30 ng/mL and 40 ng/mL, between 40 ng/mL and

50 ng/mL, and between 50 ng/mL and 100 ng/mL of OKT-3 antibody. In some embodiments, the cell culture medium does not comprise OKT-3 antibody. In some embodiments, the OKT-3 antibody is muromonab.

TABLE 3: 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 INPSRGYTN 60 NQKFKDKATL TTDKSSSTAY MQLSSLTSED SAVYYCARYY DDHYCLDYWG QGTTLTVSSA 120 KTTAPSVYPL APVCGGTTGS SVTLGCLVKG YFPEPVTLTW NSGSLSSGVH TFPAVLQSDL 180 YTLSSSVTVT SSTWPSQSIT CNVAHPASST KVDKKIEPRP KSCDKTHTCP PCPAPELLGG 240 PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN 300 STYRVVSVLT VLHQDWLNGK EYCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE 360 LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTTPPV LDSDGSFFLY SKLTVDKSRW 420 QQGNVFSCSV MHEALHNHDT QKSLSLSPGK 450
SEQ ID NO:2 Muromonab light chain	QIVLTQSPAI MSASPGEKVT MTCSASSSVS YMNWYQQKSG TSPKRWIYDT SKLASGVPAH 60 FRGSGSGTSY SLTISGMEAE DAATYYCQQW SSNPFTFGSG TKLEINRADT APTVSIFFPS 120 SEQLTSGGAS VVCFLNFFYP KDINVKWKID GSERQNGVLN SWTDQDSKDS TYSMSSTLT 180 TKDEYERHNS YTCEATHKTS TSPIVKSFNR NEC 213

[00402] In some embodiments, the cell culture medium comprises one or more TNFRSF agonists in a cell culture medium. In some embodiments, the TNFRSF agonist comprises a 4-1BB agonist. In some embodiments, the TNFRSF agonist is a 4-1BB agonist, and the 4-1BB agonist is selected from the group consisting of urelumab, utomilumab, EU-101, a fusion protein, and fragments, derivatives, variants, biosimilars, and combinations thereof. In some embodiments, the TNFRSF agonist is added at a concentration sufficient to achieve a concentration in the cell culture medium of between 0.1 µg/mL and 100 µg/mL. In some embodiments, the TNFRSF agonist is added at a concentration sufficient to achieve a concentration in the cell culture medium of between 20 µg/mL and 40 µg/mL.

[00403] In some embodiments, in addition to one or more TNFRSF agonists, the cell culture medium further comprises IL-2 at an initial concentration of about 3000 IU/mL and OKT-3 antibody at an initial concentration of about 30 ng/mL, and wherein the one or more TNFRSF agonists comprises a 4-1BB agonist.

[00404] In some embodiments, the first expansion culture medium is referred to as “CM”, an abbreviation for culture media. In some embodiments, it is referred to as CM1 (culture medium 1). In some embodiments, CM consists of RPMI 1640 with GlutaMAX, supplemented with 10% human AB serum, 25 mM Hepes, and 10 mg/mL gentamicin. In embodiments where cultures are initiated in gas-permeable flasks with a 40 mL capacity and a 10 cm² gas-permeable silicon bottom (for example, G-Rex10; Wilson Wolf Manufacturing, New Brighton, MN) (Fig. 1), each flask was loaded with 10–40×10⁶ viable tumor digest cells

or 5–30 tumor fragments in 10–40mL of CM with IL-2. Both the G-Rex10 and 24-well plates were incubated in a humidified incubator at 37°C in 5% CO₂ and 5 days after culture initiation, half the media was removed and replaced with fresh CM and IL-2 and after day 5, half the media was changed every 2–3 days. In some embodiments, the CM is the CM1 described in the Examples, *see*, Example 1. In some embodiments, the first expansion occurs in an initial cell culture medium or a first cell culture medium. In some embodiments, the initial cell culture medium or the first cell culture medium comprises IL-2.

[00405] In some embodiments, the first expansion (including processes such as for example those described in Step B of Figure 1, which can include those sometimes referred to as the pre-REP) process is shortened to 3-14 days, as discussed in the examples and figures. In some embodiments, the first expansion (including processes such as for example those described in Step B of Figure 1, which can include those sometimes referred to as the pre-REP) is shortened to 7 to 14 days, as discussed in the Examples and shown in Figures 4 and 5, as well as including for example, an expansion as described in Step B of Figure 1. In some embodiments, the first expansion of Step B is shortened to 10-14 days. In some embodiments, the first expansion is shortened to 11 days, as discussed in, for example, an expansion as described in Step B of Figure 1.

[00406] In some embodiments, the first TIL expansion can proceed for 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days. In some embodiments, the first TIL expansion can proceed for 1 day to 14 days. In some embodiments, the first TIL expansion can proceed for 2 days to 14 days. In some embodiments, the first TIL expansion can proceed for 3 days to 14 days. In some embodiments, the first TIL expansion can proceed for 4 days to 14 days. In some embodiments, the first TIL expansion can proceed for 5 days to 14 days. In some embodiments, the first TIL expansion can proceed for 6 days to 14 days. In some embodiments, the first TIL expansion can proceed for 7 days to 14 days. In some embodiments, the first TIL expansion can proceed for 8 days to 14 days. In some embodiments, the first TIL expansion can proceed for 9 days to 14 days. In some embodiments, the first TIL expansion can proceed for 10 days to 14 days. In some embodiments, the first TIL expansion can proceed for 11 days to 14 days. In some embodiments, the first TIL expansion can proceed for 12 days to 14 days. In some embodiments, the first TIL expansion can proceed for 13 days to 14 days. In some embodiments, the first TIL expansion can proceed for 14 days. In some embodiments, the

first TIL expansion can proceed for 1 day to 11 days. In some embodiments, the first TIL expansion can proceed for 2 days to 11 days. In some embodiments, the first TIL expansion can proceed for 3 days to 11 days. In some embodiments, the first TIL expansion can proceed for 4 days to 11 days. In some embodiments, the first TIL expansion can proceed for 5 days to 11 days. In some embodiments, the first TIL expansion can proceed for 6 days to 11 days. In some embodiments, the first TIL expansion can proceed for 7 days to 11 days. In some embodiments, the first TIL expansion can proceed for 8 days to 11 days. In some embodiments, the first TIL expansion can proceed for 9 days to 11 days. In some embodiments, the first TIL expansion can proceed for 10 days to 11 days. In some embodiments, the first TIL expansion can proceed for 11 days.

[00407] In some embodiments, a combination of IL-2, IL-7, IL-15, and/or IL-21 are employed as a combination during the first expansion. In some embodiments, IL-2, IL-7, IL-15, and/or IL-21 as well as any combinations thereof can be included during the first expansion, including for example during a Step B processes according to Figure 1, as well as described herein. In some embodiments, a combination of IL-2, IL-15, and IL-21 are employed as a combination during the first expansion. In some embodiments, IL-2, IL-15, and IL-21 as well as any combinations thereof can be included during Step B processes according to Figure 1 and as described herein.

[00408] In some embodiments, the first expansion (including processes referred to as the pre-REP; for example, Step B according to Figure 1) process is shortened to 3 to 14 days, as discussed in the examples and figures. In some embodiments, the first expansion of Step B is shortened to 7 to 14 days. In some embodiments, the first expansion of Step B is shortened to 10 to 14 days. In some embodiments, the first expansion is shortened to 11 days.

[00409] In some embodiments, the first expansion, for example, Step B according to Figure 1, is performed in a closed system bioreactor. In some embodiments, a closed system is employed for the TIL expansion, as described herein. In some embodiments, a single bioreactor is employed. In some embodiments, the single bioreactor employed is for example a G-REX -10 or a G-REX -100. In some embodiments, the closed system bioreactor is a single bioreactor.

C. STEP C: First Expansion to Second Expansion Transition

[00410] In some cases, the bulk TIL population obtained from the first expansion, including for example the TIL population obtained from for example, Step B as indicated in Figure 1,

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

[00411] In some embodiments, the TILs obtained from the first expansion (for example, from Step B as indicated in Figure 1) are stored until phenotyped for selection. In some embodiments, the TILs obtained from the first expansion (for example, from Step B as indicated in Figure 1) are not stored and proceed directly to the second expansion. In some embodiments, the TILs obtained from the first expansion are not cryopreserved after the first expansion and prior to the second expansion. In some embodiments, the transition from the first expansion to the second expansion occurs at about 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs at about 3 days to 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs at about 4 days to 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs at about 4 days to 10 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs at about 7 days to 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs at about 14 days from when fragmentation occurs.

[00412] In some embodiments, the transition from the first expansion to the second expansion occurs at 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 1 day to 14 days from when fragmentation occurs. In some embodiments, the first TIL expansion can proceed for 2 days to 14 days. In some embodiments, the transition from the first expansion to the second expansion occurs 3 days to 14 days from when fragmentation occurs. In some

[illegible]

to 11 days from when fragmentation occurs. In some embodiments, the transition from the first expansion to the second expansion occurs 11 days from when fragmentation occurs.

[00413] In some embodiments, the TILs are not stored after the first expansion and prior to the second expansion, and the TILs proceed directly to the second expansion (for example, in some embodiments, there is no storage during the transition from Step B to Step D as shown in Figure 1). In some embodiments, the transition occurs in closed system, as described herein. In some embodiments, the TILs from the first expansion, the second population of TILs, proceeds directly into the second expansion with no transition period.

[00414] In some embodiments, the transition from the first expansion to the second expansion, for example, Step C according to Figure 1, is performed in a closed system bioreactor. In some embodiments, a closed system is employed for the TIL expansion, as described herein. In some embodiments, a single bioreactor is employed. In some embodiments, the single bioreactor employed is for example a G-REX -10 or a G-REX -100. In some embodiments, the closed system bioreactor is a single bioreactor.

1. Cytokines

[00415] The expansion methods described herein generally use culture media with high doses of a cytokine, in particular IL-2, as is known in the art.

[00416] Alternatively, using combinations of cytokines for the rapid expansion and or second expansion of TILS is additionally possible, with combinations of two or more of IL-2, IL-15 and IL-21 as is generally outlined in International Publication No. WO 2015/189356 and W International Publication No. WO 2015/189357, hereby expressly incorporated by reference in their entirety. Thus, possible combinations include IL-2 and IL-15, IL-2 and IL-21, IL-15 and IL-21 and IL-2, IL-15 and IL-21, with the latter finding particular use in many embodiments. The use of combinations of cytokines specifically favors the generation of lymphocytes, and in particular T-cells as described therein.

TABLE 4: Amino acid sequences of interleukins.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:3 recombinant human IL-2 (rhIL-2)	MAPTSSSTKK TQLQLEHLLL DLQMLNGIN NYKNPKLTRM LTFKFYMPKK ATELKHLQCL 60 EEELKPLEEV LNLAQSKNFH LRPRDLISNI NVIVLELKGS ETTFMCEYAD ETATVEFLN 120 RWITFCQSII STL 134
SEQ ID NO:4 Aldeusleukin	PTSSSTKKTQ LQLEHLLLDL QMILNGINNY KNPKLTRMLT FKFYMPKKAT ELKHLQCLEE 60 ELKPLEEVLN LAQSKNFHLR PRDLISNINV IVLELKGSSET TFMCEYADET ATIVEFLNRW 120 ITFSQSIIST LT 132
SEQ ID NO:5	MHKCDITLQE IIKTLNSLTE QKTLCTELTV TDIFAASKNT TEKETFCAA TVLRQFYSHH 60 EKDTRCLGAT AQQFHRHKQL IRFLKRLDRN LWGLAGLNSC PVKEANQSTL ENFLERLKTI 120

recombinant human IL-4 (rhIL-4)	MREKYSKCSS	130
SEQ ID NO:6 recombinant human IL-7 (rhIL-7)	MDCDIEGKDG KQYESVLMVS IDQLLDSMKE IGSNCLNNEF NFFKRHICDA NKEGMFLFRA ARKLRQFLKM NSTGDFDLHL LKVSEGTTL LNCTGQVKGR KPAALGEAQP TKSLEENKSL KEQKKLNDLC FLKRLLEIK TCWNKILMGT KEH	60 120 153
SEQ ID NO:7 recombinant human IL-15 (rhIL-15)	MNWNVISDL KKIEDLIQSM HIDATLYTES DVHPCKVTA MKCFLLLELQV ISLESGDASI HDTVENLIIL ANNSLSSNGN VTESGCKECE ELEEKNIKEF LQSFVHIVQM FINTS	60 115
SEQ ID NO:8 recombinant human IL-21 (rhIL-21)	MQDRHMIRM QOLIDVDQLK NYVNDLVPEF LPAPEDVETN CEWSAFSCFQ KAQLKSANTG NNERIINVS I KKLKRKPPST NAGRQKHRL TCPSCDSYEK KPPKEFLERF KSLQKMIHQ HLSSRTHGSE DS	60 120 132

D. STEP D: Second Expansion

[00417] In some embodiments, the TIL cell population is expanded in number after harvest and initial bulk processing for example, after Step A and Step B, and the transition referred to as Step C, as indicated in Figure 1). This further expansion is referred to herein as the second expansion, which can include expansion processes generally referred to in the art as a rapid expansion process (REP; as well as processes as indicated in Step D of Figure 1). The second expansion is generally accomplished using a culture media comprising a number of components, including feeder cells, a cytokine source, and an anti-CD3 antibody, in a gas-permeable container.

[00418] In some embodiments, the second expansion or second TIL expansion (which can include expansions sometimes referred to as REP; as well as processes as indicated in Step D of Figure 1) of TIL can be performed using any TIL flasks or containers known by those of skill in the art. In some embodiments, the second TIL expansion can proceed for 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days. In some embodiments, the second TIL expansion can proceed for about 7 days to about 14 days. In some embodiments, the second TIL expansion can proceed for about 8 days to about 14 days. In some embodiments, the second TIL expansion can proceed for about 9 days to about 14 days. In some embodiments, the second TIL expansion can proceed for about 10 days to about 14 days. In some embodiments, the second TIL expansion can proceed for about 11 days to about 14 days. In some embodiments, the second TIL expansion can proceed for about 12 days to about 14 days. In some embodiments, the second TIL expansion can proceed for about 13 days to about 14 days. In some embodiments, the second TIL expansion can proceed for about 14 days.

[00419] In an embodiment, the second expansion can be performed in a gas permeable container using the methods of the present disclosure (including for example, expansions referred to as REP; as well as processes as indicated in Step D of Figure 1). For example, TILs can be rapidly expanded using non-specific T-cell receptor stimulation in the presence of interleukin-2 (IL-2) or interleukin-15 (IL-15). The non-specific T-cell receptor stimulus can include, for example, an anti-CD3 antibody, such as about 30 ng/ml of OKT3, a mouse monoclonal anti-CD3 antibody (commercially available from Ortho-McNeil, Raritan, NJ or Miltenyi Biotech, Auburn, CA) or UHCT-1 (commercially available from BioLegend, San Diego, CA, USA). TILs can be expanded to induce further stimulation of the TILs *in vitro* by including one or more antigens during the second expansion, including antigenic portions thereof, such as epitope(s), of the cancer, which can be optionally expressed from a vector, such as a human leukocyte antigen A2 (HLA-A2) binding peptide, *e.g.*, 0.3 μ M MART-1 :26-35 (27 L) or gpl 00:209-217 (210M), optionally in the presence of a T-cell growth factor, such as 300 IU/mL IL-2 or IL-15. Other suitable antigens may include, *e.g.*, NY-ESO-1, TRP-1, TRP-2, tyrosinase cancer antigen, MAGE-A3, SSX-2, and VEGFR2, or antigenic portions thereof. TIL may also be rapidly expanded by re-stimulation with the same antigen(s) of the cancer pulsed onto HLA-A2-expressing antigen-presenting cells. Alternatively, the TILs can be further re-stimulated with, *e.g.*, example, irradiated, autologous lymphocytes or with irradiated HLA-A2+ allogeneic lymphocytes and IL-2. In some embodiments, the re-stimulation occurs as part of the second expansion. In some embodiments, the second expansion occurs in the presence of irradiated, autologous lymphocytes or with irradiated HLA-A2+ allogeneic lymphocytes and IL-2.

[00420] In an embodiment, the cell culture medium further comprises IL-2. In some embodiments, the cell culture medium comprises about 3000 IU/mL of IL-2. In an embodiment, the cell culture medium comprises about 1000 IU/mL, about 1500 IU/mL, about 2000 IU/mL, about 2500 IU/mL, about 3000 IU/mL, about 3500 IU/mL, about 4000 IU/mL, about 4500 IU/mL, about 5000 IU/mL, about 5500 IU/mL, about 6000 IU/mL, about 6500 IU/mL, about 7000 IU/mL, about 7500 IU/mL, or about 8000 IU/mL of IL-2. In an embodiment, the cell culture medium comprises between 1000 and 2000 IU/mL, between 2000 and 3000 IU/mL, between 3000 and 4000 IU/mL, between 4000 and 5000 IU/mL, between 5000 and 6000 IU/mL, between 6000 and 7000 IU/mL, between 7000 and 8000 IU/mL, or between 8000 IU/mL of IL-2.

[00421] In an embodiment, the cell culture medium comprises OKT-3 antibody. In some embodiments, the cell culture medium comprises about 30 ng/mL of OKT-3 antibody. In an embodiment, the cell culture medium comprises about 0.1 ng/mL, about 0.5 ng/mL, about 1 ng/mL, about 2.5 ng/mL, about 5 ng/mL, about 7.5 ng/mL, about 10 ng/mL, about 15 ng/mL, about 20 ng/mL, about 25 ng/mL, about 30 ng/mL, about 35 ng/mL, about 40 ng/mL, about 50 ng/mL, about 60 ng/mL, about 70 ng/mL, about 80 ng/mL, about 90 ng/mL, about 100 ng/mL, about 200 ng/mL, about 500 ng/mL, and about 1 µg/mL of OKT-3 antibody. In an embodiment, the cell culture medium comprises between 0.1 ng/mL and 1 ng/mL, between 1 ng/mL and 5 ng/mL, between 5 ng/mL and 10 ng/mL, between 10 ng/mL and 20 ng/mL, between 20 ng/mL and 30 ng/mL, between 30 ng/mL and 40 ng/mL, between 40 ng/mL and 50 ng/mL, and between 50 ng/mL and 100 ng/mL of OKT-3 antibody. In some embodiments, the cell culture medium does not comprise OKT-3 antibody. In some embodiments, the OKT-3 antibody is muromonab.

[00422] In some embodiments, the cell culture medium comprises one or more TNFRSF agonists in a cell culture medium. In some embodiments, the TNFRSF agonist comprises a 4-1BB agonist. In some embodiments, the TNFRSF agonist is a 4-1BB agonist, and the 4-1BB agonist is selected from the group consisting of urelumab, utomilumab, EU-101, a fusion protein, and fragments, derivatives, variants, biosimilars, and combinations thereof. In some embodiments, the TNFRSF agonist is added at a concentration sufficient to achieve a concentration in the cell culture medium of between 0.1 µg/mL and 100 µg/mL. In some embodiments, the TNFRSF agonist is added at a concentration sufficient to achieve a concentration in the cell culture medium of between 20 µg/mL and 40 µg/mL.

[00423] In some embodiments, in addition to one or more TNFRSF agonists, the cell culture medium further comprises IL-2 at an initial concentration of about 3000 IU/mL and OKT-3 antibody at an initial concentration of about 30 ng/mL, and wherein the one or more TNFRSF agonists comprises a 4-1BB agonist.

[00424] In some embodiments, a combination of IL-2, IL-7, IL-15, and/or IL-21 are employed as a combination during the second expansion. In some embodiments, IL-2, IL-7, IL-15, and/or IL-21 as well as any combinations thereof can be included during the second expansion, including for example during a Step D processes according to Figure 1, as well as described herein. In some embodiments, a combination of IL-2, IL-15, and IL-21 are employed as a combination during the second expansion. In some embodiments, IL-2, IL-15,

and IL-21 as well as any combinations thereof can be included during Step D processes according to Figure 1 and as described herein.

[00425] In some embodiments, the second expansion can be conducted in a supplemented cell culture medium comprising IL-2, OKT-3, antigen-presenting feeder cells, and optionally a TNFRSF agonist. In some embodiments, the second expansion occurs in a supplemented cell culture medium. In some embodiments, the supplemented cell culture medium comprises IL-2, OKT-3, and antigen-presenting feeder cells. In some embodiments, the second cell culture medium comprises IL-2, OKT-3, and antigen-presenting cells (APCs; also referred to as antigen-presenting feeder cells). In some embodiments, the second expansion occurs in a cell culture medium comprising IL-2, OKT-3, and antigen-presenting feeder cells (*i.e.*, antigen presenting cells).

[00426] In some embodiments, the second expansion culture media comprises about 500 IU/mL of IL-15, about 400 IU/mL of IL-15, about 300 IU/mL of IL-15, about 200 IU/mL of IL-15, about 180 IU/mL of IL-15, about 160 IU/mL of IL-15, about 140 IU/mL of IL-15, about 120 IU/mL of IL-15, or about 100 IU/mL of IL-15. In some embodiments, the second expansion culture media comprises about 500 IU/mL of IL-15 to about 100 IU/mL of IL-15. In some embodiments, the second expansion culture media comprises about 400 IU/mL of IL-15 to about 100 IU/mL of IL-15. In some embodiments, the second expansion culture media comprises about 300 IU/mL of IL-15 to about 100 IU/mL of IL-15. In some embodiments, the second expansion culture media comprises about 200 IU/mL of IL-15. In some embodiments, the cell culture medium comprises about 180 IU/mL of IL-15. In an embodiment, the cell culture medium further comprises IL-15. In a preferred embodiment, the cell culture medium comprises about 180 IU/mL of IL-15.

[00427] In some embodiments, the second expansion culture media comprises about 20 IU/mL of IL-21, about 15 IU/mL of IL-21, about 12 IU/mL of IL-21, about 10 IU/mL of IL-21, about 5 IU/mL of IL-21, about 4 IU/mL of IL-21, about 3 IU/mL of IL-21, about 2 IU/mL of IL-21, about 1 IU/mL of IL-21, or about 0.5 IU/mL of IL-21. In some embodiments, the second expansion culture media comprises about 20 IU/mL of IL-21 to about 0.5 IU/mL of IL-21. In some embodiments, the second expansion culture media comprises about 15 IU/mL of IL-21 to about 0.5 IU/mL of IL-21. In some embodiments, the second expansion culture media comprises about 12 IU/mL of IL-21 to about 0.5 IU/mL of IL-21. In some embodiments, the second expansion culture media comprises about 10 IU/mL of IL-21 to about 0.5 IU/mL of IL-21. In some embodiments, the second expansion culture media

comprises about 5 IU/mL of IL-21 to about 1 IU/mL of IL-21. In some embodiments, the second expansion culture media comprises about 2 IU/mL of IL-21. In some embodiments, the cell culture medium comprises about 1 IU/mL of IL-21. In some embodiments, the cell culture medium comprises about 0.5 IU/mL of IL-21. In an embodiment, the cell culture medium further comprises IL-21. In a preferred embodiment, the cell culture medium comprises about 1 IU/mL of IL-21.

[00428] In some embodiments the antigen-presenting feeder cells (APCs) are PBMCs. In an embodiment, the ratio of TILs to PBMCs and/or antigen-presenting cells in the rapid expansion and/or the second expansion is about 1 to 25, about 1 to 50, about 1 to 100, about 1 to 125, about 1 to 150, about 1 to 175, about 1 to 200, about 1 to 225, about 1 to 250, about 1 to 275, about 1 to 300, about 1 to 325, about 1 to 350, about 1 to 375, about 1 to 400, or about 1 to 500. In an embodiment, the ratio of TILs to PBMCs in the rapid expansion and/or the second expansion is between 1 to 50 and 1 to 300. In an embodiment, the ratio of TILs to PBMCs in the rapid expansion and/or the second expansion is between 1 to 100 and 1 to 200.

[00429] In an embodiment, REP and/or the second expansion is performed in flasks with the bulk TILs being mixed with a 100- or 200-fold excess of inactivated feeder cells, 30 mg/mL OKT3 anti-CD3 antibody and 3000 IU/mL IL-2 in 150 ml media. Media replacement is done (generally 2/3 media replacement via respiration with fresh media) until the cells are transferred to an alternative growth chamber. Alternative growth chambers include G-REX flasks and gas permeable containers as more fully discussed below.

[00430] In some embodiments, the second expansion (which can include processes referred to as the REP process) is shortened to 7-14 days, as discussed in the examples and figures. In some embodiments, the second expansion is shortened to 11 days.

[00431] In an embodiment, REP and/or the second expansion may be performed using T-175 flasks and gas permeable bags as previously described (Tran, *et al.*, *J. Immunother.* **2008**, *31*, 742-51; Dudley, *et al.*, *J. Immunother.* **2003**, *26*, 332-42) or gas permeable cultureware (G-Rex flasks). In some embodiments, the second expansion (including expansions referred to as rapid expansions) is performed in T-175 flasks, and about 1×10^6 TILs suspended in 150 mL of media may be added to each T-175 flask. The TILs may be cultured in a 1 to 1 mixture of CM and AIM-V medium, supplemented with 3000 IU per mL of IL-2 and 30 ng per mL of anti-CD3. The T-175 flasks may be incubated at 37° C in 5% CO₂. Half the media may be exchanged on day 5 using 50/50 medium with 3000 IU per mL of IL-2. In some

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

[00432] In an embodiment, the second expansion (which can include expansions referred to as REP, as well as those referred to in Step D of Figure 1) may be performed in 500 mL capacity gas permeable flasks with 100 cm gas-permeable silicon bottoms (G-Rex 100, commercially available from Wilson Wolf Manufacturing Corporation, New Brighton, MN, USA), 5×10^6 or 10×10^6 TIL may be cultured with PBMCs in 400 mL of 50/50 medium, supplemented with 5% human AB serum, 3000 IU per mL of IL-2 and 30 ng per ml of anti-CD3 (OKT3). The G-Rex 100 flasks may be incubated at 37°C in 5% CO₂. On day 5, 250 mL of supernatant may be removed and placed into centrifuge bottles and centrifuged at 1500 rpm ($491 \times g$) for 10 minutes. The TIL pellets may be re-suspended with 150 mL of fresh medium with 5% human AB serum, 3000 IU per mL of IL-2, and added back to the original G-Rex 100 flasks. When TIL are expanded serially in G-Rex 100 flasks, on day 7 the TIL in each G-Rex 100 may be suspended in the 300 mL of media present in each flask and the cell suspension may be divided into 3 100 mL aliquots that may be used to seed 3 G-Rex 100 flasks. Then 150 mL of AIM-V with 5% human AB serum and 3000 IU per mL of IL-2 may be added to each flask. The G-Rex 100 flasks may be incubated at 37° C in 5% CO₂ and after 4 days 150 mL of AIM-V with 3000 IU per mL of IL-2 may be added to each G-REX 100 flask. The cells may be harvested on day 14 of culture.

[00433] In an embodiment, the second expansion (including expansions referred to as REP) is performed in flasks with the bulk TILs being mixed with a 100- or 200-fold excess of inactivated feeder cells, 30 mg/mL OKT3 anti-CD3 antibody and 3000 IU/mL IL-2 in 150 ml media. In some embodiments, media replacement is done until the cells are transferred to an alternative growth chamber. In some embodiments, 2/3 of the media is replaced by respiration with fresh media. In some embodiments, alternative growth chambers include G-REX flasks and gas permeable containers as more fully discussed below.

[00434] In an embodiment, the second expansion (including expansions referred to as REP) is performed and further comprises a step wherein TILs are selected for superior tumor reactivity. Any selection method known in the art may be used. For example, the methods described in U.S. Patent Application Publication No. 2016/0010058 A1, the disclosures of

which are incorporated herein by reference, may be used for selection of TILs for superior tumor reactivity.

[00435] Optionally, a cell viability assay can be performed after the second expansion (including expansions referred to as the REP expansion), using standard assays known in the art. For example, a trypan blue exclusion assay can be done on a sample of the bulk TILs, which selectively labels dead cells and allows a viability assessment. In some embodiments, TIL samples can be counted and viability determined using a Cellometer K2 automated cell counter (Nexcelom Bioscience, Lawrence, MA). In some embodiments, viability is determined according to the standard Cellometer K2 Image Cytometer Automatic Cell Counter protocol.

[00436] In some embodiments, the second expansion (including expansions referred to as REP) of TIL can be performed using T-175 flasks and gas-permeable bags as previously described (Tran KQ, Zhou J, Durflinger KH, et al., **2008**, *J Immunother.*, 31:742–751, and Dudley ME, Wunderlich JR, Shelton TE, et al. 2003, *J Immunother.*, 26:332–342) or gas-permeable G-Rex flasks. In some embodiments, the second expansion is performed using flasks. In some embodiments, the second expansion is performed using gas-permeable G-Rex flasks. In some embodiments, the second expansion is performed in T-175 flasks, and about 1×10^6 TIL are suspended in about 150 mL of media and this is added to each T-175 flask. The TIL are cultured with irradiated (50 Gy) allogeneic PBMC as “feeder” cells at a ratio of 1 to 100 and the cells were cultured in a 1 to 1 mixture of CM and AIM-V medium (50/50 medium), supplemented with 3000 IU/mL of IL-2 and 30 ng/mL of anti-CD3. The T-175 flasks are incubated at 37°C in 5% CO₂. In some embodiments, half the media is changed on day 5 using 50/50 medium with 3000 IU/mL of IL-2. In some embodiments, on day 7, cells from 2 T-175 flasks are combined in a 3 L bag and 300 mL of AIM-V with 5% human AB serum and 3000 IU/mL of IL-2 is added to the 300 mL of TIL suspension. The number of cells in each bag can be counted every day or two and fresh media can be added to keep the cell count between about 0.5 and about 2.0×10^6 cells/mL.

[00437] In some embodiments, the second expansion (including expansions referred to as REP) are performed in 500 mL capacity flasks with 100 cm² gas-permeable silicon bottoms (G-Rex 100, Wilson Wolf) (Fig. 1), about 5×10^6 or 10×10^6 TIL are cultured with irradiated allogeneic PBMC at a ratio of 1 to 100 in 400 mL of 50/50 medium, supplemented with 3000 IU/mL of IL-2 and 30 ng/mL of anti-CD3. The G-Rex 100 flasks are incubated at 37°C in 5% CO₂. In some embodiments, on day 5, 250mL of supernatant is removed and placed into

centrifuge bottles and centrifuged at 1500 rpm (491g) for 10 minutes. The TIL pellets can then be resuspended with 150 mL of fresh 50/50 medium with 3000 IU/ mL of IL-2 and added back to the original G-Rex 100 flasks. In embodiments where TILs are expanded serially in G-Rex 100 flasks, on day 7 the TIL in each G-Rex 100 are suspended in the 300 mL of media present in each flask and the cell suspension was divided into three 100 mL aliquots that are used to seed 3 G-Rex 100 flasks. Then 150 mL of AIM-V with 5% human AB serum and 3000 IU/mL of IL-2 is added to each flask. The G-Rex 100 flasks are incubated at 37°C in 5% CO₂ and after 4 days 150 mL of AIM-V with 3000 IU/mL of IL-2 is added to each G-Rex 100 flask. The cells are harvested on day 14 of culture.

[00438] The diverse antigen receptors of T and B lymphocytes are produced by somatic recombination of a limited, but large number of gene segments. These gene segments: V (variable), D (diversity), J (joining), and C (constant), determine the binding specificity and downstream applications of immunoglobulins and T-cell receptors (TCRs). The present invention provides a method for generating TILs which exhibit and increase the T-cell repertoire diversity. In some embodiments, the TILs obtained by the present method exhibit an increase in the T-cell repertoire diversity. In some embodiments, the TILs obtained in the second expansion exhibit an increase in the T-cell repertoire diversity. In some embodiments, the increase in diversity is an increase in the immunoglobulin diversity and/or the T-cell receptor diversity. In some embodiments, the diversity is in the immunoglobulin is in the immunoglobulin heavy chain. In some embodiments, the diversity is in the immunoglobulin is in the immunoglobulin light chain. In some embodiments, the diversity is in the T-cell receptor. In some embodiments, the diversity is in one of the T-cell receptors selected from the group consisting of alpha, beta, gamma, and delta receptors. In some embodiments, there is an increase in the expression of T-cell receptor (TCR) alpha and/or beta. In some embodiments, there is an increase in the expression of T-cell receptor (TCR) alpha. In some embodiments, there is an increase in the expression of T-cell receptor (TCR) beta. In some embodiments, there is an increase in the expression of TCRab (*i.e.*, TCR α/β).

[00439] In some embodiments, the second expansion culture medium (*e.g.*, sometimes referred to as CM2 or the second cell culture medium), comprises IL-2, OKT-3, as well as the antigen-presenting feeder cells (APCs), as discussed in more detail below.

[00440] In some embodiments, the second expansion, for example, Step D according to Figure 1, is performed in a closed system bioreactor. In some embodiments, a closed system is employed for the TIL expansion, as described herein. In some embodiments, a single

bioreactor is employed. In some embodiments, the single bioreactor employed is for example a G-REX -10 or a G-REX -100. In some embodiments, the closed system bioreactor is a single bioreactor.

1. Feeder Cells and Antigen Presenting Cells

[00441] In an embodiment, the second expansion procedures described herein (for example including expansion such as those described in Step D from Figure 1, as well as those referred to as REP) require an excess of feeder cells during REP TIL expansion and/or during the second expansion. In many embodiments, the feeder cells are peripheral blood mononuclear cells (PBMCs) obtained from standard whole blood units from healthy blood donors. The PBMCs are obtained using standard methods such as Ficoll-Paque gradient separation.

[00442] In general, the allogeneic PBMCs are inactivated, either via irradiation or heat treatment, and used in the REP procedures, as described in the examples, which provides an exemplary protocol for evaluating the replication incompetence of irradiate allogeneic PBMCs.

[00443] In some embodiments, PBMCs are considered replication incompetent and accepted for use in the TIL expansion procedures described herein if the total number of viable cells on day 14 is less than the initial viable cell number put into culture on day 0 of the REP and/or day 0 of the second expansion (*i.e.*, the start day of the second expansion).

[00444] In some embodiments, PBMCs are considered replication incompetent and accepted for use in the TIL expansion procedures described herein if the total number of viable cells, cultured in the presence of OKT3 and IL-2, on day 7 and day 14 has not increased from the initial viable cell number put into culture on day 0 of the REP and/or day 0 of the second expansion (*i.e.*, the start day of the second expansion). In some embodiments, the PBMCs are cultured in the presence of 30 ng/ml OKT3 antibody and 3000 IU/ml IL-2.

[00445] In some embodiments, PBMCs are considered replication incompetent and accepted for use in the TIL expansion procedures described herein if the total number of viable cells, cultured in the presence of OKT3 and IL-2, on day 7 and day 14 has not increased from the initial viable cell number put into culture on day 0 of the REP and/or day 0 of the second expansion (*i.e.*, the start day of the second expansion). In some embodiments, the PBMCs are cultured in the presence of 5-60 ng/ml OKT3 antibody and 1000-6000 IU/ml IL-2. In some embodiments, the PBMCs are cultured in the presence of 10-50 ng/ml OKT3 antibody and

2000-5000 IU/ml IL-2. In some embodiments, the PBMCs are cultured in the presence of 20-40 ng/ml OKT3 antibody and 2000-4000 IU/ml IL-2. In some embodiments, the PBMCs are cultured in the presence of 25-35 ng/ml OKT3 antibody and 2500-3500 IU/ml IL-2.

[00446] In some embodiments, the antigen-presenting feeder cells are PBMCs. In some embodiments, the antigen-presenting feeder cells are artificial antigen-presenting feeder cells. In an embodiment, the ratio of TILs to antigen-presenting feeder cells in the second expansion is about 1 to 25, about 1 to 50, about 1 to 100, about 1 to 125, about 1 to 150, about 1 to 175, about 1 to 200, about 1 to 225, about 1 to 250, about 1 to 275, about 1 to 300, about 1 to 325, about 1 to 350, about 1 to 375, about 1 to 400, or about 1 to 500. In an embodiment, the ratio of TILs to antigen-presenting feeder cells in the second expansion is between 1 to 50 and 1 to 300. In an embodiment, the ratio of TILs to antigen-presenting feeder cells in the second expansion is between 1 to 100 and 1 to 200.

[00447] In an embodiment, the second expansion procedures described herein require a ratio of about 2.5×10^9 feeder cells to about 100×10^6 TILs. In another embodiment, the second expansion procedures described herein require a ratio of about 2.5×10^9 feeder cells to about 50×10^6 TILs. In yet another embodiment, the second expansion procedures described herein require about 2.5×10^9 feeder cells to about 25×10^6 TILs.

[00448] In an embodiment, the second expansion procedures described herein require an excess of feeder cells during the second expansion. In many embodiments, the feeder cells are peripheral blood mononuclear cells (PBMCs) obtained from standard whole blood units from healthy blood donors. The PBMCs are obtained using standard methods such as Ficoll-Paque gradient separation. In an embodiment, artificial antigen-presenting (aAPC) cells are used in place of PBMCs.

[00449] In general, the allogenic PBMCs are inactivated, either via irradiation or heat treatment, and used in the TIL expansion procedures described herein, including the exemplary procedures described in the figures and examples.

[00450] In an embodiment, artificial antigen presenting cells are used in the second expansion as a replacement for, or in combination with, PBMCs.

2. Cytokines

[00451] The expansion methods described herein generally use culture media with high doses of a cytokine, in particular IL-2, as is known in the art.

[00452] Alternatively, using combinations of cytokines for the rapid expansion and or second expansion of TILS is additionally possible, with combinations of two or more of IL-2, IL-15 and IL-21 as is generally outlined in International Publication No. WO 2015/189356 and W International Publication No. WO 2015/189357, hereby expressly incorporated by reference in their entirety. Thus, possible combinations include IL-2 and IL-15, IL-2 and IL-21, IL-15 and IL-21 and IL-2, IL-15 and IL-21, with the latter finding particular use in many embodiments. The use of combinations of cytokines specifically favors the generation of lymphocytes, and in particular T-cells as described therein.

E. STEP E: Harvest TILS

[00453] After the second expansion step, cells can be harvested. In some embodiments the TILs are harvested after one, two, three, four or more expansion steps, for example as provided in Figure 1. In some embodiments the TILs are harvested after two expansion steps, for example as provided in Figure 1.

[00454] TILs can be harvested in any appropriate and sterile manner, including for example by centrifugation. Methods for TIL harvesting are well known in the art and any such known methods can be employed with the present process. In some embodiments, TILS are harvest using an automated system.

[00455] Cell harvesters and/or cell processing systems are commercially available from a variety of sources, including, for example, Fresenius Kabi, Tomtec Life Science, Perkin Elmer, and Inotech Biosystems International, Inc. Any cell based harvester can be employed with the present methods. In some embodiments, the cell harvester and/or cell processing systems is a membrane-based cell harvester. In some embodiments, cell harvesting is via a cell processing system, such as the LOVO system (manufactured by Fresenius Kabi). The term “LOVO cell processing system” also refers to any instrument or device manufactured by any vendor that can pump a solution comprising cells through a membrane or filter such as a spinning membrane or spinning filter in a sterile and/or closed system environment, allowing for continuous flow and cell processing to remove supernatant or cell culture media without pelletization. In some embodiments, the cell harvester and/or cell processing system can perform cell separation, washing, fluid-exchange, concentration, and/or other cell processing steps in a closed, sterile system.

[00456] In some embodiments, the harvest, for example, Step E according to Figure 1, is performed from a closed system bioreactor. In some embodiments, a closed system is

employed for the TIL expansion, as described herein. In some embodiments, a single bioreactor is employed. In some embodiments, the single bioreactor employed is for example a G-REX -10 or a G-REX -100. In some embodiments, the closed system bioreactor is a single bioreactor.

[00457] In some embodiments, Step E according to Figure 1, is performed according to the processes described in Example G. In some embodiments, the closed system is accessed via syringes under sterile conditions in order to maintain the sterility and closed nature of the system. In some embodiments, a closed system as described in Example G is employed.

[00458] In some embodiments, TILs are harvested according to the methods described in Example G. In some embodiments, TILs between days 1 and 11 are harvested using the methods as described in Section 8.5 (referred to as the Day 11 TIL harvest in Example G). In some embodiments, TILs between days 12 and 22 are harvested using the methods as described in Section 8.12 (referred to as the Day 22 TIL harvest in Example G).

F. STEP F: Final Formulation/ Transfer to Infusion Bag

[00459] After Steps A through E as provided in an exemplary order in Figure 1 and as outlined in detailed above and herein are complete, cells are transferred to a container for use in administration to a patient. In some embodiments, once a therapeutically sufficient number of TILs are obtained using the expansion methods described above, they are transferred to a container for use in administration to a patient.

[00460] In an embodiment, TILs expanded using APCs of the present disclosure are administered to a patient as a pharmaceutical composition. In an embodiment, the pharmaceutical composition is a suspension of TILs in a sterile buffer. TILs expanded using PBMCs of the present disclosure may be administered by any suitable route as known in the art. In some embodiments, the T-cells are administered as a single intra-arterial or intravenous infusion, which preferably lasts approximately 30 to 60 minutes. Other suitable routes of administration include intraperitoneal, intrathecal, and intralymphatic.

G. Optional Cell Medium Components

1. Anti-CD3 Antibodies

[00461] In some embodiments, the culture media used in expansion methods described herein (including those referred to as REP, see for example, Figure 1) also includes an anti-CD3 antibody. An anti-CD3 antibody in combination with IL-2 induces T cell activation and

cell division in the TIL population. This effect can be seen with full length antibodies as well as Fab and F(ab')₂ fragments, with the former being generally preferred; see, *e.g.*, Tsoukas *et al.*, *J. Immunol.* **1985**, 135, 1719, hereby incorporated by reference in its entirety.

[00462] As will be appreciated by those in the art, there are a number of suitable anti-human CD3 antibodies that find use in the invention, including anti-human CD3 polyclonal and monoclonal antibodies from various mammals, including, but not limited to, murine, human, primate, rat, and canine antibodies. In particular embodiments, the OKT3 anti-CD3 antibody is used (commercially available from Ortho-McNeil, Raritan, NJ or Miltenyi Biotech, Auburn, CA).

TABLE 5: 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 INPSRGYTNV 60 NQKFKDKATL TTDKSSSTAY MQLSSLTSED SAVYYCARYY DDHYCLDYWG QGTTLTVSSA 120 KTTAPSVYPL APVCGGTTGS SVTLGCLVKG YFPEPVTLTW NSGSLSSGVH TTPAVLQSDL 180 YTLSSSVTVT SSTWPSQSIT CNVAHPASST KVDKKIEPRP KSCDKTHTCP PCPAPELLGG 240 PSVFLFPPKP KDTLMISRTPEVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN 300 STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE 360 LTKNQVSLTCLVKGFYPSDI AVEWESNGQP ENNYKTTTPPV LDSDGSFFLY SKLTVDKSRW 420 QQGNVFSCSV MHEALHNHYT QKSLSLSPGK 450
SEQ ID NO:2 Muromonab light chain	QIVLTQSPAI MSASPGEKVT MTCSASSSVS YMNWYQQKSG TSPKRWIYDT SKLASGVPAH 60 FRGSGSGTSY SLTISGMEAE DAATYYCQQW SSNPFYFGSG TKLEINRADT APTVSIFFPS 120 SEQLTSGGAS VVCFLNFFYP KDINVKWKID GSERQNGVLN SWTDQDSKDS TYSMSSTLTTL 180 TKDEYERHNS YTCEATHKTS TSPIVKSFNR NEC 213

2. 4-1BB (CD137) AGONISTS

[00463] In an embodiment, the TNFRSF agonist is a 4-1BB (CD137) agonist. The 4-1BB agonist may be any 4-1BB binding molecule known in the art. The 4-1BB binding molecule may be a monoclonal antibody or fusion protein capable of binding to human or mammalian 4-1BB. The 4-1BB agonists or 4-1BB binding molecules may comprise an immunoglobulin heavy chain of any isotype (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. The 4-1BB agonist or 4-1BB binding molecule may have both a heavy and a light chain. As used herein, the term binding molecule also includes antibodies (including full length antibodies), monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), human, humanized or chimeric antibodies, and antibody fragments, *e.g.*, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, epitope-binding fragments of any of the above, and engineered forms of antibodies, *e.g.*, scFv molecules, that bind to 4-1BB. In an embodiment, the 4-1BB agonist is

an antigen binding protein that is a fully human antibody. In an embodiment, the 4-1BB agonist is an antigen binding protein that is a humanized antibody. In some embodiments, 4-1BB agonists for use in the presently disclosed methods and compositions include anti-4-1BB antibodies, human anti-4-1BB antibodies, mouse anti-4-1BB antibodies, mammalian anti-4-1BB antibodies, monoclonal anti-4-1BB antibodies, polyclonal anti-4-1BB antibodies, chimeric anti-4-1BB antibodies, anti-4-1BB adnectins, anti-4-1BB domain antibodies, single chain anti-4-1BB fragments, heavy chain anti-4-1BB fragments, light chain anti-4-1BB fragments, anti-4-1BB fusion proteins, and fragments, derivatives, conjugates, variants, or biosimilars thereof. Agonistic anti-4-1BB antibodies are known to induce strong immune responses. Lee, *et al.*, *PLOS One* **2013**, 8, e69677. In a preferred embodiment, the 4-1BB agonist is an agonistic, anti-4-1BB humanized or fully human monoclonal antibody (*i.e.*, an antibody derived from a single cell line). In an embodiment, the 4-1BB agonist is EU-101 (Eutilex Co. Ltd.), utomilumab, or urelumab, or a fragment, derivative, conjugate, variant, or biosimilar thereof. In a preferred embodiment, the 4-1BB agonist is utomilumab or urelumab, or a fragment, derivative, conjugate, variant, or biosimilar thereof.

[00464] In a preferred embodiment, the 4-1BB agonist or 4-1BB binding molecule may also be a fusion protein. In a preferred embodiment, a multimeric 4-1BB agonist, such as a trimeric or hexameric 4-1BB agonist (with three or six ligand binding domains), may induce superior receptor (4-1BBL) clustering and internal cellular signaling complex formation compared to an agonistic monoclonal antibody, which typically possesses two ligand binding domains. Trimeric (trivalent) or hexameric (or hexavalent) or greater fusion proteins comprising three TNFRSF binding domains and IgG1-Fc and optionally further linking two or more of these fusion proteins are described, *e.g.*, in Gieffers, *et al.*, *Mol. Cancer Therapeutics* **2013**, 12, 2735-47.

[00465] Agonistic 4-1BB antibodies and fusion proteins are known to induce strong immune responses. In a preferred embodiment, the 4-1BB agonist is a monoclonal antibody or fusion protein that binds specifically to 4-1BB antigen in a manner sufficient to reduce toxicity. In some embodiments, the 4-1BB agonist is an agonistic 4-1BB monoclonal antibody or fusion protein that abrogates antibody-dependent cellular toxicity (ADCC), for example NK cell cytotoxicity. In some embodiments, the 4-1BB agonist is an agonistic 4-1BB monoclonal antibody or fusion protein that abrogates antibody-dependent cell phagocytosis (ADCP). In some embodiments, the 4-1BB agonist is an agonistic 4-1BB monoclonal antibody or fusion protein that abrogates complement-dependent cytotoxicity (CDC). In some embodiments, the

4-1BB agonist is an agonistic 4-1BB monoclonal antibody or fusion protein which abrogates Fc region functionality.

[00466] In some embodiments, the 4-1BB agonists are characterized by binding to human 4-1BB (SEQ ID NO:9) with high affinity and agonistic activity. In an embodiment, the 4-1BB agonist is a binding molecule that binds to human 4-1BB (SEQ ID NO:9). In an embodiment, the 4-1BB agonist is a binding molecule that binds to murine 4-1BB (SEQ ID NO:10). The amino acid sequences of 4-1BB antigen to which a 4-1BB agonist or binding molecule binds are summarized in Table 6.

TABLE 6. Amino acid sequences of 4-1BB antigens.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:9 human 4-1BB, Tumor necrosis factor receptor superfamily, member 9 (Homo sapiens)	MGNSCYNIVA TLLLVNFER TRSLQDPCSN CPAGTFCDNN RNQICSPCPP NSFSSAGGQR 60 TCDICRQCKG VFRTRKECSS TSNAECDCTP GFHCLGAGCS MCEQDCKQGQ ELTKKGCKDC 120 CFGTFNDQKR GICRPWTNCS LDGKSVLVNG TKERDVVCGP SPADLSPGAS SVTPPAPARE 180 PGHSPQIISF FLALTSTALL FLLFFLTLEF SVVKRGRKKL LYIFKQPFMR PVQTTQEEDG 240 CSCRFPEEEE GGCEL 255
SEQ ID NO:10 murine 4-1BB, Tumor necrosis factor receptor superfamily, member 9 (Mus musculus)	MGNNCYNVVV IVLLLVGCEK VQAVQNSCDN CQPGTFCRKY NPVCKSCPPS TFSSIGGQPN 60 CNICRVCAGY FRFKKFCSS HNAECECIEG FHCLGPQCTR CEKDCRPGQE LTKQGCKTCS 120 LGTFNDQNGT GVCRPWTNCS LDGRSVLKTG TTEKDVVCGP PVVSFSPSTT ISVTPEGGPG 180 GHSLQVLTLE LALTSALLLA LIFITLLEFSV LKWIRKKFPH IFKQPFKKT GAAQEEDACS 240 CRCPQEEEGG GGGYEL 256

[00467] In some embodiments, the compositions, processes and methods described include a 4-1BB agonist that binds human or murine 4-1BB with a K_D of about 100 pM or lower, binds human or murine 4-1BB with a K_D of about 90 pM or lower, binds human or murine 4-1BB with a K_D of about 80 pM or lower, binds human or murine 4-1BB with a K_D of about 70 pM or lower, binds human or murine 4-1BB with a K_D of about 60 pM or lower, binds human or murine 4-1BB with a K_D of about 50 pM or lower, binds human or murine 4-1BB with a K_D of about 40 pM or lower, or binds human or murine 4-1BB with a K_D of about 30 pM or lower.

[00468] In some embodiments, the compositions, processes and methods described include a 4-1BB agonist that binds to human or murine 4-1BB with a k_{assoc} of about 7.5×10^5 1/M·s or faster, binds to human or murine 4-1BB with a k_{assoc} of about 7.5×10^5 1/M·s or faster, binds to human or murine 4-1BB with a k_{assoc} of about 8×10^5 1/M·s or faster, binds to human or murine 4-1BB with a k_{assoc} of about 8.5×10^5 1/M·s or faster, binds to human or murine 4-1BB with a k_{assoc} of about 9×10^5 1/M·s or faster, binds to human or murine 4-1BB with a k_{assoc} of about 9.5×10^5 1/M·s or faster, or binds to human or murine 4-1BB with a k_{assoc} of about 1×10^6 1/M·s or faster.

[00469] In some embodiments, the compositions, processes and methods described include a 4-1BB agonist that binds to human or murine 4-1BB with a k_{dissoc} of about 2×10^{-5} 1/s or slower, binds to human or murine 4-1BB with a k_{dissoc} of about 2.1×10^{-5} 1/s or slower, binds to human or murine 4-1BB with a k_{dissoc} of about 2.2×10^{-5} 1/s or slower, binds to human or murine 4-1BB with a k_{dissoc} of about 2.3×10^{-5} 1/s or slower, binds to human or murine 4-1BB with a k_{dissoc} of about 2.4×10^{-5} 1/s or slower, binds to human or murine 4-1BB with a k_{dissoc} of about 2.5×10^{-5} 1/s or slower, binds to human or murine 4-1BB with a k_{dissoc} of about 2.6×10^{-5} 1/s or slower or binds to human or murine 4-1BB with a k_{dissoc} of about 2.7×10^{-5} 1/s or slower, binds to human or murine 4-1BB with a k_{dissoc} of about 2.8×10^{-5} 1/s or slower, binds to human or murine 4-1BB with a k_{dissoc} of about 2.9×10^{-5} 1/s or slower, or binds to human or murine 4-1BB with a k_{dissoc} of about 3×10^{-5} 1/s or slower.

[00470] In some embodiments, the compositions, processes and methods described include a 4-1BB agonist that binds to human or murine 4-1BB with an IC_{50} of about 10 nM or lower, binds to human or murine 4-1BB with an IC_{50} of about 9 nM or lower, binds to human or murine 4-1BB with an IC_{50} of about 8 nM or lower, binds to human or murine 4-1BB with an IC_{50} of about 7 nM or lower, binds to human or murine 4-1BB with an IC_{50} of about 6 nM or lower, binds to human or murine 4-1BB with an IC_{50} of about 5 nM or lower, binds to human or murine 4-1BB with an IC_{50} of about 4 nM or lower, binds to human or murine 4-1BB with an IC_{50} of about 3 nM or lower, binds to human or murine 4-1BB with an IC_{50} of about 2 nM or lower, or binds to human or murine 4-1BB with an IC_{50} of about 1 nM or lower.

[00471] In a preferred embodiment, the 4-1BB agonist is utomilumab, also known as PF-05082566 or MOR-7480, or a fragment, derivative, variant, or biosimilar thereof.

Utomilumab is available from Pfizer, Inc. Utomilumab is an immunoglobulin G2-lambda, anti-[*Homo sapiens* TNFRSF9 (tumor necrosis factor receptor (TNFR) superfamily member 9, 4-1BB, T cell antigen ILA, CD137)], *Homo sapiens* (fully human) monoclonal antibody. The amino acid sequences of utomilumab are set forth in Table EE. Utomilumab comprises glycosylation sites at Asn59 and Asn292; heavy chain intrachain disulfide bridges at positions 22-96 (V_H - V_L), 143-199 (C_H1 - C_L), 256-316 (C_H2) and 362-420 (C_H3); light chain intrachain disulfide bridges at positions 22'-87' (V_H - V_L) and 136'-195' (C_H1 - C_L); interchain heavy chain-heavy chain disulfide bridges at IgG2A isoform positions 218-218, 219-219, 222-222, and 225-225, at IgG2A/B isoform positions 218-130, 219-219, 222-222, and 225-225, and at IgG2B isoform positions 219-130 (2), 222-222, and 225-225; and interchain heavy chain-light chain disulfide bridges at IgG2A isoform positions 130-213' (2), IgG2A/B

isoform positions 218-213' and 130-213', and at IgG2B isoform positions 218-213' (2). The preparation and properties of utomilumab and its variants and fragments are described in U.S. Patent Nos. 8,821,867; 8,337,850; and 9,468,678, and International Patent Application Publication No. WO 2012/032433 A1, the disclosures of each of which are incorporated by reference herein. Preclinical characteristics of utomilumab are described in Fisher, *et al.*, *Cancer Immunolog. & Immunother.* **2012**, *61*, 1721-33. Current clinical trials of utomilumab in a variety of hematological and solid tumor indications include U.S. National Institutes of Health clinicaltrials.gov identifiers NCT02444793, NCT01307267, NCT02315066, and NCT02554812.

[00472] In an embodiment, a 4-1BB agonist comprises a heavy chain given by SEQ ID NO:11 and a light chain given by SEQ ID NO:12. In an embodiment, a 4-1BB agonist comprises heavy and light chains having the sequences shown in SEQ ID NO:11 and SEQ ID NO:12, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In an embodiment, a 4-1BB agonist comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:11 and SEQ ID NO:12, respectively. In an embodiment, a 4-1BB agonist comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:11 and SEQ ID NO:12, respectively. In an embodiment, a 4-1BB agonist comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:11 and SEQ ID NO:12, respectively. In an embodiment, a 4-1BB agonist comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:11 and SEQ ID NO:12, respectively. In an embodiment, a 4-1BB agonist comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:11 and SEQ ID NO:12, respectively.

[00473] In an embodiment, the 4-1BB agonist comprises the heavy and light chain CDRs or variable regions (VRs) of utomilumab. In an embodiment, the 4-1BB agonist heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:13, and the 4-1BB agonist light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:14, and conservative amino acid substitutions thereof. In an embodiment, a 4-1BB agonist comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:13 and SEQ ID NO:14, respectively. In an embodiment, a 4-1BB agonist comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:13 and SEQ ID NO:14, respectively. In an embodiment, a 4-1BB agonist comprises V_H and V_L

regions that are each at least 97% identical to the sequences shown in SEQ ID NO:13 and SEQ ID NO:14, respectively. In an embodiment, a 4-1BB agonist comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:13 and SEQ ID NO:14, respectively. In an embodiment, a 4-1BB agonist comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:13 and SEQ ID NO:14, respectively. In an embodiment, a 4-1BB agonist comprises an scFv antibody comprising V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:13 and SEQ ID NO:14.

[00474] In an embodiment, a 4-1BB agonist comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:18, SEQ ID NO:19, and SEQ ID NO:20, respectively, and conservative amino acid substitutions thereof.

[00475] In an embodiment, the 4-1BB agonist is a 4-1BB agonist biosimilar monoclonal antibody approved by drug regulatory authorities with reference to utomilumab. In an embodiment, the biosimilar monoclonal antibody comprises an 4-1BB antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is utomilumab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is a 4-1BB agonist antibody authorized or submitted for authorization, wherein the 4-1BB agonist antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is utomilumab. The 4-1BB agonist antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is utomilumab. In some embodiments, the biosimilar is provided as a composition which further

comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is utomilumab.

TABLE 7. Amino acid sequences for 4-1BB agonist antibodies related to utomilumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:11 heavy chain for utomilumab	EVQLVQSGAE VKKPGESLRI SCKGSGYSFS TYWISWVRQM PGKGLEWMGK IYPGDSYTN 60 SPSFQGGVTI SADKSISTAY LQWSSLKASD TAMYYCARGY GIFDYWGQGT LVTVSSASTK 120 GPSVFPLAPC SRSTSESTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS 180 LSSVTVFPSS NFGTQTYTCN VDHKPSNTKV DKTVERKCCV ECPPCAPPV AGPSVFLFPP 240 KPKDTLMISR TPEVTCVVD VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVSV 300 LTVVHQDWLN GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL 360 TCLVKGFYPS DIAVEWESNG QPENNYKTP PMLDSGGSFF LYSKLTVDKS RWQQGNVFSC 420 SVMHEALHNH YTKSLSLSP G 441
SEQ ID NO:12 light chain for utomilumab	SYELTQPPSV SVSPGQTASI TCSGDNIGDQ YAHWYQQKPG QSPVLVIYQD KNRPSGIPER 60 FSGSNSGNTA TLTISGTQAM DEADYYCATY TGFGLAVFG GGTKLTVLQ PKAAPSVTLF 120 PPSSEELQAN KATLVCLISD FYPGAIVTAV KADSSPVKAG VETTTSPSKQS NNKYAASSYL 180 SLTPEQWKSH RSYSCQVTHE GSTVEKTVAP TECS 214
SEQ ID NO:13 heavy chain variable region for utomilumab	EVQLVQSGAE VKKPGESLRI SCKGSGYSFS TYWISWVRQM PGKGLEWMG K IYPGDSYTN 60 YSPSFQGGVT ISADKSISTA YLQWSSLKAS DTAMYYCARG YGIFDYWGQ GTLTVTVSS 118
SEQ ID NO:14 light chain variable region for utomilumab	SYELTQPPSV SVSPGQTASI TCSGDNIGDQ YAHWYQQKPG QSPVLVIYQD KNRPSGIPER 60 FSGSNSGNTA TLTISGTQAM DEADYYCATY TGFGLAVFG GGTKLTVL 108
SEQ ID NO:15 heavy chain CDR1 for utomilumab	STYWIS 6
SEQ ID NO:16 heavy chain CDR2 for utomilumab	KIYPGDSYTN YSPSFQG 17
SEQ ID NO:17 heavy chain CDR3 for utomilumab	RGYGIFDY 8
SEQ ID NO:18 light chain CDR1 for utomilumab	SGDNIGDQYA H 11
SEQ ID NO:19 light chain CDR2 for utomilumab	QDKNRPS 7
SEQ ID NO:20 light chain CDR3 for utomilumab	ATYTGFGSLA V 11

[00476] In a preferred embodiment, the 4-1BB agonist is the monoclonal antibody urelumab, also known as BMS-663513 and 20H4.9.h4a, or a fragment, derivative, variant, or biosimilar thereof. Urelumab is available from Bristol-Myers Squibb, Inc., and Creative Biolabs, Inc. Urelumab is an immunoglobulin G4-kappa, anti-*[Homo sapiens* TNFRSF9 (tumor necrosis factor receptor superfamily member 9, 4-1BB, T cell antigen ILA, CD137)], *Homo sapiens* (fully human) monoclonal antibody. The amino acid sequences of urelumab are set forth in Table EE. Urelumab comprises N-glycosylation sites at positions 298 (and 298''); heavy chain intrachain disulfide bridges at positions 22-95 (V_H-V_L), 148-204 (C_{H1}-C_L), 262-322 (C_{H2}) and 368-426 (C_{H3}) (and at positions 22''-95'', 148''-204'', 262''-322'', and 368''-426''); light chain intrachain disulfide bridges at positions 23'-88' (V_H-V_L) and 136'-196'

(C_{H1}-C_L) (and at positions 23'''-88''' and 136'''-196'''); interchain heavy chain-heavy chain disulfide bridges at positions 227-227'' and 230-230''; and interchain heavy chain-light chain disulfide bridges at 135-216' and 135''-216'''. The preparation and properties of urelumab and its variants and fragments are described in U.S. Patent Nos. 7,288,638 and 8,962,804, the disclosures of which are incorporated by reference herein. The preclinical and clinical characteristics of urelumab are described in Segal, *et al.*, *Clin. Cancer Res.* **2016**, available at <http://dx.doi.org/10.1158/1078-0432.CCR-16-1272>. Current clinical trials of urelumab in a variety of hematological and solid tumor indications include U.S. National Institutes of Health clinicaltrials.gov identifiers NCT01775631, NCT02110082, NCT02253992, and NCT01471210.

[00477] In an embodiment, a 4-1BB agonist comprises a heavy chain given by SEQ ID NO:21 and a light chain given by SEQ ID NO:22. In an embodiment, a 4-1BB agonist comprises heavy and light chains having the sequences shown in SEQ ID NO:21 and SEQ ID NO:22, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In an embodiment, a 4-1BB agonist comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:21 and SEQ ID NO:22, respectively. In an embodiment, a 4-1BB agonist comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:21 and SEQ ID NO:22, respectively. In an embodiment, a 4-1BB agonist comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:21 and SEQ ID NO:22, respectively. In an embodiment, a 4-1BB agonist comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:21 and SEQ ID NO:22, respectively. In an embodiment, a 4-1BB agonist comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:21 and SEQ ID NO:22, respectively.

[00478] In an embodiment, the 4-1BB agonist comprises the heavy and light chain CDRs or variable regions (VRs) of urelumab. In an embodiment, the 4-1BB agonist heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:23, and the 4-1BB agonist light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:24, and conservative amino acid substitutions thereof. In an embodiment, a 4-1BB agonist comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:23 and SEQ ID NO:24, respectively. In an embodiment, a 4-1BB agonist comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:23

and SEQ ID NO:24, respectively. In an embodiment, a 4-1BB agonist comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:23 and SEQ ID NO:24, respectively. In an embodiment, a 4-1BB agonist comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:23 and SEQ ID NO:24, respectively. In an embodiment, a 4-1BB agonist comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:23 and SEQ ID NO:24, respectively. In an embodiment, a 4-1BB agonist comprises an scFv antibody comprising V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:23 and SEQ ID NO:24.

[00479] In an embodiment, a 4-1BB agonist comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, respectively, and conservative amino acid substitutions thereof.

[00480] In an embodiment, the 4-1BB agonist is a 4-1BB agonist biosimilar monoclonal antibody approved by drug regulatory authorities with reference to urelumab. In an embodiment, the biosimilar monoclonal antibody comprises an 4-1BB antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is urelumab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is a 4-1BB agonist antibody authorized or submitted for authorization, wherein the 4-1BB agonist antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is urelumab. The 4-1BB agonist antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is urelumab.

In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is urelumab.

TABLE 8: Amino acid sequences for 4-1BB agonist antibodies related to urelumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:21 heavy chain for urelumab	QVQLQQWGAG LLKPSETLSL TCAVYGGSF S GYWSWIRQS PEKGLEWIGE INHGGYVTYN 60 PSLESRV TIS VDTSKNQFSL KLSSVTAADT AVYYCARDYG PGNYDWYFDL WGRGTLVTVS 120 SASTKGPSVF PLAPCSRSTS ESTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS 180 SGLYSLSSVV TVPSSSLGK TYTCNV DHPK SNTKVDKRVE SKYGPPCPPC PAPEFLGGPS 240 VFLFPKPKD TLMISRTPEV TCVVDVSQE DPEVQFNWYV DGEVHNHAKT KPREEQFNST 300 YRVSVLTVL HQDWLNGKEY KCKVSNKGLP SSIEKTISKA KGQPREPQVY TLPPSQEEMT 360 KNQVSLTCLV KGFYP SDAIV EWESNGQPEN NYKTPPVLD SDGSFFLYSR LTVDKSRWQE 420 GNVFSCSVMH EALHNHYTQK SLSLSLGK 448
SEQ ID NO:22 light chain for urelumab	EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA 60 RFGSGSGTD FTLTISSLEP EDFAVYYCQQ RSNWPPALTF CGGKVEIKR TVAAPS VFIF 120 PPSDEQLKSG TASVVC LLNN FYPREAKVQW KVDNALQSGN SQESVTEQDS KDSTYLSST 180 LTLKADYEK HKVYACEVTH QGLSSPVTKS FNRGEC 216
SEQ ID NO:23 variable heavy chain for urelumab	MKHLWFFLL VAA PRWVLSQ VQLQQWGAGL LKPSETLSLT CAVYGGSFSG YYWSWIRQSP 60 EKGLEWIGEI NHGGYVTYNP SLESRV TISV DTSKNQFSLK LSSVTAADTA VYYCARDYGP 120
SEQ ID NO:24 variable light chain for urelumab	MEAPAQLLFL LLLWLPD TTG EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP 60 GQAPRLLIYD ASNRATGIPA RFGSGSGTD FTLTISSLEP EDFAVYYCQQ 110
SEQ ID NO:25 heavy chain CDR1 for urelumab	GYYS 5
SEQ ID NO:26 heavy chain CDR2 for urelumab	EINHGGYVTY NPSLES 16
SEQ ID NO:27 heavy chain CDR3 for urelumab	DYGP GNYDWY FDL 13
SEQ ID NO:28 light chain CDR1 for urelumab	RASQSVSSYL A 11
SEQ ID NO:29 light chain CDR2 for urelumab	DASNRAT 7
SEQ ID NO:30 light chain CDR3 for urelumab	QQRSDWPPAL T 11

[00481] In an embodiment, the 4-1BB agonist is selected from the group consisting of 1D8, 3Elor, 4B4 (BioLegend 309809), H4-1BB-M127 (BD Pharmingen 552532), BBK2 (Thermo Fisher MS621PABX), 145501 (Leinco Technologies B591), the antibody produced by cell line deposited as ATCC No. HB-11248 and disclosed in U.S. Patent No. 6,974,863, 5F4 (BioLegend 31 1503), C65-485 (BD Pharmingen 559446), antibodies disclosed in U.S. Patent Application Publication No. US 2005/0095244, antibodies disclosed in U.S. Patent No. 7,288,638 (such as 20H4.9-IgG1 (BMS-663031)), antibodies disclosed in U.S. Patent No. 6,887,673 (such as 4E9 or BMS-554271), antibodies disclosed in U.S. Patent No. 7,214,493, antibodies disclosed in U.S. Patent No. 6,303,121, antibodies disclosed in U.S. Patent No. 6,569,997, antibodies disclosed in U.S. Patent No. 6,905,685 (such as 4E9 or BMS-554271),

antibodies disclosed in U.S. Patent No. 6,362,325 (such as 1D8 or BMS-469492; 3H3 or BMS-469497; or 3E1), antibodies disclosed in U.S. Patent No. 6,974,863 (such as 53A2); antibodies disclosed in U.S. Patent No. 6,210,669 (such as 1D8, 3B8, or 3E1), antibodies described in U.S. Patent No. 5,928,893, antibodies disclosed in U.S. Patent No. 6,303,121, antibodies disclosed in U.S. Patent No. 6,569,997, antibodies disclosed in International Patent Application Publication Nos. WO 2012/177788, WO 2015/119923, and WO 2010/042433, and fragments, derivatives, conjugates, variants, or biosimilars thereof, wherein the disclosure of each of the foregoing patents or patent application publications is incorporated by reference here.

[00482] In an embodiment, the 4-1BB agonist is a 4-1BB agonistic fusion protein described in International Patent Application Publication Nos. WO 2008/025516 A1, WO 2009/007120 A1, WO 2010/003766 A1, WO 2010/010051 A1, and WO 2010/078966 A1; U.S. Patent Application Publication Nos. US 2011/0027218 A1, US 2015/0126709 A1, US 2011/0111494 A1, US 2015/0110734 A1, and US 2015/0126710 A1; and U.S. Patent Nos. 9,359,420, 9,340,599, 8,921,519, and 8,450,460, the disclosures of which are incorporated by reference herein.

[00483] In an embodiment, the 4-1BB agonist is a 4-1BB agonistic fusion protein as depicted in Structure I-A (C-terminal Fc-antibody fragment fusion protein) or Structure I-B (N-terminal Fc-antibody fragment fusion protein), or a fragment, derivative, conjugate, variant, or biosimilar thereof, as provided in Figure 10.

[00484] In 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 C_H3 and C_H2 domains) is then used to link two of the trivalent proteins together through disulfide bonds (small elongated ovals), stabilizing the structure and providing an agonists capable of bringing together the intracellular signaling domains of the six receptors and signaling proteins to form a signaling complex. The TNFRSF binding domains denoted as cylinders may be scFv domains comprising, *e.g.*, a V_H and a V_L chain connected by a linker that may comprise hydrophilic residues and Gly and Ser sequences for flexibility, as well as Glu and Lys for solubility. Any scFv domain design may be used, such as those described in de Marco, *Microbial Cell Factories*, **2011**, 10, 44; Ahmad, *et al.*, *Clin. & Dev. Immunol.* **2012**, 980250; Monnier, *et al.*, *Antibodies*, **2013**, 2, 193-208; or in references incorporated

elsewhere herein. Fusion protein structures of this form are described in U.S. Patent Nos. 9,359,420, 9,340,599, 8,921,519, and 8,450,460, the disclosures of which are incorporated by reference herein.

[00485] Amino acid sequences for the other polypeptide domains of structure I-A are given in Table GG. The Fc domain preferably comprises a complete constant domain (amino acids 17-230 of SEQ ID NO:31) the complete hinge domain (amino acids 1-16 of SEQ ID NO:31) or a portion of the hinge domain (*e.g.*, amino acids 4-16 of SEQ ID NO:31). Preferred linkers for connecting a C-terminal Fc-antibody may be selected from the embodiments given in SEQ ID NO:32 to SEQ ID NO:41, including linkers suitable for fusion of additional polypeptides.

TABLE 9: Amino acid sequences for TNFRSF fusion proteins, including 4-1BB fusion proteins, with C-terminal Fc-antibody fragment fusion protein design (structure I-A).

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:31 Fc domain	KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNW 60 YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS 120 KAKGQPREPQ VYTLPPSREE MTKNQVSLTLC LVKGFFPSDI AVEWESNGQP ENNYKTTTPPV 180 LDSDGSFFLY SKLTVDKSRW QQGNVVFSCSV MHEALHNHYT QKSLSLSPGK 230
SEQ ID NO:32 linker	GGPGSSKSCD KTHTCPPCPA PE 22
SEQ ID NO:33 linker	GGSGSSKSCD KTHTCPPCPA PE 22
SEQ ID NO:34 linker	GGPGSSSSSS SKSCDKTHTC PPCPAPE 27
SEQ ID NO:35 linker	GGSGSSSSSS SKSCDKTHTC PPCPAPE 27
SEQ ID NO:36 linker	GGPGSSSSSS SSSKSCDKTH TCPPCPAPE 29
SEQ ID NO:37 linker	GGSGSSSSSS SSSKSCDKTH TCPPCPAPE 29
SEQ ID NO:38 linker	GGPGSSSGSGS SDKTHTCPPC PAPE 24
SEQ ID NO:39 linker	GGPGSSSGSGS DKTHTCPPCP APE 23
SEQ ID NO:40 linker	GGPSSSGSDK THTCPPCPAP E 21
SEQ ID NO:41 linker	GGSSSSSSSS GSDKTHTCPP CPAPE 25

[00486] Amino acid sequences for the other polypeptide domains of structure I-B are given in Table HH. If an Fc antibody fragment is fused to the N-terminus of an TNFRSF fusion protein as in structure I-B, the sequence of the Fc module is preferably that shown in SEQ ID NO:42, and the linker sequences are preferably selected from those embodiments set forth in SEQ ID NO:43 to SEQ ID NO:45.

TABLE 10: Amino acid sequences for TNFRSF fusion proteins, including 4-1BB fusion proteins, with N-terminal Fc-antibody fragment fusion protein design (structure I-B).

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:42	METDTLLLV LLLWVPAGNG DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT 60

Fc domain	CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPG	120 180 240 246
SEQ ID NO:43 linker	SGSGSGSGSG S	11
SEQ ID NO:44 linker	SSSSSSGSGS GS	12
SEQ ID NO:45 linker	SSSSSSGSGS GSGSGS	16

[00487] In an embodiment, a 4-1BB agonist fusion protein according to structures I-A or I-B comprises one or more 4-1BB binding domains selected from the group consisting of a variable heavy chain and variable light chain of utomilumab, a variable heavy chain and variable light chain of urelumab, a variable heavy chain and variable light chain of utomilumab, a variable heavy chain and variable light chain selected from the variable heavy chains and variable light chains described in Table GG, any combination of a variable heavy chain and variable light chain of the foregoing, and fragments, derivatives, conjugates, variants, and biosimilars thereof.

[00488] In an embodiment, a 4-1BB agonist fusion protein according to structures I-A or I-B comprises one or more 4-1BB binding domains comprising a 4-1BBL sequence. In an embodiment, a 4-1BB agonist fusion protein according to structures I-A or I-B comprises one or more 4-1BB binding domains comprising a sequence according to SEQ ID NO:46. In an embodiment, a 4-1BB agonist fusion protein according to structures I-A or I-B comprises one or more 4-1BB binding domains comprising a soluble 4-1BBL sequence. In an embodiment, a 4-1BB agonist fusion protein according to structures I-A or I-B comprises one or more 4-1BB binding domains comprising a sequence according to SEQ ID NO:47.

[00489] In an embodiment, a 4-1BB agonist fusion protein according to structures I-A or I-B comprises one or more 4-1BB binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:13 and SEQ ID NO:14, respectively, wherein the V_H and V_L domains are connected by a linker. In an embodiment, a 4-1BB agonist fusion protein according to structures I-A or I-B comprises one or more 4-1BB binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:23 and SEQ ID NO:24, respectively, wherein the V_H and V_L domains are connected by a linker. In an embodiment, a 4-1BB agonist fusion protein according to structures I-A or I-B comprises one or more 4-1BB binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the V_H and V_L sequences given in Table 11, wherein the V_H and V_L domains are connected by a linker.

TABLE 11: Additional polypeptide domains useful as 4-1BB binding domains in fusion proteins or as scFv 4-1BB agonist antibodies.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:46 4-1BBL	MEYASDASLD PEAPWPPAPR ARACRVLPWA LVAGLLLLLL LAAACAVFLA CPWAVSGARA 60 SPGSAASPRL REGPELSPDD PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL 120 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA 180 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVLHTEA RARHAWQLTQ GATVLGLFRV 240 TPEIPAGLPS PRSE 254
SEQ ID NO:47 4-1BBL soluble domain	LRQGMFAQLV AQNVLLIDGP LSWYSDPGLA GVSLTGGLSY KEDTKELVVA KAGVYYVFFQ 60 LELRRVAGE GSGSVSLALH LQPLRSAAGA AALALTVDLP PASSEARNSA FGFQGRLLHL 120 SAGQLRGVHL HTEARARHAW QLTQGATVLG LFRVTPEIPA GLPSPRSE 168
SEQ ID NO:48 variable heavy chain for 4B4-1-1 version 1	QVQLQQPGAE LVKPGASVKL SCKASGYTFS SYWMHWVKQR PGQVLEWIGE INPGNGHTNY 60 NEKFKSKATL TVDKSSSTAY MQLSSLTSED SAVYYCARSF TTARGFAYWG QGTLVTVS 118
SEQ ID NO:49 variable light chain for 4B4-1-1 version 1	DIVMTQSPAT QSVTPGDRVS LSCRASQTIS DYLHWYQQKS HESPRLLIKY ASQSISGIPS 60 RFGSGSGSD FTLSINSVEP EDVGVYYCQD GHSFPPTFGG GTKLEIK 107
SEQ ID NO:50 variable heavy chain for 4B4-1-1 version 2	QVQLQQPGAE LVKPGASVKL SCKASGYTFS SYWMHWVKQR PGQVLEWIGE INPGNGHTNY 60 NEKFKSKATL TVDKSSSTAY MQLSSLTSED SAVYYCARSF TTARGFAYWG QGTLVTVSA 119
SEQ ID NO:51 variable light chain for 4B4-1-1 version 2	DIVMTQSPAT QSVTPGDRVS LSCRASQTIS DYLHWYQQKS HESPRLLIKY ASQSISGIPS 60 RFGSGSGSD FTLSINSVEP EDVGVYYCQD GHSFPPTFGG GTKLEIKR 108
SEQ ID NO:52 variable heavy chain for H39E3-2	MDWTWRILFL VAAATGAHSE VQLVESGGGL VQPGGSLRLS CAASGFTFSD YWMSWVRQAP 60 GKGLEWVADI KNDGSYTNYA PSLTNRTIS RDNAKNSLYL QMNSLRAEDT AVYYCARELT 120
SEQ ID NO:53 variable light chain for H39E3-2	MEAPAQLLFL LLLWLPDTHG DIVMTQSPDS LAVSLGERAT INCKSSQSLL SSGNQKNYL 60 WYQQKPGQPP KLLIYYASTR QSGVPDRFSG SSGTDFTLT ISSLQAEDVA 110

[00490] In an embodiment, the 4-1BB agonist is a 4-1BB agonistic single-chain fusion polypeptide comprising (i) a first soluble 4-1BB binding domain, (ii) a first peptide linker, (iii) a second soluble 4-1BB binding domain, (iv) a second peptide linker, and (v) a third soluble 4-1BB binding domain, further comprising an additional domain at the N-terminal and/or C-terminal end, and wherein the additional domain is a Fab or Fc fragment domain. In an embodiment, the 4-1BB agonist is a 4-1BB agonistic single-chain fusion polypeptide comprising (i) a first soluble 4-1BB binding domain, (ii) a first peptide linker, (iii) a second soluble 4-1BB binding domain, (iv) a second peptide linker, and (v) a third soluble 4-1BB binding domain, further comprising an additional domain at the N-terminal and/or C-terminal end, wherein the additional domain is a Fab or Fc fragment domain, wherein each of the soluble 4-1BB domains lacks a stalk region (which contributes to trimerisation and provides a certain distance to the cell membrane, but is not part of the 4-1BB binding domain) and the first and the second peptide linkers independently have a length of 3-8 amino acids.

[00491] In an embodiment, the 4-1BB agonist is a 4-1BB agonistic single-chain fusion polypeptide comprising (i) a first soluble tumor necrosis factor (TNF) superfamily cytokine domain, (ii) a first peptide linker, (iii) a second soluble TNF superfamily cytokine domain,

(iv) a second peptide linker, and (v) a third soluble TNF superfamily cytokine domain, wherein each of the soluble TNF superfamily cytokine domains lacks a stalk region and the first and the second peptide linkers independently have a length of 3-8 amino acids, and wherein each TNF superfamily cytokine domain is a 4-1BB binding domain.

[00492] In an embodiment, the 4-1BB agonist is a 4-1BB agonistic scFv antibody comprising any of the foregoing V_H domains linked to any of the foregoing V_L domains.

[00493] In an embodiment, the 4-1BB agonist is BPS Bioscience 4-1BB agonist antibody catalog no. 79097-2, commercially available from BPS Bioscience, San Diego, CA, USA. In an embodiment, the 4-1BB agonist is Creative Biolabs 4-1BB agonist antibody catalog no. MOM-18179, commercially available from Creative Biolabs, Shirley, NY, USA.

3. OX40 (CD134) AGONISTS

[00494] In an embodiment, the TNFRSF agonist is an OX40 (CD134) agonist. The OX40 agonist may be any OX40 binding molecule known in the art. The OX40 binding molecule may be a monoclonal antibody or fusion protein capable of binding to human or mammalian OX40. The OX40 agonists or OX40 binding molecules may comprise an immunoglobulin heavy chain of any isotype (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. The OX40 agonist or OX40 binding molecule may have both a heavy and a light chain. As used herein, the term binding molecule also includes antibodies (including full length antibodies), monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), human, humanized or chimeric antibodies, and antibody fragments, *e.g.*, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, epitope-binding fragments of any of the above, and engineered forms of antibodies, *e.g.*, scFv molecules, that bind to OX40. In an embodiment, the OX40 agonist is an antigen binding protein that is a fully human antibody. In an embodiment, the OX40 agonist is an antigen binding protein that is a humanized antibody. In some embodiments, OX40 agonists for use in the presently disclosed methods and compositions include anti-OX40 antibodies, human anti-OX40 antibodies, mouse anti-OX40 antibodies, mammalian anti-OX40 antibodies, monoclonal anti-OX40 antibodies, polyclonal anti-OX40 antibodies, chimeric anti-OX40 antibodies, anti-OX40 adnectins, anti-OX40 domain antibodies, single chain anti-OX40 fragments, heavy chain anti-OX40 fragments, light chain anti-OX40 fragments, anti-OX40 fusion proteins, and fragments, derivatives, conjugates, variants, or

biosimilars thereof. In a preferred embodiment, the OX40 agonist is an agonistic, anti-OX40 humanized or fully human monoclonal antibody (*i.e.*, an antibody derived from a single cell line).

[00495] In a preferred embodiment, the OX40 agonist or OX40 binding molecule may also be a fusion protein. OX40 fusion proteins comprising an Fc domain fused to OX40L are described, for example, in Sadun, *et al.*, *J. Immunother.* **2009**, *182*, 1481-89. In a preferred embodiment, a multimeric OX40 agonist, such as a trimeric or hexameric OX40 agonist (with three or six ligand binding domains), may induce superior receptor (OX40L) clustering and internal cellular signaling complex formation compared to an agonistic monoclonal antibody, which typically possesses two ligand binding domains. Trimeric (trivalent) or hexameric (or hexavalent) or greater fusion proteins comprising three TNFRSF binding domains and IgG1-Fc and optionally further linking two or more of these fusion proteins are described, *e.g.*, in Gieffers, *et al.*, *Mol. Cancer Therapeutics* **2013**, *12*, 2735-47.

[00496] Agonistic OX40 antibodies and fusion proteins are known to induce strong immune responses. Curti, *et al.*, *Cancer Res.* **2013**, *73*, 7189-98. In a preferred embodiment, the OX40 agonist is a monoclonal antibody or fusion protein that binds specifically to OX40 antigen in a manner sufficient to reduce toxicity. In some embodiments, the OX40 agonist is an agonistic OX40 monoclonal antibody or fusion protein that abrogates antibody-dependent cellular toxicity (ADCC), for example NK cell cytotoxicity. In some embodiments, the OX40 agonist is an agonistic OX40 monoclonal antibody or fusion protein that abrogates antibody-dependent cell phagocytosis (ADCP). In some embodiments, the OX40 agonist is an agonistic OX40 monoclonal antibody or fusion protein that abrogates complement-dependent cytotoxicity (CDC). In some embodiments, the OX40 agonist is an agonistic OX40 monoclonal antibody or fusion protein which abrogates Fc region functionality.

[00497] In some embodiments, the OX40 agonists are characterized by binding to human OX40 (SEQ ID NO:54) with high affinity and agonistic activity. In an embodiment, the OX40 agonist is a binding molecule that binds to human OX40 (SEQ ID NO:54). In an embodiment, the OX40 agonist is a binding molecule that binds to murine OX40 (SEQ ID NO:55). The amino acid sequences of OX40 antigen to which an OX40 agonist or binding molecule binds are summarized in Table 12.

TABLE 12: Amino acid sequences of OX40 antigens.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:54 human OX40 (Homo sapiens)	MCVGARRLGR GPCAALLLLG LGLSTVTGLH CVGDTYPSND RCCHECRPGN GMVSRCSRSQ 60 NTVCRPCGPG FYNDVVSSKP CKPCTWCNLR SGERKQLCT ATQDTCRCR AGTQPLDSYK 120 PGVDCAPCPP GHFSPGDNQA CKPWTNCTLA GKHTLQPASN SSDAICEDRD PPATQPQETQ 180 GPPARPITVQ PTEAWPRTSQ GPSTRPVEVP GGRAVAAILG LGLVLGLLGP LAILLALYLL 240 RRDQRLPPDA HKPPGGGSFR TPIQEEQADA HSTLAKI 277
SEQ ID NO:55 murine OX40 (Mus musculus)	MYVWVQQPTA LLLLGLTLGV TARRLNCVKH TYP SGHKCCR ECQPGHGMVS RCDHTRDTLC 60 HPCETGFYNE AVNYDTCKQC TQCNHRSGSE LKQNCPTPTQD TVCRCRPGTQ PRQDSGYKLG 120 VDCVPCPPGH FSPGNNQACK PWTNCTLSGK QTRHPASDSL DAVCEDRSL ATLLWETQRP 180 TFRPTTVQST TVWPRTSELP SPPTLVTPQG PAFAVLLGLG LGLLAPLTVL LALYLLRKAW 240 RLPNTFKPCW GNSFRTPIQE EHTDAHFTLA KI 272

[00498] In some embodiments, the compositions, processes and methods described include a OX40 agonist that binds human or murine OX40 with a K_D of about 100 pM or lower, binds human or murine OX40 with a K_D of about 90 pM or lower, binds human or murine OX40 with a K_D of about 80 pM or lower, binds human or murine OX40 with a K_D of about 70 pM or lower, binds human or murine OX40 with a K_D of about 60 pM or lower, binds human or murine OX40 with a K_D of about 50 pM or lower, binds human or murine OX40 with a K_D of about 40 pM or lower, or binds human or murine OX40 with a K_D of about 30 pM or lower.

[00499] In some embodiments, the compositions, processes and methods described include a OX40 agonist that binds to human or murine OX40 with a k_{assoc} of about 7.5×10^5 1/M·s or faster, binds to human or murine OX40 with a k_{assoc} of about 7.5×10^5 1/M·s or faster, binds to human or murine OX40 with a k_{assoc} of about 8×10^5 1/M·s or faster, binds to human or murine OX40 with a k_{assoc} of about 8.5×10^5 1/M·s or faster, binds to human or murine OX40 with a k_{assoc} of about 9×10^5 1/M·s or faster, binds to human or murine OX40 with a k_{assoc} of about 9.5×10^5 1/M·s or faster, or binds to human or murine OX40 with a k_{assoc} of about 1×10^6 1/M·s or faster.

[00500] In some embodiments, the compositions, processes and methods described include a OX40 agonist that binds to human or murine OX40 with a k_{dissoc} of about 2×10^{-5} 1/s or slower, binds to human or murine OX40 with a k_{dissoc} of about 2.1×10^{-5} 1/s or slower, binds to human or murine OX40 with a k_{dissoc} of about 2.2×10^{-5} 1/s or slower, binds to human or murine OX40 with a k_{dissoc} of about 2.3×10^{-5} 1/s or slower, binds to human or murine OX40 with a k_{dissoc} of about 2.4×10^{-5} 1/s or slower, binds to human or murine OX40 with a k_{dissoc} of about 2.5×10^{-5} 1/s or slower, binds to human or murine OX40 with a k_{dissoc} of about 2.6×10^{-5} 1/s or slower or binds to human or murine OX40 with a k_{dissoc} of about 2.7×10^{-5} 1/s or slower, binds to human or murine OX40 with a k_{dissoc} of about 2.8×10^{-5} 1/s or slower, binds to human or murine OX40 with a k_{dissoc} of about 2.9×10^{-5} 1/s or slower, or binds to human or murine OX40 with a k_{dissoc} of about 3×10^{-5} 1/s or slower.

[00501] In some embodiments, the compositions, processes and methods described include OX40 agonist that binds to human or murine OX40 with an IC₅₀ of about 10 nM or lower, binds to human or murine OX40 with an IC₅₀ of about 9 nM or lower, binds to human or murine OX40 with an IC₅₀ of about 8 nM or lower, binds to human or murine OX40 with an IC₅₀ of about 7 nM or lower, binds to human or murine OX40 with an IC₅₀ of about 6 nM or lower, binds to human or murine OX40 with an IC₅₀ of about 5 nM or lower, binds to human or murine OX40 with an IC₅₀ of about 4 nM or lower, binds to human or murine OX40 with an IC₅₀ of about 3 nM or lower, binds to human or murine OX40 with an IC₅₀ of about 2 nM or lower, or binds to human or murine OX40 with an IC₅₀ of about 1 nM or lower.

[00502] In some embodiments, the OX40 agonist is tavolixizumab, also known as MEDI0562 or MEDI-0562. Tavolixizumab is available from the MedImmune subsidiary of AstraZeneca, Inc. Tavolixizumab is immunoglobulin G1-kappa, anti-[*Homo sapiens* TNFRSF4 (tumor necrosis factor receptor (TNFR) superfamily member 4, OX40, CD134)], humanized and chimeric monoclonal antibody. The amino acid sequences of tavolixizumab are set forth in Table KK. Tavolixizumab comprises N-glycosylation sites at positions 301 and 301'', with fucosylated complex bi-antennary CHO-type glycans; heavy chain intrachain disulfide bridges at positions 22-95 (V_H-V_L), 148-204 (C_{H1}-C_L), 265-325 (C_{H2}) and 371-429 (C_{H3}) (and at positions 22''-95'', 148''-204'', 265''-325'', and 371''-429''); light chain intrachain disulfide bridges at positions 23'-88' (V_H-V_L) and 134'-194' (C_{H1}-C_L) (and at positions 23'''-88''' and 134'''-194'''); interchain heavy chain-heavy chain disulfide bridges at positions 230-230'' and 233-233''; and interchain heavy chain-light chain disulfide bridges at 224-214' and 224''-214''. Current clinical trials of tavolixizumab in a variety of solid tumor indications include U.S. National Institutes of Health clinicaltrials.gov identifiers NCT02318394 and NCT02705482.

[00503] In an embodiment, a OX40 agonist comprises a heavy chain given by SEQ ID NO:56 and a light chain given by SEQ ID NO:57. In an embodiment, a OX40 agonist comprises heavy and light chains having the sequences shown in SEQ ID NO:56 and SEQ ID NO:57, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In an embodiment, a OX40 agonist comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:56 and SEQ ID NO:57, respectively. In an embodiment, a OX40 agonist comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:56 and SEQ ID NO:57, respectively. In an embodiment, a OX40 agonist

comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:56 and SEQ ID NO:57, respectively. In an embodiment, a OX40 agonist comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:56 and SEQ ID NO:57, respectively. In an embodiment, a OX40 agonist comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:56 and SEQ ID NO:57, respectively.

[00504] In an embodiment, the OX40 agonist comprises the heavy and light chain CDRs or variable regions (VRs) of tavolixizumab. In an embodiment, the OX40 agonist heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:58, and the OX40 agonist light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:59, and conservative amino acid substitutions thereof. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:58 and SEQ ID NO:59, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:58 and SEQ ID NO:59, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:58 and SEQ ID NO:59, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:58 and SEQ ID NO:59, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:58 and SEQ ID NO:59, respectively. In an embodiment, an OX40 agonist comprises an scFv antibody comprising V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:58 and SEQ ID NO:59.

[00505] In an embodiment, a OX40 agonist comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, respectively, and conservative amino acid substitutions thereof.

[00506] In an embodiment, the OX40 agonist is a OX40 agonist biosimilar monoclonal antibody approved by drug regulatory authorities with reference to tavolixizumab. In an embodiment, the biosimilar monoclonal antibody comprises an OX40 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or

reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is tavolixizumab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is a OX40 agonist antibody authorized or submitted for authorization, wherein the OX40 agonist antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is tavolixizumab. The OX40 agonist antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is tavolixizumab. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is tavolixizumab.

TABLE 13: Amino acid sequences for OX40 agonist antibodies related to tavolixizumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:56 heavy chain for tavolixizumab	QVQLQESGPG LVKPSQTLST TCAVYGGSFSGYWNWIRKH PGKGLLEYIGY ISYNGITYHN 60 PSLKSRITIN RDTSKNQYSL QLNSTVPEDT AVYYCARYKY DYDGGHAMDY WGQGTPLVTVS 120 SASTKGPSVF PLAPSSKSTS GGTAAALGCLV KDYFPEPVTW SWNSGALTSG VHTFPAVLQS 180 SGLYSLSSV TVPSSSLGTQ TYICNVNHKP SNTKVDKRVK PKSCDKTHTC PPCPAPELLG 240 GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY 300 NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRE 360 EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTTP VLDSDGSFFL YSKLTVDKSR 420 WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K 451
SEQ ID NO:57 light chain for tavolixizumab	DIQMTQSPSS LSASVGDRVT ITCRASQDIS NYLNWYQQKP GKAPKLLIYY TSKLHSGVPS 60 RFGSGSGTD YTLTISSLQP EDFATYYCQQ GSALPWFPGQ GTKVEIKRTV AAPSVEIFPP 120 SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSLT 180 LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEK 214
SEQ ID NO:58 heavy chain variable region for tavolixizumab	QVQLQESGPG LVKPSQTLST TCAVYGGSFSGYWNWIRKH PGKGLLEYIGY ISYNGITYHN 60 PSLKSRITIN RDTSKNQYSL QLNSTVPEDT AVYYCARYKY DYDGGHAMDY WGQGTPLV 118
SEQ ID NO:59 light chain variable region for tavolixizumab	DIQMTQSPSS LSASVGDRVT ITCRASQDIS NYLNWYQQKP GKAPKLLIYY TSKLHSGVPS 60 RFGSGSGTD YTLTISSLQP EDFATYYCQQ GSALPWFPGQ GTKVEIKR 108
SEQ ID NO:60 heavy chain CDR1 for tavolixizumab	GSFSSGYWN 9

SEQ ID NO:61 heavy chain CDR2 for tavolixizumab	YIGYISYNGI TYH	13
SEQ ID NO:62 heavy chain CDR3 for tavolixizumab	RYKYDYDGGH AMDY	14
SEQ ID NO:63 light chain CDR1 for tavolixizumab	QDISNYLN	8
SEQ ID NO:64 light chain CDR2 for tavolixizumab	LLIYYTSKLH S	11
SEQ ID NO:65 light chain CDR3 for tavolixizumab	QQGSALPW	8

[00507] In some embodiments, the OX40 agonist is 11D4, which is a fully human antibody available from Pfizer, Inc. The preparation and properties of 11D4 are described in U.S. Patent Nos. 7,960,515; 8,236,930; and 9,028,824, the disclosures of which are incorporated by reference herein. The amino acid sequences of 11D4 are set forth in Table LL.

[00508] In an embodiment, a OX40 agonist comprises a heavy chain given by SEQ ID NO:66 and a light chain given by SEQ ID NO:67. In an embodiment, a OX40 agonist comprises heavy and light chains having the sequences shown in SEQ ID NO:66 and SEQ ID NO:67, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In an embodiment, a OX40 agonist comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:66 and SEQ ID NO:67, respectively. In an embodiment, a OX40 agonist comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:66 and SEQ ID NO:67, respectively. In an embodiment, a OX40 agonist comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:66 and SEQ ID NO:67, respectively. In an embodiment, a OX40 agonist comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:66 and SEQ ID NO:67, respectively. In an embodiment, a OX40 agonist comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:66 and SEQ ID NO:67, respectively.

[00509] In an embodiment, the OX40 agonist comprises the heavy and light chain CDRs or variable regions (VRs) of 11D4. In an embodiment, the OX40 agonist heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:68, and the OX40 agonist light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:69, and conservative amino acid substitutions thereof. In an embodiment, a OX40 agonist comprises

V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:68 and SEQ ID NO:69, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:68 and SEQ ID NO:69, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:68 and SEQ ID NO:69, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:68 and SEQ ID NO:69, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:68 and SEQ ID NO:69, respectively.

[00510] In an embodiment, a OX40 agonist comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:70, SEQ ID NO:71, and SEQ ID NO:72, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:73, SEQ ID NO:74, and SEQ ID NO:75, respectively, and conservative amino acid substitutions thereof.

[00511] In an embodiment, the OX40 agonist is a OX40 agonist biosimilar monoclonal antibody approved by drug regulatory authorities with reference to 11D4. In an embodiment, the biosimilar monoclonal antibody comprises an OX40 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is 11D4. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is a OX40 agonist antibody authorized or submitted for authorization, wherein the OX40 agonist antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is 11D4. The OX40 agonist antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product,

wherein the reference medicinal product or reference biological product is 11D4. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is 11D4.

TABLE 14: Amino acid sequences for OX40 agonist antibodies related to 11D4.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:66 heavy chain for 11D4	EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYSMNWVRQA PGKGLEWVS Y ISSSSSTIDY 60 ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARES GWYLFDYWGQ GTLTVTVSSAS 120 TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPTVSWN SGALTSGVHT FPAVLQSSGL 180 YSLSSVVTVP SSNFGTQTYT CNVDHKPSNT KVDKTVKRC CVECPCPCAP PVAGPSVFLF 240 PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV QFNWYVDGVE VHNAKTKPRE EQFNSTFRVV 300 SVLTVVHQDW LNGKEYKCKV SNKGLPAPIE KTISKTKGQP REPQVYTLPP SREEMTKNQV 360 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPMLDSGDS FFLYSKLTVD KSRWQQGNVF 420 SCSMHEALH NHYTQKSLSL SPGK 444
SEQ ID NO:67 light chain for 11D4	DIQMTQSPSS LSASVGDRVT ITCRASQGIS SWLAWYQQKP EKAPKSLIYA ASSLQSGVPS 60 RFGSGSGTD FTLTISSLQP EDFATYYCQQ YNSYPPTFGG GTKVEIKRTV AAPSVFIFPP 120 SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSLT 180
SEQ ID NO:68 heavy chain variable region for 11D4	LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC 214
SEQ ID NO:69 light chain variable region for 11D4	EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYSMNWVRQA PGKGLEWVS Y ISSSSSTIDY 60 ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARES GWYLFDYWGQ GTLTVTVSS 118
SEQ ID NO:70 heavy chain CDR1 for 11D4	DIQMTQSPSS LSASVGDRVT ITCRASQGIS SWLAWYQQKP EKAPKSLIYA ASSLQSGVPS 60 RFGSGSGTD FTLTISSLQP EDFATYYCQQ YNSYPPTFGG GTKVEIK 107
SEQ ID NO:71 heavy chain CDR2 for 11D4	SYSMN 5
SEQ ID NO:72 heavy chain CDR3 for 11D4	YISSSSSTID YADSVKG 17
SEQ ID NO:73 light chain CDR1 for 11D4	ESGWYLFDY 9
SEQ ID NO:74 light chain CDR2 for 11D4	RASQGISSWL A 11
SEQ ID NO:75 light chain CDR3 for 11D4	AASSLQS 7
SEQ ID NO:76 heavy chain CDR1 for 11D4	QQYNSYPPT 9

[00512] In some embodiments, the OX40 agonist is 18D8, which is a fully human antibody available from Pfizer, Inc. The preparation and properties of 18D8 are described in U.S. Patent Nos. 7,960,515; 8,236,930; and 9,028,824, the disclosures of which are incorporated by reference herein. The amino acid sequences of 18D8 are set forth in Table MM.

[00513] In an embodiment, a OX40 agonist comprises a heavy chain given by SEQ ID NO:76 and a light chain given by SEQ ID NO:77. In an embodiment, a OX40 agonist comprises heavy and light chains having the sequences shown in SEQ ID NO:76 and SEQ ID NO:77, respectively, or antigen binding fragments, Fab fragments, single-chain variable

fragments (scFv), variants, or conjugates thereof. In an embodiment, a OX40 agonist comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:76 and SEQ ID NO:77, respectively. In an embodiment, a OX40 agonist comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:76 and SEQ ID NO:77, respectively. In an embodiment, a OX40 agonist comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:76 and SEQ ID NO:77, respectively. In an embodiment, a OX40 agonist comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:76 and SEQ ID NO:77, respectively. In an embodiment, a OX40 agonist comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:76 and SEQ ID NO:77, respectively.

[00514] In an embodiment, the OX40 agonist comprises the heavy and light chain CDRs or variable regions (VRs) of 18D8. In an embodiment, the OX40 agonist heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:78, and the OX40 agonist light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:79, and conservative amino acid substitutions thereof. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:78 and SEQ ID NO:79, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:78 and SEQ ID NO:79, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:78 and SEQ ID NO:79, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:78 and SEQ ID NO:79, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:78 and SEQ ID NO:79, respectively.

[00515] In an embodiment, a OX40 agonist comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:80, SEQ ID NO:81, and SEQ ID NO:82, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:83, SEQ ID NO:84, and SEQ ID NO:85, respectively, and conservative amino acid substitutions thereof.

[00516] In an embodiment, the OX40 agonist is a OX40 agonist biosimilar monoclonal antibody approved by drug regulatory authorities with reference to 18D8. In an embodiment,

the biosimilar monoclonal antibody comprises an OX40 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is 18D8. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is a OX40 agonist antibody authorized or submitted for authorization, wherein the OX40 agonist antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is 18D8. The OX40 agonist antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is 18D8. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is 18D8.

TABLE 15: Amino acid sequences for OX40 agonist antibodies related to 18D8.

Identifier	Sequence (One-Letter Amino Acid Symbols)							
SEQ ID NO:76 heavy chain for 18D8	EVQLVESGGG	LVQPGRSLRL	SCAASGFTFD	DYAMHWVRQA	PGKGLEWVSG	ISWNSGSIGY		60
	ADSVKGRFTI	SRDNAKNSLY	LQMNSLRAED	TALYYCAKDQ	STADYYFYYG	MDVWGQGTTV		120
	TVSSASTKGP	SVFPLAPCSR	STSESTAALG	CLVKDYFPEP	VTVSWNSGAL	TSGVHTFPAV		180
	LQSSGLYSLS	SVTVPPSSNF	GTQTYTCNVD	HKPSNTKVDK	TVERKCCVEC	PPCPAPPVAG		240
	PSVFLFPPKP	KDTLMISRTP	EVTCTVVDVS	HEDPEVQFNW	YVDGVEVHNA	KTKPREEQFN		300
	STFRVVSFLT	VVHQDWLNGK	EYKCKVSNKG	LPAPIEKTIS	KTKGQPREPQ	VYTLPPSREE		360
	MTKNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKTTPPM	LDSGGSFFLY	SKLTVDKSRW		420
	QQGNVFSCSV	MHEALHNHYT	QKSLSLSPGK					450
	EIVVTQSPAT	LSLSPGERAT	LSCRASQSVS	SYLAWYQQKP	GQAPRLLIYD	ASNRATGIPA		60
	RFSGSGSGTD	FTLTISLLEP	EDFAVYYCQQ	RSNWPTFGQG	TKVEIKRTVA	APSVFIFPPS		120
SEQ ID NO:77 light chain for 18D8	DEQLKSGTAS	VVCLLNNFYP	REAKVQWKVD	NALQSGNSQE	SVTEQDSKDS	TYSLSSTLTTL		180
	SKADYEKHKV	YACEVTHQGL	SSPVTKSEFN	GEC				213
SEQ ID NO:78 heavy chain variable region for 18D8	EVQLVESGGG	LVQPGRSLRL	SCAASGFTFD	DYAMHWVRQA	PGKGLEWVSG	ISWNSGSIGY		60
	ADSVKGRFTI	SRDNAKNSLY	LQMNSLRAED	TALYYCAKDQ	STADYYFYYG	MDVWGQGTTV		120
	TVSS							124
SEQ ID NO:79 light chain variable region for 18D8	EIVVTQSPAT	LSLSPGERAT	LSCRASQSVS	SYLAWYQQKP	GQAPRLLIYD	ASNRATGIPA		60
	RFSGSGSGTD	FTLTISLLEP	EDFAVYYCQQ	RSNWPTFGQG	TKVEIK			106

SEQ ID NO:80 heavy chain CDR1 for 18D8	DYAMH	5
SEQ ID NO:81 heavy chain CDR2 for 18D8	GISWNSGSIG YADSVKG	17
SEQ ID NO:82 heavy chain CDR3 for 18D8	DQSTADYYFY YGMDV	15
SEQ ID NO:83 light chain CDR1 for 18D8	RASQSVSSYL A	11
SEQ ID NO:84 light chain CDR2 for 18D8	DASNRAT	7
SEQ ID NO:85 light chain CDR3 for 18D8	QQRSNWPT	8

[00517] In some embodiments, the OX40 agonist is Hu119-122, which is a humanized antibody available from GlaxoSmithKline plc. The preparation and properties of Hu119-122 are described in U.S. Patent Nos. 9,006,399 and 9,163,085, and in International Patent Publication No. WO 2012/027328, the disclosures of which are incorporated by reference herein. The amino acid sequences of Hu119-122 are set forth in Table NN.

[00518] In an embodiment, the OX40 agonist comprises the heavy and light chain CDRs or variable regions (VRs) of Hu119-122. In an embodiment, the OX40 agonist heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:86, and the OX40 agonist light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:87, and conservative amino acid substitutions thereof. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:86 and SEQ ID NO:87, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:86 and SEQ ID NO:87, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:86 and SEQ ID NO:87, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:86 and SEQ ID NO:87, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:86 and SEQ ID NO:87, respectively.

[00519] In an embodiment, a OX40 agonist comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:88, SEQ ID NO:89, and SEQ ID NO:90, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:91, SEQ ID NO:92, and SEQ ID NO:93, respectively, and conservative amino acid substitutions thereof.

[00520] In an embodiment, the OX40 agonist is a OX40 agonist biosimilar monoclonal antibody approved by drug regulatory authorities with reference to Hu119-122. In an embodiment, the biosimilar monoclonal antibody comprises an OX40 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is Hu119-122. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is a OX40 agonist antibody authorized or submitted for authorization, wherein the OX40 agonist antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is Hu119-122. The OX40 agonist antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is Hu119-122. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is Hu119-122.

TABLE 16: Amino acid sequences for OX40 agonist antibodies related to Hu119-122.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:86 heavy chain variable region for Hu119-122	EVQLVESGGG LVQPGGSLRL SCAASEYEFP SHDMSWVRQA PGKGLELVAA INSDGGSTYY 60 PDTMERRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARHY DDYYAWFAYW GQGTMTVSS 120
SEQ ID NO:87 light chain variable region for Hu119-122	EIVLTQSPAT LSLSPGERAT LSCRASKSVS TSGYSYMHWY QQKPGQAPRL LIYLASNLES 60 GVPARFSGSG SGTDFTLTIS SLEPEDFAVY YCQHSRELPL TFGGGTKVEI K 111
SEQ ID NO:88 heavy chain CDR1 for Hu119-122	SHDMS 5
SEQ ID NO:89 heavy chain CDR2 for Hu119-122	AINSDGGSTY YPDTMER 17

SEQ ID NO:90 heavy chain CDR3 for Hu119-122	HYDDYYAWFA Y	11
SEQ ID NO:91 light chain CDR1 for Hu119-122	RASKSVSTSG YSYM	15
SEQ ID NO:92 light chain CDR2 for Hu119-122	LASNLES	7
SEQ ID NO:93 light chain CDR3 for Hu119-122	QHSRELPLT	9

[00521] In some embodiments, the OX40 agonist is Hu106-222, which is a humanized antibody available from GlaxoSmithKline plc. The preparation and properties of Hu106-222 are described in U.S. Patent Nos. 9,006,399 and 9,163,085, and in International Patent Publication No. WO 2012/027328, the disclosures of which are incorporated by reference herein. The amino acid sequences of Hu106-222 are set forth in Table OO.

[00522] In an embodiment, the OX40 agonist comprises the heavy and light chain CDRs or variable regions (VRs) of Hu106-222. In an embodiment, the OX40 agonist heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:94, and the OX40 agonist light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:95, and conservative amino acid substitutions thereof. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:94 and SEQ ID NO:95, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:94 and SEQ ID NO:95, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:94 and SEQ ID NO:95, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:94 and SEQ ID NO:95, respectively. In an embodiment, a OX40 agonist comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:94 and SEQ ID NO:95, respectively.

[00523] In an embodiment, a OX40 agonist comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:96, SEQ ID NO:97, and SEQ ID NO:98, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:99, SEQ ID NO:100, and SEQ ID NO:101, respectively, and conservative amino acid substitutions thereof.

[00524] In an embodiment, the OX40 agonist is a OX40 agonist biosimilar monoclonal antibody approved by drug regulatory authorities with reference to Hu106-222. In an embodiment, the biosimilar monoclonal antibody comprises an OX40 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is Hu106-222. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is a OX40 agonist antibody authorized or submitted for authorization, wherein the OX40 agonist antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is Hu106-222. The OX40 agonist antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is Hu106-222. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is Hu106-222.

TABLE 17: Amino acid sequences for OX40 agonist antibodies related to Hu106-222.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:94 heavy chain variable region for Hu106-222	QVQLVQSGSE LKKPGASVKV SCKASGYTFT DYSMHWVRQA PGQGLKWMGW INTETGEPTY 60 ADDFKGRFVF SLDTSVSTAY LQISLKAED TAVYYCANPY YDYVSYAMD YWGQGTITVTV 120 SS 122
SEQ ID NO:95 light chain variable region for Hu106-222	DIQMTQSPSS LSASVGDRVT ITCKASQDVS TAVAWYQQKP GKAPKLLIYS ASYLYTGVPS 60 RFSGSGSGTD FTTISLQF EDIATYYCQQ HYSTPRTFGQ GTKLEIK 107
SEQ ID NO:96 heavy chain CDR1 for Hu106-222	DYSMH 5
SEQ ID NO:97 heavy chain CDR2 for Hu106-222	WINTETGEPT YADDFKG 17

SEQ ID NO:98 heavy chain CDR3 for Hu106-222	PYYDYVSYA MDY	13
SEQ ID NO:99 light chain CDR1 for Hu106-222	KASQDVSTAV A	11
SEQ ID NO:100 light chain CDR2 for Hu106-222	SASYLYT	7
SEQ ID NO:101 light chain CDR3 for Hu106-222	QQHYSTPRT	9

[00525] In some embodiments, the OX40 agonist antibody is MEDI6469 (also referred to as 9B12). MEDI6469 is a murine monoclonal antibody. Weinberg, *et al.*, *J. Immunother.* **2006**, 29, 575-585. In some embodiments the OX40 agonist is an antibody produced by the 9B12 hybridoma, deposited with Biovest Inc. (Malvern, MA, USA), as described in Weinberg, *et al.*, *J. Immunother.* **2006**, 29, 575-585, the disclosure of which is hereby incorporated by reference in its entirety. In some embodiments, the antibody comprises the CDR sequences of MEDI6469. In some embodiments, the antibody comprises a heavy chain variable region sequence and/or a light chain variable region sequence of MEDI6469.

[00526] In an embodiment, the OX40 agonist is L106 BD (Pharmingen Product #340420). In some embodiments, the OX40 agonist comprises the CDRs of antibody L106 (BD Pharmingen Product #340420). In some embodiments, the OX40 agonist comprises a heavy chain variable region sequence and/or a light chain variable region sequence of antibody L106 (BD Pharmingen Product #340420). In an embodiment, the OX40 agonist is ACT35 (Santa Cruz Biotechnology, Catalog #20073). In some embodiments, the OX40 agonist comprises the CDRs of antibody ACT35 (Santa Cruz Biotechnology, Catalog #20073). In some embodiments, the OX40 agonist comprises a heavy chain variable region sequence and/or a light chain variable region sequence of antibody ACT35 (Santa Cruz Biotechnology, Catalog #20073). In an embodiment, the OX40 agonist is the murine monoclonal antibody anti-mCD134/mOX40 (clone OX86), commercially available from InVivoMAb, BioXcell Inc, West Lebanon, NH.

[00527] In an embodiment, the OX40 agonist is selected from the OX40 agonists described in International Patent Application Publication Nos. WO 95/12673, WO 95/21925, WO 2006/121810, WO 2012/027328, WO 2013/028231, WO 2013/038191, and WO 2014/148895; European Patent Application EP 0672141; U.S. Patent Application Publication Nos. US 2010/136030, US 2014/377284, US 2015/190506, and US 2015/132288 (including clones 20E5 and 12H3); and U.S. Patent Nos. 7,504,101, 7,550,140, 7,622,444, 7,696,175,

7,960,515, 7,961,515, 8,133,983, 9,006,399, and 9,163,085, the disclosure of each of which is incorporated herein by reference in its entirety.

[00528] In an embodiment, the OX40 agonist is an OX40 agonistic fusion protein as depicted in Structure I-A (C-terminal Fc-antibody fragment fusion protein) or Structure I-B (N-terminal Fc-antibody fragment fusion protein), or a fragment, derivative, conjugate, variant, or biosimilar thereof. The properties of structures I-A and I-B are described above and in U.S. Patent Nos. 9,359,420, 9,340,599, 8,921,519, and 8,450,460, the disclosures of which are incorporated by reference herein. Amino acid sequences for the polypeptide domains of structure I-A are given in Table GG. The Fc domain preferably comprises a complete constant domain (amino acids 17-230 of SEQ ID NO:31) the complete hinge domain (amino acids 1-16 of SEQ ID NO:31) or a portion of the hinge domain (*e.g.*, amino acids 4-16 of SEQ ID NO:31). Preferred linkers for connecting a C-terminal Fc-antibody may be selected from the embodiments given in SEQ ID NO:32 to SEQ ID NO:41, including linkers suitable for fusion of additional polypeptides. Likewise, amino acid sequences for the polypeptide domains of structure I-B are given in Table HH. If an Fc antibody fragment is fused to the N-terminus of an TNFRSF fusion protein as in structure I-B, the sequence of the Fc module is preferably that shown in SEQ ID NO:42, and the linker sequences are preferably selected from those embodiments set forth in SEQ ID NO:43 to SEQ ID NO:45.

[00529] In an embodiment, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains selected from the group consisting of a variable heavy chain and variable light chain of tavolixizumab, a variable heavy chain and variable light chain of 11D4, a variable heavy chain and variable light chain of 18D8, a variable heavy chain and variable light chain of Hu119-122, a variable heavy chain and variable light chain of Hu106-222, a variable heavy chain and variable light chain selected from the variable heavy chains and variable light chains described in Table OO, any combination of a variable heavy chain and variable light chain of the foregoing, and fragments, derivatives, conjugates, variants, and biosimilars thereof.

[00530] In an embodiment, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains comprising an OX40L sequence. In an embodiment, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains comprising a sequence according to SEQ ID NO:102. In an embodiment, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains comprising a soluble OX40L sequence. In an

embodiment, a OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains comprising a sequence according to SEQ ID NO:103. In an embodiment, a OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains comprising a sequence according to SEQ ID NO:104.

[00531] In an embodiment, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:58 and SEQ ID NO:59, respectively, wherein the V_H and V_L domains are connected by a linker. In an embodiment, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:68 and SEQ ID NO:69, respectively, wherein the V_H and V_L domains are connected by a linker. In an embodiment, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:78 and SEQ ID NO:79, respectively, wherein the V_H and V_L domains are connected by a linker. In an embodiment, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:86 and SEQ ID NO:87, respectively, wherein the V_H and V_L domains are connected by a linker. In an embodiment, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:94 and SEQ ID NO:95, respectively, wherein the V_H and V_L domains are connected by a linker. In an embodiment, an OX40 agonist fusion protein according to structures I-A or I-B comprises one or more OX40 binding domains that is a scFv domain comprising V_H and V_L regions that are each at least 95% identical to the V_H and V_L sequences given in Table 18, wherein the V_H and V_L domains are connected by a linker.

TABLE 18: Additional polypeptide domains useful as OX40 binding domains in fusion proteins (*e.g.*, structures I-A and I-B) or as scFv OX40 agonist antibodies.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:102 OX40L	MERVQPLEEN VGNAARPRFE RNKLLLVASV IQGLGLLLCF TYICLHFSAL QVSHRYPRIQ 60 SIKVQFTEYK KEGFILTQ KEDEIMKVQN NSVIINCDGF YLISLKGYS QEVNISLHYQ 120 KDEEPLFQLK KRSVNSLMV ASLTYKDKVY LNVTTDNTSL DDFHVNNGEL ILIHQNPGEF 180 CVL 183

SEQ ID NO:103 OX40L soluble domain	SHRYPRIQSI KVQFTEYKKE KGFILTSQKE DEIMKVQNN S VIINCDGFYL ISLKGYSQSE VNISLHYQKD EEPLFQLKKV RSVNSLMVAS LTYKDKVYLN VTDTNTSLDD FHVNGGELIL IHQNPGEFCV L	60 120 131
SEQ ID NO:104 OX40L soluble domain (alternative)	YPRIQSIKVQ FTEYKKEKGF ILTSQKEDI MKVQNN SVII NCDGFYLISL KGYFSQEVNI SLHYQKDEEP LFQLKKVRSV NSLMVASLTY KDKVYLVNVT DNTSLDDFHV NGGELILIHQ NPGEFCVL	60 120 128
SEQ ID NO:105 variable heavy chain for 008	EVQLVESGGG LVQPGGSLRL SCAASGFTFS NYTMNWVRQA PGKGLEWVSA ISGSGGSTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKDR YSQVHYALDY WGQGLTVTVS	60 120
SEQ ID NO:106 variable light chain for 008	DIVMTQSPDS LPVTPGEPAS ISCRSSQSLL HSNNGYNLDW YLQKAGQSPQ LLIYLGSNRA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCQQYYNHP TTFGQGTK	60 108
SEQ ID NO:107 variable heavy chain for 011	EVQLVESGGG VVQPGSLRL SCAASGFTFS DYTMNWVRQA PGKGLEWVSS ISGGSTYYAD SRKGRFTISR DNSKNTLYLQ MNLSLRAEDTA VYYCARDRYF RQQNAFDYWG QGTLTVTVSSA	60 120
SEQ ID NO:108 variable light chain for 011	DIVMTQSPDS LPVTPGEPAS ISCRSSQSLL HSNNGYNLDW YLQKAGQSPQ LLIYLGSNRA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCQQYYNHP TTFGQGTK	60 108
SEQ ID NO:109 variable heavy chain for 021	EVQLVESGGG LVQPRGSLRL SCAASGFTFS SYAMNWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKDR YITLNPALDY WGQGLTVTVS	60 120
SEQ ID NO:110 variable light chain for 021	DIQMTQSPVS LPVTPGEPAS ISCRSSQSLL HSNNGYNLDW YLQKPGQSPQ LLIYLGSNRA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCQQYKSNP PTFGQGTK	60 108
SEQ ID NO:111 variable heavy chain for 023	EVQLVESGGG LVHPGGSLRL SCAGSGFTFS SYAMHWVRQA PGKGLEWVSA IGTGGGTYYA DSVMGRFTIS RDNSKNTLYL QMNSLRAEDT AVYYCARYDN VMGLYWFYDW GQGLTVTVSS	60 120
SEQ ID NO:112 variable light chain for 023	EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLIYD ASNRATGIPA RFSGSGSGTD FTLTISLLEP EDFAVYYCQQ RSNWPPAFGG GTKVEIKR	60 108
SEQ ID NO:113 heavy chain variable region	EVQLQQSGPE LVKPGASVKM SCKASGYTFT SYVMHWVKQK PGQGLEWIGY INPYNDGTKY NEKFKGKATL TSDKSSSTAY MELSSLTSED SAVYYCANY GSSLMDYWG QGTSVTVSS	60 119
SEQ ID NO:114 light chain variable region	DIQMTQTTS LSASLGDRVT ISCRASQDIS NYLWYQQKP DGTVKLLIYY TSRLHSGVPS RFSGSGSGTD YSLTISNLEQ EDIATYFCQQ GNTLPWTFGG GTKLEIKR	60 108
SEQ ID NO:115 heavy chain variable region	EVQLQQSGPE LVKPGASVKI SCKTSGYTFK DYTMHWVKQS HGKSLEWIGG IYPNNGGSTY NQNFKDKATL TVDKSSSTAY MEFRSLTSED SAVYYCARMG YHGPHLDFDV WGAGTIVTVS P	60 120 121
SEQ ID NO:116 light chain variable region	DIVMTQSHKF MSTSLGDRVS ITCKASQDVG AAVAWYQQKP GQSPKLLIYW ASTRHTGVDP RFTGGSGTD FTLTISNVQS EDLTDYFCQQ YINYPITFGG GTKLEIKR	60 108
SEQ ID NO:117 heavy chain variable region of humanized antibody	QIQLVQSGPE LKKPGETVKI SCKASGYTFT DYSMHWVKQA PGKGLKWMGW INTETGEPTY ADDFKGRFAF SLETSASTAY LQINNKNED TATYFCANPY YDYVSYAMD YWGHGTSVTV SS	60 120 122
SEQ ID NO:118 heavy chain variable region of humanized antibody	QVQLVQSGSE LKKPGASVKV SCKASGYTFT DYSMHWVRQA PGQGLKWMGW INTETGEPTY ADDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCANPY YDYVSYAMD YWQGTVTVTV SS	60 120 122
SEQ ID NO:119 light chain variable region of humanized antibody	DIVMTQSHKF MSTSVRDRVS ITCKASQDVS TAVAWYQQKP GQSPKLLIYS ASYLYTGVPD RFTGSGSGTD FTFITSSVQA EDLAVYYCQQ HYSTPRTFGG GTKLEIK	60 107
SEQ ID NO:120 light chain variable region of humanized antibody	DIVMTQSHKF MSTSVRDRVS ITCKASQDVS TAVAWYQQKP GQSPKLLIYS ASYLYTGVPD RFTGSGSGTD FTFITSSVQA EDLAVYYCQQ HYSTPRTFGG GTKLEIK	60 107
SEQ ID NO:121 heavy chain variable region of humanized antibody	EVQLVESGGG LVQPGESLKL SCESNEYEFF SHDMSWVRKT PEKRLVLVAA INSDGGSTYY PDTMERRFII SRDNTKKTLY LQMSSLRSED TALYYCARHY DDYAWFAYW GQGLTVTVSA	60 120
SEQ ID NO:122 heavy chain variable region of humanized antibody	EVQLVESGGG LVQPGGSLRL SCAASEYEFF SHDMSWVRQA PGKGLELVAA INSDGGSTYY PDTMERRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARHY DDYAWFAYW GQGTMTVTVSS	60 120
SEQ ID NO:123 light chain	DIVLTQSPAS LAVSLGQRAT ISCRASKSVS TSGYSYMHY QKPGQPPKL LIYLASNLES GVPARFSGSG SGTDFTLNIH PVEEEDAATY YCQHSRELPL TFGAGTKLEL K	60 111

variable region of humanized antibody		
SEQ ID NO:124 light chain variable region of humanized antibody	EIVLTQSPAT LSLSPGERAT LSCRASKSVS TSGYSYMHWW QQKPGQAPRL LIYLASNLES GVPARFSGSG SGTDFTLTIS SLEPEDFAVY YCQHSRELPL TFGGGTKVEI K	60 111
SEQ ID NO:125 heavy chain variable region	MYLGLNYVFI VFLNGVQSE VKLEESGGGL VQPGGSMKLS CAASGFTFSD AWMWVRQSP EKGLEWVAEI RSKANNHATY YAESVNGRFT ISRDDSKSSV YLQMNSLRAE DTGIYYCTWG EVFYFDYWGQ GTTLTVSS	60 120 138
SEQ ID NO:126 light chain variable region	MRPSIQFLGL LLEWLHGAQC DIQMTQSPSS LSASLGKVT ITCKSSQDIN KYIAWYQHQP GKGPRLLIHY TSTLQPGIPS RFGSGSGGRD YSFSISNLEP EDIATYYCLQ YDNLLTFGAG TKLELK	60 120 126

[00532] In an embodiment, the OX40 agonist is a OX40 agonistic single-chain fusion polypeptide comprising (i) a first soluble OX40 binding domain, (ii) a first peptide linker, (iii) a second soluble OX40 binding domain, (iv) a second peptide linker, and (v) a third soluble OX40 binding domain, further comprising an additional domain at the N-terminal and/or C-terminal end, and wherein the additional domain is a Fab or Fc fragment domain. In an embodiment, the OX40 agonist is a OX40 agonistic single-chain fusion polypeptide comprising (i) a first soluble OX40 binding domain, (ii) a first peptide linker, (iii) a second soluble OX40 binding domain, (iv) a second peptide linker, and (v) a third soluble OX40 binding domain, further comprising an additional domain at the N-terminal and/or C-terminal end, wherein the additional domain is a Fab or Fc fragment domain wherein each of the soluble OX40 binding domains lacks a stalk region (which contributes to trimerisation and provides a certain distance to the cell membrane, but is not part of the OX40 binding domain) and the first and the second peptide linkers independently have a length of 3-8 amino acids.

[00533] In an embodiment, the OX40 agonist is an OX40 agonistic single-chain fusion polypeptide comprising (i) a first soluble tumor necrosis factor (TNF) superfamily cytokine domain, (ii) a first peptide linker, (iii) a second soluble TNF superfamily cytokine domain, (iv) a second peptide linker, and (v) a third soluble TNF superfamily cytokine domain, wherein each of the soluble TNF superfamily cytokine domains lacks a stalk region and the first and the second peptide linkers independently have a length of 3-8 amino acids, and wherein the TNF superfamily cytokine domain is an OX40 binding domain.

[00534] In some embodiments, the OX40 agonist is MEDI6383. MEDI6383 is an OX40 agonistic fusion protein and can be prepared as described in U.S. Patent No. 6,312,700, the disclosure of which is incorporated by reference herein.

[00535] In an embodiment, the OX40 agonist is an OX40 agonistic scFv antibody comprising any of the foregoing V_H domains linked to any of the foregoing V_L domains.

[00536] In an embodiment, the OX40 agonist is Creative Biolabs OX40 agonist monoclonal antibody MOM-18455, commercially available from Creative Biolabs, Inc., Shirley, NY, USA.

[00537] In an embodiment, the OX40 agonist is OX40 agonistic antibody clone Ber-ACT35 commercially available from BioLegend, Inc., San Diego, CA, USA.

H. Optional Cell Viability Analyses

[00538] Optionally, a cell viability assay can be performed after the first expansion (sometimes referred to as the initial bulk expansion), using standard assays known in the art. For example, a trypan blue exclusion assay can be done on a sample of the bulk TILs, which selectively labels dead cells and allows a viability assessment. Other assays for use in testing viability can include but are not limited to the Alamar blue assay; and the MTT assay.

1. Cell Counts, Viability, Flow Cytometry

[00539] In some embodiments, cell counts and/or viability are measured. The expression of markers such as but not limited CD3, CD4, CD8, and CD56, as well as any other disclosed or described herein, can be measured by flow cytometry with antibodies, for example but not limited to those commercially available from BD Bio-sciences (BD Biosciences, San Jose, CA) using a FACSCanto™ flow cytometer (BD Biosciences). The cells can be counted manually using a disposable c-chip hemocytometer (VWR, Batavia, IL) and viability can be assessed using any method known in the art, including but not limited to trypan blue staining. The cell viability can also be assayed based on USSN 15/863,634, incorporated by reference herein in its entirety.

[00540] In some cases, the bulk TIL population can be cryopreserved immediately, using the protocols discussed below. Alternatively, the bulk TIL population can be subjected to REP and then cryopreserved as discussed below. Similarly, in the case where genetically modified TILs will be used in therapy, the bulk or REP TIL populations can be subjected to genetic modifications for suitable treatments.

[00541] According to the present disclosure, a method for assaying TILs for viability and/or further use in administration to a subject. In some embodiments, the method for assay tumor infiltrating lymphocytes (TILs) comprises:

- (i) obtaining a first population of TILs;

- (ii) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, to produce a second population of TILs; and
- (iii) 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 third population of TILs is at least 50-fold greater in number than the second population of TILs;
- (iv) harvesting, washing, and cryopreserving the third population of TILs;
- (v) storing the cryopreserved TILs at a cryogenic temperature;
- (vi) thawing the third population of TILs to provide a thawed third population of TILs; and
- (vii) performing an additional second expansion of a portion of the thawed third population of TILs by supplementing the cell culture medium of the third population with IL-2, OKT-3, and APCs for an additional expansion period (sometimes referred to as a reREP period) of at least 3 days, wherein the third expansion is performed to obtain a fourth population of TILs, wherein the number of TILs in the fourth population of TILs is compared to the number of TILs in the third population of TILs to obtain a ratio;
- (viii) determining based on the ratio in step (vii) whether the thawed population of TILs is suitable for administration to a patient;
- (ix) administering a therapeutically effective dosage of the thawed third population of TILs to the patient when the ratio of the number of TILs in the fourth population of TILs to the number of TILs in the third population of TILs is determined to be greater than 5:1 in step (viii).

[00542] In some embodiments, the TILs are assayed for viability after step (vii).

[00543] The present disclosure also provides further methods for assaying TILs. In some embodiments, the disclosure provides a method for assaying TILs comprising:

- (i) obtaining a portion of a first population of cryopreserved TILs;
- (ii) thawing the portion of the first population of cryopreserved TILs;
- (iii) performing a first expansion by culturing the portion of the first population of

TILs in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) for an additional expansion period (sometimes referred to as a reREP period) of at least 3 days, to produce a second population of TILs, wherein the portion from the first population of TILs is compared to the second population of TILs to obtain a ratio of the number of TILs, wherein the ratio of the number of TILs in the second population of TILs to the number of TILs in the portion of the first population of TILs is greater than 5:1;

(iv) determining based on the ratio in step (iii) whether the first population of TILs is suitable for use in therapeutic administration to a patient;

(v) determining the first population of TILs is suitable for use in therapeutic administration when the ratio of the number of TILs in the second population of TILs to the number of TILs in the first population of TILs is determined to be greater than 5:1 in step (iv).

[00544] In some embodiments, the ratio of the number of TILs in the second population of TILs to the number of TILs in the portion of the first population of TILs is greater than 50:1.

[00545] In some embodiments, the method further comprises performing expansion of the entire first population of cryopreserved TILs from step (i) according to the methods as described in any of the embodiments provided herein.

[00546] In some embodiments, the method further comprises administering the entire first population of cryopreserved TILs from step (i) to the patient.

2. Cell Cultures

[00547] In an embodiment, a method for expanding TILs, including those discussed above as well as exemplified in Figure 1, may include using about 5,000 mL to about 25,000 mL of cell medium, about 5,000 mL to about 10,000 mL of cell medium, or about 5,800 mL to about 8,700 mL of cell medium. In some embodiments, the media is a serum free medium. In some embodiments, the media in the first expansion is serum free. In some embodiments, the media in the second expansion is serum free. . In some embodiments, the media in the first expansion and the second are both serum free. In an embodiment, expanding the number of TILs uses no more than one type of cell culture medium. Any suitable cell culture medium may be used, *e.g.*, AIM-V cell medium (L-glutamine, 50 μ M streptomycin sulfate, and 10 μ M gentamicin sulfate) cell culture medium (Invitrogen, Carlsbad CA). In this regard, the

inventive methods advantageously reduce the amount of medium and the number of types of medium required to expand the number of TIL. In an embodiment, expanding the number of TIL may comprise feeding the cells no more frequently than every third or fourth day. Expanding the number of cells in a gas permeable container simplifies the procedures necessary to expand the number of cells by reducing the feeding frequency necessary to expand the cells.

[00548] In an embodiment, the cell medium in the first and/or second gas permeable container is unfiltered. The use of unfiltered cell medium may simplify the procedures necessary to expand the number of cells. In an embodiment, the cell medium in the first and/or second gas permeable container lacks beta-mercaptoethanol (BME).

[00549] In an embodiment, the duration of the method comprising obtaining a tumor tissue sample from the mammal; culturing the tumor tissue sample in a first gas permeable container containing cell medium therein; obtaining TILs from the tumor tissue sample; expanding the number of TILs in a second gas permeable container containing cell medium for a duration of about 7 to 14 days, *e.g.*, about 11 days. In some embodiments pre-REP is about 7 to 14 days, *e.g.*, about 11 days. In some embodiments, REP is about 7 to 14 days, *e.g.*, about 11 days.

[00550] In an embodiment, TILs are expanded in gas-permeable containers. Gas-permeable containers have been used to expand TILs using PBMCs using methods, compositions, and devices known in the art, including those described in U.S. Patent Application Publication No. 2005/0106717 A1, the disclosures of which are incorporated herein by reference. In an embodiment, TILs are expanded in gas-permeable bags. In an embodiment, TILs are expanded using a cell expansion system that expands TILs in gas permeable bags, such as the Xuri Cell Expansion System W25 (GE Healthcare). In an embodiment, TILs are expanded using a cell expansion system that expands TILs in gas permeable bags, such as the WAVE Bioreactor System, also known as the Xuri Cell Expansion System W5 (GE Healthcare). In an embodiment, the cell expansion system includes a gas permeable cell bag with a volume selected from the group consisting of about 100 mL, about 200 mL, about 300 mL, about 400 mL, about 500 mL, about 600 mL, about 700 mL, about 800 mL, about 900 mL, about 1 L, about 2 L, about 3 L, about 4 L, about 5 L, about 6 L, about 7 L, about 8 L, about 9 L, and about 10 L.

[00551] In an embodiment, TILs can be expanded in G-Rex flasks (commercially available from Wilson Wolf Manufacturing). Such embodiments allow for cell populations to expand from about 5×10^5 cells/cm² to between 10×10^6 and 30×10^6 cells/cm². In an embodiment this is without feeding. In an embodiment, this is without feeding so long as medium resides at a height of about 10 cm in the G-Rex flask. In an embodiment this is without feeding but with the addition of one or more cytokines. In an embodiment, the cytokine can be added as a bolus without any need to mix the cytokine with the medium. Such containers, devices, and methods are known in the art and have been used to expand TILs, and include those described in U.S. Patent Application Publication No. US 2014/0377739A1, International Publication No. WO 2014/210036 A1, U.S. Patent Application Publication No. US 2013/0115617 A1, International Publication No. WO 2013/188427 A1, U.S. Patent Application Publication No. US 2011/0136228 A1, U.S. Patent No. US 8,809,050 B2, International publication No. WO 2011/072088 A2, U.S. Patent Application Publication No. US 2016/0208216 A1, U.S. Patent Application Publication No. US 2012/0244133 A1, International Publication No. WO 2012/129201 A1, U.S. Patent Application Publication No. US 2013/0102075 A1, U.S. Patent No. US 8,956,860 B2, International Publication No. WO 2013/173835 A1, U.S. Patent Application Publication No. US 2015/0175966 A1, the disclosures of which are incorporated herein by reference. Such processes are also described in Jin *et al.*, *J. Immunotherapy*, **2012**, 35:283-292.

I. Optional Genetic Engineering of TILs

[00552] In some embodiments, the TILs are optionally genetically engineered to include additional functionalities, including, but not limited to, a high-affinity T cell receptor (TCR), *e.g.*, a TCR targeted at a tumor-associated antigen such as MAGE-1, HER2, or NY-ESO-1, or a chimeric antigen receptor (CAR) which binds to a tumor-associated cell surface molecule (*e.g.*, mesothelin) or lineage-restricted cell surface molecule (*e.g.*, CD19).

J. Optional Cryopreservation of TILs

[00553] As discussed above, and exemplified in Steps A through E as provided in Figure 1, cryopreservation can occur at numerous points throughout the TIL expansion process. In some embodiments, the expanded population of TILs after the second expansion (as provided for example, according to Step D of Figure 1) can be cryopreserved. Cryopreservation can be generally accomplished by placing the TIL population into a freezing solution, *e.g.*, 85% complement inactivated AB serum and 15% dimethyl sulfoxide (DMSO). The cells in

solution are placed into cryogenic vials and stored for 24 hours at -80 °C, with optional transfer to gaseous nitrogen freezers for cryopreservation. *See, Sadeghi, et al., Acta Oncologica* **2013**, 52, 978-986. In some embodiments, the TILs are cryopreserved in 5% DMSO. In some embodiments, the TILs are cryopreserved in cell culture media plus 5% DMSO. In some embodiments, the TILs are cryopreserved according to the methods provided in Examples F and G.

[00554] When appropriate, the cells are removed from the freezer and thawed in a 37 °C water bath until approximately 4/5 of the solution is thawed. The cells are generally resuspended in complete media and optionally washed one or more times. In some embodiments, the thawed TILs can be counted and assessed for viability as is known in the art.

K. Closed Systems for TIL Manufacturing

[00555] The present invention provides for the use of closed systems during the TIL culturing process. Such closed systems allow for preventing and/or reducing microbial contamination, allow for the use of fewer flasks, and allow for cost reductions. In some embodiments, the closed system uses two containers.

[00556] Such closed systems are well-known in the art and can be found, for example, at <http://www.fda.gov/cber/guidelines.htm> and <https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm076779.htm>.

[00557] Sterile connecting devices (STCDs) produce sterile welds between two pieces of compatible tubing. This procedure permits sterile connection of a variety of containers and tube diameters. In some embodiments, the closed systems include luer lock and heat sealed systems as described in for example, Example G. In some embodiments, the closed system is accessed via syringes under sterile conditions in order to maintain the sterility and closed nature of the system. In some embodiments, a closed system as described in Example G is employed. In some embodiments, the TILs are formulated into a final product formulation container according to the method described in Example G, section “Final Formulation and Fill”.

[00558] In some embodiments, the closed system uses one container from the time the tumor fragments are obtained until the TILs are ready for administration to the patient or cryopreserving. In some embodiments when two containers are used, the first container is a

closed G-container and the population of TILs is centrifuged and transferred to an infusion bag without opening the first closed G-container. In some embodiments, when two containers are used, the infusion bag is a HypoThermosol-containing infusion bag. A closed system or closed TIL cell culture system is characterized in that once the tumor sample and/or tumor fragments have been added, the system is tightly sealed from the outside to form a closed environment free from the invasion of bacteria, fungi, and/or any other microbial contamination.

[00559] In some embodiments, the reduction in microbial contamination is between about 5% and about 100%. In some embodiments, the reduction in microbial contamination is between about 5% and about 95%. In some embodiments, the reduction in microbial contamination is between about 5% and about 90%. In some embodiments, the reduction in microbial contamination is between about 10% and about 90%. In some embodiments, the reduction in microbial contamination is between about 15% and about 85%. In some embodiments, the reduction in microbial contamination is about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 97%, about 98%, about 99%, or about 100%.

[00560] The closed system allows for TIL growth in the absence and/or with a significant reduction in microbial contamination.

[00561] Moreover, pH, carbon dioxide partial pressure and oxygen partial pressure of the TIL cell culture environment each vary as the cells are cultured. Consequently, even though a medium appropriate for cell culture is circulated, the closed environment still needs to be constantly maintained as an optimal environment for TIL proliferation. To this end, it is desirable that the physical factors of pH, carbon dioxide partial pressure and oxygen partial pressure within the culture liquid of the closed environment be monitored by means of a sensor, the signal whereof is used to control a gas exchanger installed at the inlet of the culture environment, and the that gas partial pressure of the closed environment be adjusted in real time according to changes in the culture liquid so as to optimize the cell culture environment. In some embodiments, the present invention provides a closed cell culture system which incorporates at the inlet to the closed environment a gas exchanger equipped with a monitoring device which measures the pH, carbon dioxide partial pressure and oxygen partial pressure of the closed environment, and optimizes the cell culture environment by automatically adjusting gas concentrations based on signals from the monitoring device.

[00562] In some embodiments, the pressure within the closed environment is continuously or intermittently controlled. That is, the pressure in the closed environment can be varied by means of a pressure maintenance device for example, thus ensuring that the space is suitable for growth of TILs in a positive pressure state, or promoting exudation of fluid in a negative pressure state and thus promoting cell proliferation. By applying negative pressure intermittently, moreover, it is possible to uniformly and efficiently replace the circulating liquid in the closed environment by means of a temporary shrinkage in the volume of the closed environment.

[00563] In some embodiments, optimal culture components for proliferation of the TILs can be substituted or added, and including factors such as IL-2 and/or OKT3, as well as combination, can be added.

L. Optional Cryopreservation of TILs

[00564] Either the bulk TIL population or the expanded population of TILs can be optionally cryopreserved. In some embodiments, cryopreservation occurs on therapeutic TIL population. In some embodiments, cryopreservation occurs on the TILs harvested after the second expansion. In some embodiments, cryopreservation occurs on the TILs in exemplary Step F of Figure 1. In some embodiments, the TILs are cryopreserved in the infusion bag. In some embodiments, the TILs are cryopreserved prior to placement in an infusion bag. In some embodiments, the TILs are cryopreserved and not placed in an infusion bag. In some embodiments, cryopreservation is performed using a cryopreservation medium. In some embodiments, the cryopreservation media contains dimethylsulfoxide (DMSO). This is generally accomplished by putting the TIL population into a freezing solution, e.g. 85% complement inactivated AB serum and 15% dimethyl sulfoxide (DMSO). The cells in solution are placed into cryogenic vials and stored for 24 hours at -80 °C, with optional transfer to gaseous nitrogen freezers for cryopreservation. See, Sadeghi, *et al.*, *Acta Oncologica* **2013**, 52, 978-986.

[00565] When appropriate, the cells are removed from the freezer and thawed in a 37 °C water bath until approximately 4/5 of the solution is thawed. The cells are generally resuspended in complete media and optionally washed one or more times. In some embodiments, the thawed TILs can be counted and assessed for viability as is known in the art.

[00566] In a preferred embodiment, a population of TILs is cryopreserved using CS10 cryopreservation media (CryoStor 10, BioLife Solutions). In a preferred embodiment, a population of TILs is cryopreserved using a cryopreservation media containing dimethylsulfoxide (DMSO). In a preferred embodiment, a population of TILs is cryopreserved using a 1:1 (vol:vol) ratio of CS10 and cell culture media. In a preferred embodiment, a population of TILs is cryopreserved using about a 1:1 (vol:vol) ratio of CS10 and cell culture media, further comprising additional IL-2.

[00567] As discussed above in Steps A through E, cryopreservation can occur at numerous points throughout the TIL expansion process. In some embodiments, the bulk TIL population after the first expansion according to Step B or the expanded population of TILs after the one or more second expansions according to Step D can be cryopreserved. Cryopreservation can be generally accomplished by placing the TIL population into a freezing solution, *e.g.*, 85% complement inactivated AB serum and 15% dimethyl sulfoxide (DMSO). The cells in solution are placed into cryogenic vials and stored for 24 hours at -80 °C, with optional transfer to gaseous nitrogen freezers for cryopreservation. See Sadeghi, *et al.*, *Acta Oncologica* **2013**, 52, 978-986.

[00568] When appropriate, the cells are removed from the freezer and thawed in a 37 °C water bath until approximately 4/5 of the solution is thawed. The cells are generally resuspended in complete media and optionally washed one or more times. In some embodiments, the thawed TILs can be counted and assessed for viability as is known in the art.

[00569] In some cases, the Step B TIL population can be cryopreserved immediately, using the protocols discussed below. Alternatively, the bulk TIL population can be subjected to Step C and Step D and then cryopreserved after Step D. Similarly, in the case where genetically modified TILs will be used in therapy, the Step B or Step D TIL populations can be subjected to genetic modifications for suitable treatments.

IV. Pharmaceutical Compositions, Dosages, and Dosing Regimens

[00570] In an embodiment, TILs expanded using the methods of the present disclosure are administered to a patient as a pharmaceutical composition. In an embodiment, the pharmaceutical composition is a suspension of TILs in a sterile buffer. TILs expanded using PBMCs of the present disclosure may be administered by any suitable route as known in the art. In some embodiments, the T-cells are administered as a single intra-arterial or

intravenous infusion, which preferably lasts approximately 30 to 60 minutes. Other suitable routes of administration include intraperitoneal, intrathecal, and intralymphatic administration.

[00571] Any suitable dose of TILs can be administered. In some embodiments, from about 2.3×10^{10} to about 13.7×10^{10} TILs are administered, with an average of around 7.8×10^{10} TILs, particularly if the cancer is NSCLC. In an embodiment, about 1.2×10^{10} to about 4.3×10^{10} of TILs are administered. In some embodiments, about 3×10^{10} to about 12×10^{10} TILs are administered. In some embodiments, about 4×10^{10} to about 10×10^{10} TILs are administered. In some embodiments, about 5×10^{10} to about 8×10^{10} TILs are administered. In some embodiments, about 6×10^{10} to about 8×10^{10} TILs are administered. In some embodiments, about 7×10^{10} to about 8×10^{10} TILs are administered. In some embodiments, therapeutically effective dosage is about 2.3×10^{10} to about 13.7×10^{10} . In some embodiments, therapeutically effective dosage is about 7.8×10^{10} TILs, particularly of the cancer is NSCLC. In some embodiments, therapeutically effective dosage is about 1.2×10^{10} to about 4.3×10^{10} of TILs. In some embodiments, therapeutically effective dosage is about 3×10^{10} to about 12×10^{10} TILs. In some embodiments, therapeutically effective dosage is about 4×10^{10} to about 10×10^{10} TILs. In some embodiments, therapeutically effective dosage is about 5×10^{10} to about 8×10^{10} TILs. In some embodiments, therapeutically effective dosage is about 6×10^{10} to about 8×10^{10} TILs. In some embodiments, therapeutically effective dosage is about 7×10^{10} to about 8×10^{10} TILs.

[00572] In some embodiments, from about 1×10^9 to about 150×10^9 TILs are administered, particularly if the cancer is NSCLC. In some embodiments, therapeutically effective dosage is about 1×10^9 to about 150×10^9 TILs. In some embodiments, therapeutically effective dosage is about 1×10^9 to about 140×10^9 TILs. In some embodiments, therapeutically effective dosage is about 1×10^9 to about 130×10^9 TILs. In some embodiments, therapeutically effective dosage is about 1×10^9 to about 120×10^9 TILs. In some embodiments, therapeutically effective dosage is about 1×10^9 to about 110×10^9 TILs. In some embodiments, therapeutically effective dosage is about 1×10^9 to about 100×10^9 TILs. In some embodiments, therapeutically effective dosage is about 1×10^9 to about 90×10^9 TILs. In some embodiments, therapeutically effective dosage is about 1×10^9 to about 80×10^9 TILs. In some embodiments, therapeutically effective dosage is about 1×10^9 to about 70×10^9 TILs. In some embodiments, therapeutically effective dosage is about 1×10^9 to about 60×10^9 TILs. In some embodiments, therapeutically effective dosage is about 1×10^9 to about 50×10^9 TILs. In some embodiments, therapeutically effective dosage

is about 1×10^9 to about 40×10^9 TILs. In some embodiments, therapeutically effective dosage is about 1×10^9 to about 30×10^9 TILs. In some embodiments, therapeutically effective dosage is about 1×10^9 to about 20×10^9 TILs. In some embodiments, therapeutically effective dosage is about 1×10^9 to about 10×10^9 TILs. In some embodiments, therapeutically effective dosage is about 1×10^9 to about 5×10^9 TILs.

[00573] In some embodiments, the number of the TILs provided in the pharmaceutical compositions of the invention is about 1×10^6 , 2×10^6 , 3×10^6 , 4×10^6 , 5×10^6 , 6×10^6 , 7×10^6 , 8×10^6 , 9×10^6 , 1×10^7 , 2×10^7 , 3×10^7 , 4×10^7 , 5×10^7 , 6×10^7 , 7×10^7 , 8×10^7 , 9×10^7 , 1×10^8 , 2×10^8 , 3×10^8 , 4×10^8 , 5×10^8 , 6×10^8 , 7×10^8 , 8×10^8 , 9×10^8 , 1×10^9 , 2×10^9 , 3×10^9 , 4×10^9 , 5×10^9 , 6×10^9 , 7×10^9 , 8×10^9 , 9×10^9 , 1×10^{10} , 2×10^{10} , 3×10^{10} , 4×10^{10} , 5×10^{10} , 6×10^{10} , 7×10^{10} , 8×10^{10} , 9×10^{10} , 1×10^{11} , 2×10^{11} , 3×10^{11} , 4×10^{11} , 5×10^{11} , 6×10^{11} , 7×10^{11} , 8×10^{11} , 9×10^{11} , 1×10^{12} , 2×10^{12} , 3×10^{12} , 4×10^{12} , 5×10^{12} , 6×10^{12} , 7×10^{12} , 8×10^{12} , 9×10^{12} , 1×10^{13} , 2×10^{13} , 3×10^{13} , 4×10^{13} , 5×10^{13} , 6×10^{13} , 7×10^{13} , 8×10^{13} , and 9×10^{13} . In an embodiment, the number of the TILs provided in the pharmaceutical compositions of the invention is in the range of 1×10^6 to 5×10^6 , 5×10^6 to 1×10^7 , 1×10^7 to 5×10^7 , 5×10^7 to 1×10^8 , 1×10^8 to 5×10^8 , 5×10^8 to 1×10^9 , 1×10^9 to 5×10^9 , 5×10^9 to 1×10^{10} , 1×10^{10} to 5×10^{10} , 5×10^{10} to 1×10^{11} , 5×10^{11} to 1×10^{12} , 1×10^{12} to 5×10^{12} , and 5×10^{12} to 1×10^{13} .

[00574] In some embodiments, the concentration of the TILs provided in the pharmaceutical compositions of the invention is less than, for example, 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.09%, 0.08%, 0.07%, 0.06%, 0.05%, 0.04%, 0.03%, 0.02%, 0.01%, 0.009%, 0.008%, 0.007%, 0.006%, 0.005%, 0.004%, 0.003%, 0.002%, 0.001%, 0.0009%, 0.0008%, 0.0007%, 0.0006%, 0.0005%, 0.0004%, 0.0003%, 0.0002% or 0.0001% w/w, w/v or v/v of the pharmaceutical composition.

[00575] In some embodiments, the concentration of the TILs provided in the pharmaceutical compositions of the invention is greater than 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 19.75%, 19.50%, 19.25%, 19%, 18.75%, 18.50%, 18.25%, 18%, 17.75%, 17.50%, 17.25%, 17%, 16.75%, 16.50%, 16.25%, 16%, 15.75%, 15.50%, 15.25%, 15%, 14.75%, 14.50%, 14.25%, 14%, 13.75%, 13.50%, 13.25%, 13%, 12.75%, 12.50%, 12.25%, 12%, 11.75%, 11.50%, 11.25%, 11%, 10.75%, 10.50%, 10.25%, 10%, 9.75%, 9.50%, 9.25%, 9%, 8.75%, 8.50%, 8.25%, 8%, 7.75%, 7.50%, 7.25%, 7%, 6.75%, 6.50%, 6.25%, 6%, 5.75%, 5.50%, 5.25%, 5%, 4.75%, 4.50%, 4.25%, 4%, 3.75%, 3.50%, 3.25%, 3%, 2.75%, 2.50%, 2.25%, 2%, 1.75%, 1.50%, 1.25%, 1%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.09%, 0.08%, 0.07%,

0.06%, 0.05%, 0.04%, 0.03%, 0.02%, 0.01%, 0.009%, 0.008%, 0.007%, 0.006%, 0.005%, 0.004%, 0.003%, 0.002%, 0.001%, 0.0009%, 0.0008%, 0.0007%, 0.0006%, 0.0005%, 0.0004%, 0.0003%, 0.0002% or 0.0001% w/w, w/v, or v/v of the pharmaceutical composition.

[00576] In some embodiments, the concentration of the TILs provided in the pharmaceutical compositions of the invention is in the range from about 0.0001% to about 50%, about 0.001% to about 40%, about 0.01% to about 30%, about 0.02% to about 29%, about 0.03% to about 28%, about 0.04% to about 27%, about 0.05% to about 26%, about 0.06% to about 25%, about 0.07% to about 24%, about 0.08% to about 23%, about 0.09% to about 22%, about 0.1% to about 21%, about 0.2% to about 20%, about 0.3% to about 19%, about 0.4% to about 18%, about 0.5% to about 17%, about 0.6% to about 16%, about 0.7% to about 15%, about 0.8% to about 14%, about 0.9% to about 12% or about 1% to about 10% w/w, w/v or v/v of the pharmaceutical composition.

[00577] In some embodiments, the concentration of the TILs provided in the pharmaceutical compositions of the invention is in the range from about 0.001% to about 10%, about 0.01% to about 5%, about 0.02% to about 4.5%, about 0.03% to about 4%, about 0.04% to about 3.5%, about 0.05% to about 3%, about 0.06% to about 2.5%, about 0.07% to about 2%, about 0.08% to about 1.5%, about 0.09% to about 1%, about 0.1% to about 0.9% w/w, w/v or v/v of the pharmaceutical composition.

[00578] In some embodiments, the amount of the TILs provided in the pharmaceutical compositions of the invention is equal to or less than 10 g, 9.5 g, 9.0 g, 8.5 g, 8.0 g, 7.5 g, 7.0 g, 6.5 g, 6.0 g, 5.5 g, 5.0 g, 4.5 g, 4.0 g, 3.5 g, 3.0 g, 2.5 g, 2.0 g, 1.5 g, 1.0 g, 0.95 g, 0.9 g, 0.85 g, 0.8 g, 0.75 g, 0.7 g, 0.65 g, 0.6 g, 0.55 g, 0.5 g, 0.45 g, 0.4 g, 0.35 g, 0.3 g, 0.25 g, 0.2 g, 0.15 g, 0.1 g, 0.09 g, 0.08 g, 0.07 g, 0.06 g, 0.05 g, 0.04 g, 0.03 g, 0.02 g, 0.01 g, 0.009 g, 0.008 g, 0.007 g, 0.006 g, 0.005 g, 0.004 g, 0.003 g, 0.002 g, 0.001 g, 0.0009 g, 0.0008 g, 0.0007 g, 0.0006 g, 0.0005 g, 0.0004 g, 0.0003 g, 0.0002 g, or 0.0001 g.

[00579] In some embodiments, the amount of the TILs provided in the pharmaceutical compositions of the invention is more than 0.0001 g, 0.0002 g, 0.0003 g, 0.0004 g, 0.0005 g, 0.0006 g, 0.0007 g, 0.0008 g, 0.0009 g, 0.001 g, 0.0015 g, 0.002 g, 0.0025 g, 0.003 g, 0.0035 g, 0.004 g, 0.0045 g, 0.005 g, 0.0055 g, 0.006 g, 0.0065 g, 0.007 g, 0.0075 g, 0.008 g, 0.0085 g, 0.009 g, 0.0095 g, 0.01 g, 0.015 g, 0.02 g, 0.025 g, 0.03 g, 0.035 g, 0.04 g, 0.045 g, 0.05 g, 0.055 g, 0.06 g, 0.065 g, 0.07 g, 0.075 g, 0.08 g, 0.085 g, 0.09 g, 0.095 g, 0.1 g, 0.15 g, 0.2 g,

0.25 g, 0.3 g, 0.35 g, 0.4 g, 0.45 g, 0.5 g, 0.55 g, 0.6 g, 0.65 g, 0.7 g, 0.75 g, 0.8 g, 0.85 g, 0.9 g, 0.95 g, 1 g, 1.5 g, 2 g, 2.5 g, 3 g, 3.5 g, 4 g, 4.5 g, 5 g, 5.5 g, 6 g, 6.5 g, 7 g, 7.5 g, 8 g, 8.5 g, 9 g, 9.5 g, or 10 g.

[00580] The TILs provided in the pharmaceutical compositions of the invention are effective over a wide dosage range. The exact dosage will depend upon the route of administration, the form in which the compound is administered, the gender and age of the subject to be treated, the body weight of the subject to be treated, and the preference and experience of the attending physician. The clinically-established dosages of the TILs may also be used if appropriate. The amounts of the pharmaceutical compositions administered using the methods herein, such as the dosages of TILs, will be dependent on the human or mammal being treated, the severity of the disorder or condition, the rate of administration, the disposition of the active pharmaceutical ingredients and the discretion of the prescribing physician.

[00581] In some embodiments, TILs may be administered in a single dose. Such administration may be by injection, *e.g.*, intravenous injection. In some embodiments, TILs may be administered in multiple doses. Dosing may be once, twice, three times, four times, five times, six times, or more than six times per year. Dosing may be once a month, once every two weeks, once a week, or once every other day. Administration of TILs may continue as long as necessary.

[00582] In some embodiments, an effective dosage of TILs is about 1×10^6 , 2×10^6 , 3×10^6 , 4×10^6 , 5×10^6 , 6×10^6 , 7×10^6 , 8×10^6 , 9×10^6 , 1×10^7 , 2×10^7 , 3×10^7 , 4×10^7 , 5×10^7 , 6×10^7 , 7×10^7 , 8×10^7 , 9×10^7 , 1×10^8 , 2×10^8 , 3×10^8 , 4×10^8 , 5×10^8 , 6×10^8 , 7×10^8 , 8×10^8 , 9×10^8 , 1×10^9 , 2×10^9 , 3×10^9 , 4×10^9 , 5×10^9 , 6×10^9 , 7×10^9 , 8×10^9 , 9×10^9 , 1×10^{10} , 2×10^{10} , 3×10^{10} , 4×10^{10} , 5×10^{10} , 6×10^{10} , 7×10^{10} , 8×10^{10} , 9×10^{10} , 1×10^{11} , 2×10^{11} , 3×10^{11} , 4×10^{11} , 5×10^{11} , 6×10^{11} , 7×10^{11} , 8×10^{11} , 9×10^{11} , 1×10^{12} , 2×10^{12} , 3×10^{12} , 4×10^{12} , 5×10^{12} , 6×10^{12} , 7×10^{12} , 8×10^{12} , 9×10^{12} , 1×10^{13} , 2×10^{13} , 3×10^{13} , 4×10^{13} , 5×10^{13} , 6×10^{13} , 7×10^{13} , 8×10^{13} , and 9×10^{13} . In some embodiments, an effective dosage of TILs is in the range of 1×10^6 to 5×10^6 , 5×10^6 to 1×10^7 , 1×10^7 to 5×10^7 , 5×10^7 to 1×10^8 , 1×10^8 to 5×10^8 , 5×10^8 to 1×10^9 , 1×10^9 to 5×10^9 , 5×10^9 to 1×10^{10} , 1×10^{10} to 5×10^{10} , 5×10^{10} to 1×10^{11} , 5×10^{11} to 1×10^{12} , 1×10^{12} to 5×10^{12} , and 5×10^{12} to 1×10^{13} .

[00583] In some embodiments, an effective dosage of TILs is in the range of about 0.01 mg/kg to about 4.3 mg/kg, about 0.15 mg/kg to about 3.6 mg/kg, about 0.3 mg/kg to about

3.2 mg/kg, about 0.35 mg/kg to about 2.85 mg/kg, about 0.15 mg/kg to about 2.85 mg/kg, about 0.3 mg to about 2.15 mg/kg, about 0.45 mg/kg to about 1.7 mg/kg, about 0.15 mg/kg to about 1.3 mg/kg, about 0.3 mg/kg to about 1.15 mg/kg, about 0.45 mg/kg to about 1 mg/kg, about 0.55 mg/kg to about 0.85 mg/kg, about 0.65 mg/kg to about 0.8 mg/kg, about 0.7 mg/kg to about 0.75 mg/kg, about 0.7 mg/kg to about 2.15 mg/kg, about 0.85 mg/kg to about 2 mg/kg, about 1 mg/kg to about 1.85 mg/kg, about 1.15 mg/kg to about 1.7 mg/kg, about 1.3 mg/kg to about 1.6 mg/kg, about 1.35 mg/kg to about 1.5 mg/kg, about 2.15 mg/kg to about 3.6 mg/kg, about 2.3 mg/kg to about 3.4 mg/kg, about 2.4 mg/kg to about 3.3 mg/kg, about 2.6 mg/kg to about 3.15 mg/kg, about 2.7 mg/kg to about 3 mg/kg, about 2.8 mg/kg to about 3 mg/kg, or about 2.85 mg/kg to about 2.95 mg/kg.

[00584] In some embodiments, an effective dosage of TILs is in the range of about 1 mg to about 500 mg, about 10 mg to about 300 mg, about 20 mg to about 250 mg, about 25 mg to about 200 mg, about 1 mg to about 50 mg, about 5 mg to about 45 mg, about 10 mg to about 40 mg, about 15 mg to about 35 mg, about 20 mg to about 30 mg, about 23 mg to about 28 mg, about 50 mg to about 150 mg, about 60 mg to about 140 mg, about 70 mg to about 130 mg, about 80 mg to about 120 mg, about 90 mg to about 110 mg, or about 95 mg to about 105 mg, about 98 mg to about 102 mg, about 150 mg to about 250 mg, about 160 mg to about 240 mg, about 170 mg to about 230 mg, about 180 mg to about 220 mg, about 190 mg to about 210 mg, about 195 mg to about 205 mg, or about 198 to about 207 mg.

[00585] An effective amount of the TILs may be administered in either single or multiple doses by any of the accepted modes of administration of agents having similar utilities, including intranasal and transdermal routes, by intra-arterial injection, intravenously, intraperitoneally, parenterally, intramuscularly, subcutaneously, topically, by transplantation, or by inhalation.

V. Methods of Treating Patients

[00586] Methods of treatment begin with the initial TIL collection and culture of TILs. Such methods have been both described in the art by, for example, Jin *et al.*, *J. Immunotherapy*, **2012**, 35(3):283-292, incorporated by reference herein in its entirety. Embodiments of methods of treatment are described throughout the sections below, including the Examples.

[00587] The expanded TILs produced according the methods described herein, including for example as described in Steps A through F above or according to Steps A through F above (also as shown, for example, in Figure 1) find particular use in the treatment of patients with

cancer (for example, as described in Goff, et al., *J. Clinical Oncology*, **2016**, 34(20):2389-239, as well as the supplemental content; incorporated by reference herein in its entirety). In some embodiments, TIL were grown from resected deposits of metastatic melanoma as previously described (see, Dudley, et al., *J Immunother.*, **2003**, 26:332-342; incorporated by reference herein in its entirety). Fresh tumor can be dissected under sterile conditions. A representative sample can be collected for formal pathologic analysis. Single fragments of 2 mm³ to 3 mm³ may be used. In some embodiments, 5, 10, 15, 20, 25 or 30 samples per patient are obtained. In some embodiments, 20, 25, or 30 samples per patient are obtained. In some embodiments, 20, 22, 24, 26, or 28 samples per patient are obtained. In some embodiments, 24 samples per patient are obtained. Samples can be placed in individual wells of a 24-well plate, maintained in growth media with high-dose IL-2 (6,000 IU/mL), and monitored for destruction of tumor and/or proliferation of TIL. Any tumor with viable cells remaining after processing can be enzymatically digested into a single cell suspension and cryopreserved, as described herein.

[00588] In some embodiments, successfully grown TIL can be sampled for phenotype analysis (CD3, CD4, CD8, and CD56) and tested against autologous tumor when available. TIL can be considered reactive if overnight coculture yielded interferon-gamma (IFN- γ) levels > 200 pg/mL and twice background. (Goff, et al., *J Immunother.*, **2010**, 33:840-847; incorporated by reference herein in its entirety). In some embodiments, cultures with evidence of autologous reactivity or sufficient growth patterns can be selected for a second expansion (for example, a second expansion as provided in according to Step D of Figure 1), including second expansions that are sometimes referred to as rapid expansion (REP). In some embodiments, expanded TILs with high autologous reactivity (for example, high proliferation during a second expansion), are selected for an additional second expansion. In some embodiments, TILs with high autologous reactivity (for example, high proliferation during second expansion as provided in Step D of Figure 1), are selected for an additional second expansion according to Step D of Figure 1.

[00589] Cell phenotypes of cryopreserved samples of infusion bag TIL can be analyzed by flow cytometry (e.g., FlowJo) for surface markers CD3, CD4, CD8, CCR7, and CD45RA (BD BioSciences), as well as by any of the methods described herein. Serum cytokines were measured by using standard enzyme-linked immunosorbent assay techniques. A rise in serum IFN-g was defined as >100 pg/mL and greater than 4 3 baseline levels.

[00590] In some embodiments, the TILs produced by the methods provided herein, for example those exemplified in Figure 1, provide for a surprising improvement in clinical efficacy of the TILs. In some embodiments, the TILs produced by the methods provided herein, for example those exemplified in Figure 1, exhibit increased clinical efficacy as compared to TILs produced by methods other than those described herein, including for example, methods other than those exemplified in Figure 1. In some embodiments, the methods other than those described herein include methods referred to as process 1C and/or Generation 1 (Gen 1). In some embodiments, the increased efficacy is measured by DCR, ORR, and/or other clinical responses. In some embodiments, the TILs produced by the methods provided herein, for example those exemplified in Figure 1, exhibit a similar time to response and safety profile compared to TILs produced by methods other than those described herein, including for example, methods other than those exemplified in Figure 1, for example the Gen 1 process.

[00591] In some embodiments, IFN-gamma (IFN- γ) is indicative of treatment efficacy and/or increased clinical efficacy. In some embodiments, IFN- γ in the blood of subjects treated with TILs is indicative of active TILs. In some embodiments, a potency assay for IFN- γ production is employed. IFN- γ production is another measure of cytotoxic potential. IFN- γ production can be measured by determining the levels of the cytokine IFN- γ in the blood, serum, or TILs *ex vivo* of a subject treated with TILs prepared by the methods of the present invention, including those as described for example in Figure 1. In some embodiments, an increase in IFN- γ is indicative of treatment efficacy in a patient treated with the TILs produced by the methods of the present invention. In some embodiments, IFN- γ is increased one-fold, two-fold, three-fold, four-fold, or five-fold or more as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, IFN- γ secretion is increased one-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, IFN- γ secretion is increased two-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, IFN- γ secretion is increased three-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other

methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, IFN- γ secretion is increased four-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, IFN- γ secretion is increased five-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, IFN- γ is measured using a Quantikine ELISA kit. In some embodiments, IFN- γ is measured in TILs *ex vivo* of a subject treated with TILs prepared by the methods of the present invention, including those as described for example in Figure 1. In some embodiments, IFN- γ is measured in blood of a subject treated with TILs prepared by the methods of the present invention, including those as described for example in Figure 1. In some embodiments, IFN- γ is measured in TILs serum of a subject treated with TILs prepared by the methods of the present invention, including those as described for example in Figure 1.

[00592] In some embodiments, the TILs prepared by the methods of the present invention, including those as described for example in Figure 1, exhibit increased polyclonality as compared to TILs produced by other methods, including those not exemplified in Figure 1, such as for example, methods referred to as process 1C methods. In some embodiments, significantly improved polyclonality and/or increased polyclonality is indicative of treatment efficacy and/or increased clinical efficacy. In some embodiments, polyclonality refers to the T-cell repertoire diversity. In some embodiments, an increase in polyclonality can be indicative of treatment efficacy with regard to administration of the TILs produced by the methods of the present invention. In some embodiments, polyclonality is increased one-fold, two-fold, ten-fold, 100-fold, 500-fold, or 1000-fold as compared to TILs prepared using methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, polyclonality is increased one-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, polyclonality is increased two-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, polyclonality is increased ten-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other

methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, polyclonality is increased 100-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, polyclonality is increased 500-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, polyclonality is increased 1000-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including for example, methods other than those embodied in Figure 1.

[00593] Measures of efficacy can include the disease control rate (DCR) as well as overall response rate (ORR), as known in the art as well as described herein.

1. **Methods of Treating NSCLC**

[00594] The compositions and methods described herein can be used in a method for treating non-small-cell lung cancer (NSCLC), wherein the NSCLC is refractory to treatment with an anti-PD-1 antibody. In some embodiments the anti-PD-1 antibody includes, *e.g.*, but is not limited to nivolumab (BMS-936558, Bristol-Myers Squibb; Opdivo®), pembrolizumab (lambrolizumab, MK03475 or MK-3475, Merck; Keytruda®), ipilimumab (Yervoy®), humanized anti-PD-1 antibody JS001 (ShangHai JunShi), monoclonal anti-PD-1 antibody TSR-042 (Tesaro, Inc.), Pidilizumab (anti-PD-1 mAb CT-011, Medivation), anti-PD-1 monoclonal Antibody BGB-A317 (BeiGene), and/or anti-PD-1 antibody SHR-1210 (ShangHai HengRui), human monoclonal antibody REGN2810 (Regeneron), human monoclonal antibody MDX-1106 (Bristol-Myers Squibb), and/or humanized anti-PD-1 IgG4 antibody PDR001 (Novartis). In some embodiments, the PD-1 antibody is from clone: RMP1-14 (rat IgG) - BioXcell cat# BP0146. Other suitable antibodies suitable for use in co-administration methods with TILs produced according to Steps A through F as described herein are anti-PD-1 antibodies disclosed in U.S. Patent No. 8,008,449, herein incorporated by reference. In some embodiments, the antibody or antigen-binding portion thereof binds specifically to PD-L1 and inhibits its interaction with PD-1, thereby increasing immune activity. Any antibodies known in the art which bind to PD-L1 and disrupt the interaction between the PD-1 and PD-L1, and stimulates an anti-tumor immune response, are include.

For example, antibodies that target PD-L1 and are in clinical trials, include BMS-936559 (Bristol-Myers Squibb) and MPDL3280A (Genentech). Other suitable antibodies that target PD-L1 are disclosed in U.S. Patent No. 7,943,743, herein incorporated by reference. It will be understood by one of ordinary skill that any antibody which binds to PD-1 or PD-L1, disrupts the PD-1/PD-L1 interaction, and stimulates an anti-tumor immune response, are included.

[00595] In some embodiments, the NSCLC has been treated with an anti-PD-1 antibody. In some embodiments, the NSCLC has been treated with an anti-PD-L1 antibody. In some embodiments, the NSCLC subject is treatment naïve. In some embodiments, the NSCLC has not been treated with an anti-PD-1 antibody. In some embodiments, the NSCLC has not been treated with an anti-PD-L1 antibody. In some embodiments, the NSCLC has been previously treated with a chemotherapeutic agent. In some embodiments, the NSCLC has been previously treated with a chemotherapeutic agent but is no longer being treated with the chemotherapeutic agent. In some embodiments, the NSCLC patient is anti-PD-1/PD-L1 naïve. In some embodiments, the NSCLC subject has low expression of PD-L1. In some embodiments, the NSCLC subject has treatment naïve NSCLC or is post-chemotherapeutic treatment but anti-PD-1/PD-L1 naïve. In some embodiments, the NSCLC subject is treatment naïve NSCLC or post-chemotherapeutic treatment but anti-PD-1/PD-L1 naïve and has low expression of PD-L1. In some embodiments, the NSCLC subject has bulky disease at baseline. In some embodiments, the subject has bulky disease at baseline and has low expression of PD-L1. In some embodiments, the NSCLC subject has treatment naïve NSCLC or post chemotherapy but anti-PD-1/PD-L1 naïve who have low expression of PD-L1 and/or have bulky disease at baseline. In some embodiments, bulky disease is indicated where the maximal tumor diameter is greater than 7 cm measured in either the transverse or coronal plane. In some embodiments, bulky disease is indicated when there are swollen lymph nodes with a short-axis diameter of 20 mm or greater. In some embodiments, the chemotherapeutic includes a standard of care therapeutic for NSCLC.

[00596] In some embodiments, the TILs prepared by the methods of the present invention, including those as described for example in Figure 1, exhibit increased polyclonality as compared to TILs produced by other methods, including those not exemplified in Figure 1, such as for example, methods referred to as process 1C methods. In some embodiments, significantly improved polyclonality and/or increased polyclonality is indicative of treatment efficacy and/or increased clinical efficacy for cancer treatment. In some embodiments, polyclonality refers to the T-cell repertoire diversity. In some embodiments, an increase in

polyclonality can be indicative of treatment efficacy with regard to administration of the TILs produced by the methods of the present invention. In some embodiments, polyclonality is increased one-fold, two-fold, ten-fold, 100-fold, 500-fold, or 1000-fold as compared to TILs prepared using methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, polyclonality is increased one-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, polyclonality is increased two-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, polyclonality is increased ten-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, polyclonality is increased 100-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, polyclonality is increased 500-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including for example, methods other than those embodied in Figure 1. In some embodiments, polyclonality is increased 1000-fold as compared to an untreated patient and/or as compared to a patient treated with TILs prepared using other methods than those provide herein including for example, methods other than those embodied in Figure 1.

[00597] In some embodiments, the subject to be treated has Stage III or Stage IV NSCLC (squamous, adenocarcinoma, large cell carcinoma). In some embodiments, the subject to be treated has Stage III NSCLC (squamous, adenocarcinoma, large cell carcinoma). In some embodiments, the subject to be treated has Stage IV NSCLC (squamous, adenocarcinoma, large cell carcinoma). In some embodiments, the subject to be treated has an oncogene-driven tumor and had been treated with at least one effective targeted therapy directed toward the oncogene.

[00598] In some embodiments, the subject to be treated has Stage III or Stage IV NSCLC (squamous, adenocarcinoma, large cell carcinoma) and were previously treated with for example, a PD-1 inhibitor or PD-L1 inhibitor, such as for example, for example, anti-PD-

1 and/or anti-PD-L1. In some embodiments, the subject to be treated has Stage III NSCLC (squamous, adenocarcinoma, large cell carcinoma) and were previously treated with systemic therapy including for example, anti-PD-1 and/or anti-PD-L1. In some embodiments, the subject to be treated has Stage IV NSCLC (squamous, adenocarcinoma, large cell carcinoma) and were previously treated with systemic therapy including for example, anti-PD-1 and/or anti-PD-L1. In some embodiments, the subject to be treated has Stage III NSCLC (squamous, adenocarcinoma, large cell carcinoma) and were previously treated with systemic therapy including anti-PD-1. In some embodiments, the subject to be treated has Stage IV NSCLC (squamous, adenocarcinoma, large cell carcinoma) and were previously treated with systemic therapy including anti-PD-1. In some embodiments, the subject to be treated has Stage III NSCLC (squamous, adenocarcinoma, large cell carcinoma) and were previously treated with systemic therapy including anti-PD-L1. In some embodiments, the subject to be treated has Stage IV NSCLC (squamous, adenocarcinoma, large cell carcinoma) and were previously treated with systemic therapy including anti-PD-L1. In some embodiments, the subject to be treated has an oncogene-driven tumor and had been treated with at least one effective targeted therapy directed toward the oncogene.

[00599] In some embodiments, the subject to be treated has a histologically or pathologically confirmed diagnosis of Stage III or Stage IV NSCLC (squamous, nonsquamous, adenocarcinoma, large cell carcinoma).

[00600] In some embodiments, the subject to be treated are immunotherapy naïve. In some embodiments, the subject to be treated has may have received up to 3 prior systemic anticancer therapies, including for example, systemic therapy in the adjuvant or neoadjuvant setting, or as part of definitive chemoradiotherapy. In some embodiments, the subject to be treated has oncogene mutations, including for example mutations in EGFR, ALK, and/or ROS.

[00601] In some embodiments, the subject to be treated had previously received systemic therapy with for example, a PD-1 inhibitor or PD-L1 inhibitor, such as for example, anti-PD-1 and/or anti-PD-L1, as part of ≤ 3 prior lines of systemic therapy. In some embodiments, the subject to be treated has oncogene mutations, including for example mutations in EGFR, ALK, and/or ROS.

[00602] In some embodiments, the subject to be treated has at least 1 resectable lesion (or aggregate lesions) of at least about 1.5 cm in diameter post-resection for use in TIL preparation.

[00603] In some embodiments, the subject to be treated had a washout period from one or more prior anticancer therapies of a minimum duration, prior to the first study treatment (*i.e.*, start of nonmyeloablative lymphodepletion (NMA-LD) or pembrolizumab)

[00604] In some embodiments, the subject to be treated had prior targeted therapy with an epidermal growth factor receptor (EGFR), MEK, BRAF, ALK, ROS1 and/or other-targeted agents (including, for example, erlotinib, afatinib, dacomitinib, osimertinib, crizotinib, ceritinib, and/or lorlatinib) and a minimum washout of prior treatment of at least 14 days prior to the start of TIL treatment.

[00605] In some embodiments, the subject to be treated had adjuvant, neoadjuvant or definitive chemotherapy and/or chemoradiation and a minimum washout of prior treatment of at least 21 days prior to the start of treatment.

[00606] In some embodiments, the subject to be treated had prior checkpoint-targeted therapy with for example, a PD-1 inhibitor or PD-L1 inhibitor, such as for example, an anti-PD-1, and anti-PD-L1, other mAbs, and/or vaccines and a minimum washout period of greater than or equal to 21 days before the start of nonmyeloablative lymphodepletion (NMA-LD).

a. PD-1 and PD-L1 Inhibitors

[0001] Programmed death 1 (PD-1) is a 288-amino acid transmembrane immunocheckpoint receptor protein expressed by T cells, B cells, natural killer (NK) T cells, activated monocytes, and dendritic cells. PD-1, which is also known as CD279, belongs to the CD28 family, and in humans is encoded by the *Pdcd1* gene on chromosome 2. PD-1 consists of one immunoglobulin (Ig) superfamily domain, a transmembrane region, and an intracellular domain containing an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). PD-1 and its ligands (PD-L1 and PD-L2) are known to play a key role in immune tolerance, as described in Keir, *et al.*, *Annu. Rev. Immunol.* **2008**, 26, 677-704. PD-1 provides inhibitory signals that negatively regulate T cell immune responses. PD-L1 (also known as B7-H1 or CD274) and PD-L2 (also known as B7-DC or CD273) are expressed on tumor cells and stromal cells, which may be encountered by activated T cells expressing PD-1, leading to immunosuppression of the T

cells. PD-L1 is a 290 amino acid transmembrane protein encoded by the *Cd274* gene on human chromosome 9. Blocking the interaction between PD-1 and its ligands PD-L1 and PD-L2 by use of a PD-1 inhibitor, a PD-L1 inhibitor, and/or a PD-L2 inhibitor can overcome immune resistance, as demonstrated in recent clinical studies, such as that described in Topalian, *et al.*, *N. Eng. J. Med.* **2012**, 366, 2443-54. PD-L1 is expressed on many tumor cell lines, while PD-L2 is expressed mostly on dendritic cells and a few tumor lines. In addition to T cells (which inducibly express PD-1 after activation), PD-1 is also expressed on B cells, natural killer cells, macrophages, activated monocytes, and dendritic cells.

[0002] In an embodiment, the PD-1 inhibitor may be any PD-1 inhibitor or PD-1 blocker known in the art. In particular, it is one of the PD-1 inhibitors or blockers described in more detail in the following paragraphs. The terms “inhibitor,” “antagonist,” and “blocker” are used interchangeably herein in reference to PD-1 inhibitors. For avoidance of doubt, references herein to a PD-1 inhibitor that is an antibody may refer to a compound or antigen-binding fragments, variants, conjugates, or biosimilars thereof. For avoidance of doubt, references herein to a PD-1 inhibitor may also refer to a small molecule compound or a pharmaceutically acceptable salt, ester, solvate, hydrate, cocrystal, or prodrug thereof.

[0003] In a preferred embodiment, the PD-1 inhibitor is an antibody (*i.e.*, an anti-PD-1 antibody), a fragment thereof, including Fab fragments, or a single-chain variable fragment (scFv) thereof. In some embodiments the PD-1 inhibitor is a polyclonal antibody. In a preferred embodiment, the PD-1 inhibitor is a monoclonal antibody. In some embodiments, the PD-1 inhibitor competes for binding with PD-1, and/or binds to an epitope on PD-1. In an embodiment, the antibody competes for binding with PD-1, and/or binds to an epitope on PD-1.

[0004] In some embodiments, the PD-1 inhibitor is one that binds human PD-1 with a K_D of about 100 pM or lower, binds human PD-1 with a K_D of about 90 pM or lower, binds human PD-1 with a K_D of about 80 pM or lower, binds human PD-1 with a K_D of about 70 pM or lower, binds human PD-1 with a K_D of about 60 pM or lower, binds human PD-1 with a K_D of about 50 pM or lower, binds human PD-1 with a K_D of about 40 pM or lower, binds human PD-1 with a K_D of about 30 pM or lower, binds human PD-1 with a K_D of about 20 pM or lower, binds human PD-1 with a K_D of about 10 pM or lower, or binds human PD-1 with a K_D of about 1 pM or lower.

[0005] In some embodiments, the PD-1 inhibitor is one that binds to human PD-1 with a

k_{assoc} of about 7.5×10^5 1/M·s or faster, binds to human PD-1 with a k_{assoc} of about 7.5×10^5 1/M·s or faster, binds to human PD-1 with a k_{assoc} of about 8×10^5 1/M·s or faster, binds to human PD-1 with a k_{assoc} of about 8.5×10^5 1/M·s or faster, binds to human PD-1 with a k_{assoc} of about 9×10^5 1/M·s or faster, binds to human PD-1 with a k_{assoc} of about 9.5×10^5 1/M·s or faster, or binds to human PD-1 with a k_{assoc} of about 1×10^6 1/M·s or faster.

[0006] In some embodiments, the PD-1 inhibitor is one that binds to human PD-1 with a k_{dissoc} of about 2×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.1×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.2×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.3×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.4×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.5×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.6×10^{-5} 1/s or slower or binds to human PD-1 with a k_{dissoc} of about 2.7×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.8×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.9×10^{-5} 1/s or slower, or binds to human PD-1 with a k_{dissoc} of about 3×10^{-5} 1/s or slower.

[0007] In some embodiments, the PD-1 inhibitor is one that blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 10 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 9 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 8 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 7 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 6 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 5 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 4 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 3 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 2 nM or lower, or blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 1 nM or lower.

[0008] In an embodiment, the PD-1 inhibitor is nivolumab (commercially available as OPDIVO from Bristol-Myers Squibb Co.), or biosimilars, antigen-binding fragments, conjugates, or variants thereof. Nivolumab is a fully human IgG4 antibody blocking the PD-1 receptor. In an embodiment, the anti-PD-1 antibody is an immunoglobulin G4 kappa, anti-(human CD274) antibody. Nivolumab is assigned Chemical Abstracts Service (CAS)

registry number 946414-94-4 and is also known as 5C4, BMS-936558, MDX-1106, and ONO-4538. The preparation and properties of nivolumab are described in U.S. Patent No. 8,008,449 and International Patent Publication No. WO 2006/121168, the disclosures of which are incorporated by reference herein. The clinical safety and efficacy of nivolumab in various forms of cancer has been described in Wang, *et al.*, *Cancer Immunol Res.* **2014**, *2*, 846-56; Page, *et al.*, *Ann. Rev. Med.*, **2014**, *65*, 185-202; and Weber, *et al.*, *J. Clin. Oncology*, **2013**, *31*, 4311-4318, the disclosures of which are incorporated by reference herein. The amino acid sequences of nivolumab are set forth in Table 48. Nivolumab has intra-heavy chain disulfide linkages at 22-96, 140-196, 254-314, 360-418, 22"-96", 140"-196", 254"-314", and 360"-418"; intra-light chain disulfide linkages at 23'-88', 134'-194', 23'''-88''', and 134'''-194'''; inter-heavy-light chain disulfide linkages at 127-214', 127"-214'", inter-heavy-heavy chain disulfide linkages at 219-219" and 222-222"; and N-glycosylation sites (H CH₂ 84.4) at 290, 290".

[0009] In an embodiment, a PD-1 inhibitor comprises a heavy chain given by SEQ ID NO:463 and a light chain given by SEQ ID NO:128. In an embodiment, a PD-1 inhibitor comprises heavy and light chains having the sequences shown in SEQ ID NO:463 and SEQ ID NO:128, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In an embodiment, a PD-1 inhibitor comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:463 and SEQ ID NO:128, respectively. In an embodiment, a PD-1 inhibitor comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:463 and SEQ ID NO:128, respectively. In an embodiment, a PD-1 inhibitor comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:463 and SEQ ID NO:128, respectively. In an embodiment, a PD-1 inhibitor comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:463 and SEQ ID NO:128, respectively. In an embodiment, a PD-1 inhibitor comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:463 and SEQ ID NO:128, respectively.

[0010] In an embodiment, the PD-1 inhibitor comprises the heavy and light chain CDRs or variable regions (VRs) of nivolumab. In an embodiment, the PD-1 inhibitor heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:129, and the PD-1 inhibitor light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:130, and conservative amino acid substitutions thereof. In an embodiment, a PD-1 inhibitor

comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:129 and SEQ ID NO:130, respectively. In an embodiment, a PD-1 inhibitor comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:129 and SEQ ID NO:130, respectively. In an embodiment, a PD-1 inhibitor comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:129 and SEQ ID NO:130, respectively. In an embodiment, a PD-1 inhibitor comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:129 and SEQ ID NO:130, respectively. In an embodiment, a PD-1 inhibitor comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:129 and SEQ ID NO:130, respectively.

[0011] In an embodiment, a PD-1 inhibitor comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:131, SEQ ID NO:132, and SEQ ID NO:133, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:134, SEQ ID NO:135, and SEQ ID NO:136, respectively, and conservative amino acid substitutions thereof. In an embodiment, the antibody competes for binding with, and/or binds to the same epitope on PD-1 as any of the aforementioned antibodies.

[0012] In an embodiment, the PD-1 inhibitor is an anti-PD-1 biosimilar monoclonal antibody approved by drug regulatory authorities with reference to nivolumab. In an embodiment, the biosimilar comprises an anti-PD-1 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is nivolumab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is an anti-PD-1 antibody authorized or submitted for authorization, wherein the anti-PD-1 antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is nivolumab. The anti-PD-1 antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one

or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is nivolumab. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is nivolumab.

TABLE 19. Amino acid sequences for PD-1 inhibitors related to nivolumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:463 nivolumab heavy chain	QVQLVESGGG VVQPGSRSLRL DCKASGITFS NSGMHWVRQA PGKGLEWVAV IWYDGSKRYY 60 ADSVKGRFTI SRDNSKNTLF LQMNSLRAED TAVYYCATND DYWGQGTLLV VSSASTKGPS 120 VFPLAPCSRS TSESTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS 180 VVTVPSSSLG TKTYTCNVDH KPSNTKVDKR VESKYGPPCP PCPAPEFLGG PSVFLFPPKP 240 KDTLMISRTP EVTCVVVDVS QEDPEVQFNW YVDGVEVHNA KTKPREEQFN STYRVVSVLT 300 VLHQDWLNGK EYKCKVSNKG LPSSIEKTIS KAKGQPREPQ VYTLPPSQEE MTKNQVSLTC 360 LVKGFYPSDI AVEWESNGQP ENNYKTTTPPV LDSDGSFFLY SRLTVDKSRW QEGNVFSCSV 420 MHEALHNHYT QKSLSLSLGK 440
SEQ ID NO:128 nivolumab light chain	EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA 60 RFGSGSGTD FTLTISLEP EDFAVYYCQQ SSNWPRTFGQ GTKVEIKRTV AAPSVFIFPP 120 SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSLT 180 LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEK 214
SEQ ID NO:129 nivolumab variable heavy chain	QVQLVESGGG VVQPGSRSLRL DCKASGITFS NSGMHWVRQA PGKGLEWVAV IWYDGSKRYY 60 ADSVKGRFTI SRDNSKNTLF LQMNSLRAED TAVYYCATND DYWGQGTLLV VSS 113
SEQ ID NO:130 nivolumab variable light chain	EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA 60 RFGSGSGTD FTLTISLEP EDFAVYYCQQ SSNWPRTFGQ GTKVEIK 107
SEQ ID NO:131 nivolumab heavy chain CDR1	NSGMH 5
SEQ ID NO:132 nivolumab heavy chain CDR2	VIWYDGSKRY YADSVKG 17
SEQ ID NO:133 nivolumab heavy chain CDR3	NDDY 4
SEQ ID NO:134 nivolumab light chain CDR1	RASQSVSSYL A 11
SEQ ID NO:135 nivolumab light chain CDR2	DASNRAT 7
SEQ ID NO:136 nivolumab light chain CDR3	QQSSNWPRT 9

[0013] In another embodiment, the PD-1 inhibitor comprises pembrolizumab (commercially available as KEYTRUDA from Merck & Co., Inc., Kenilworth, NJ, USA), or antigen-binding fragments, conjugates, or variants thereof. Pembrolizumab is assigned CAS

registry number 1374853-91-4 and is also known as pembrolizumab, MK-3475, and SCH-900475. Pembrolizumab has an immunoglobulin G4, anti-(human protein PDCD1 (programmed cell death 1)) (human-Mus musculus monoclonal heavy chain), disulfide with human-Mus musculus monoclonal light chain, dimer structure. The structure of pembrolizumab may also be described as immunoglobulin G4, anti-(human programmed cell death 1); humanized mouse monoclonal [228-L-proline(H10-S>P)] γ 4 heavy chain (134-218')-disulfide with humanized mouse monoclonal κ light chain dimer (226-226":229-229")-bisdisulfide. The properties, uses, and preparation of pembrolizumab are described in International Patent Publication No. WO 2008/156712 A1, U.S. Patent No. 8,354,509 and U.S. Patent Application Publication Nos. US 2010/0266617 A1, US 2013/0108651 A1, and US 2013/0109843 A2, the disclosures of which are incorporated herein by reference. The clinical safety and efficacy of pembrolizumab in various forms of cancer is described in Fuerst, *Oncology Times*, **2014**, *36*, 35-36; Robert, *et al.*, *Lancet*, **2014**, *384*, 1109-17; and Thomas, *et al.*, *Exp. Opin. Biol. Ther.*, **2014**, *14*, 1061-1064. The amino acid sequences of pembrolizumab are set forth in Table 49. Pembrolizumab includes the following disulfide bridges: 22-96, 22"-96", 23'-92', 23'''-92"', 134-218', 134"-218"', 138'-198', 138'''-198"', 147-203, 147"-203", 226-226", 229-229", 261-321, 261"-321", 367-425, and 367"-425", and the following glycosylation sites (N): Asn-297 and Asn-297". Pembrolizumab is an IgG4/kappa isotype with a stabilizing S228P mutation in the Fc region; insertion of this mutation in the IgG4 hinge region prevents the formation of half molecules typically observed for IgG4 antibodies. Pembrolizumab is heterogeneously glycosylated at Asn297 within the Fc domain of each heavy chain, yielding a molecular weight of approximately 149 kDa for the intact antibody. The dominant glycoform of pembrolizumab is the fucosylated agalactodiantennary glycan form (G0F).

[0014] In an embodiment, a PD-1 inhibitor comprises a heavy chain given by SEQ ID NO:137 and a light chain given by SEQ ID NO:138. In an embodiment, a PD-1 inhibitor comprises heavy and light chains having the sequences shown in SEQ ID NO:137 and SEQ ID NO:138, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In an embodiment, a PD-1 inhibitor comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:137 and SEQ ID NO:138, respectively. In an embodiment, a PD-1 inhibitor comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:137 and SEQ ID NO:138, respectively. In an embodiment, a PD-1 inhibitor

comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:137 and SEQ ID NO:138, respectively. In an embodiment, a PD-1 inhibitor comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:137 and SEQ ID NO:138, respectively. In an embodiment, a PD-1 inhibitor comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:137 and SEQ ID NO:138, respectively.

[0015] In an embodiment, the PD-1 inhibitor comprises the heavy and light chain CDRs or variable regions (VRs) of pembrolizumab. In an embodiment, the PD-1 inhibitor heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:139, and the PD-1 inhibitor light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:140, and conservative amino acid substitutions thereof. In an embodiment, a PD-1 inhibitor comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:139 and SEQ ID NO:140, respectively. In an embodiment, a PD-1 inhibitor comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:139 and SEQ ID NO:140, respectively. In an embodiment, a PD-1 inhibitor comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:139 and SEQ ID NO:140, respectively. In an embodiment, a PD-1 inhibitor comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:139 and SEQ ID NO:140, respectively. In an embodiment, a PD-1 inhibitor comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:139 and SEQ ID NO:140, respectively.

[0016] In an embodiment, a PD-1 inhibitor comprises the heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:141, SEQ ID NO:142, and SEQ ID NO:143, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:144, SEQ ID NO:145, and SEQ ID NO:146, respectively, and conservative amino acid substitutions thereof. In an embodiment, the antibody competes for binding with, and/or binds to the same epitope on PD-1 as any of the aforementioned antibodies.

[0017] In an embodiment, the PD-1 inhibitor is an anti-PD-1 biosimilar monoclonal antibody approved by drug regulatory authorities with reference to pembrolizumab. In an embodiment, the biosimilar comprises an anti-PD-1 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological

product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is pembrolizumab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is an anti-PD-1 antibody authorized or submitted for authorization, wherein the anti-PD-1 antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is pembrolizumab. The anti-PD-1 antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is pembrolizumab. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is pembrolizumab.

TABLE 20. Amino acid sequences for PD-1 inhibitors related to pembrolizumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:137 pembrolizumab heavy chain	QVQLVQSGVE VKKPGASVKV SCKASGYTFT NYYMYWVRQA PGQGLEWMGG INPSNGGTNF 60 NEKFKNRVTL TTDSSTTTAY MELKSLQFDD TAVYYCARRD YRFDMGFQYW GQGT'TVTVSS 120 ASTKGPSVFAP LAPSRSSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS 180 GLYSLSSVVT VPSSSLGKT YTCNVDHKPS NTKVDKRVES KYGPPCPPCP APEFLGGPSV 240 FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY 300 RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK 360 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPPVLD SGSFFLYSRL TVDKSRWQEG 420 NVFSCSVME ALHNHYTQKS LSLSLGK 447
SEQ ID NO:138 pembrolizumab light chain	EIVLTQSPAT LSLSPGERAT LSCRASKGVS TSGYSYLHWY QKPGQAPRL LIYLAAYLES 60 GVPARFSGSG SGTDFTLTIS SLEPEDFAVY YCQHSRDLPL TFGGGTKVEI KRTVAAPSVE 120 IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS 180 STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC 218
SEQ ID NO:139 pembrolizumab variable heavy chain	QVQLVQSGVE VKKPGASVKV SCKASGYTFT NYYMYWVRQA PGQGLEWMGG INPSNGGTNF 60 NEKFKNRVTL TTDSSTTTAY MELKSLQFDD TAVYYCARRD YRFDMGFQYW GQGT'TVTVSS 120
SEQ ID NO:140 pembrolizumab variable light chain	EIVLTQSPAT LSLSPGERAT LSCRASKGVS TSGYSYLHWY QKPGQAPRL LIYLAAYLES 60 GVPARFSGSG SGTDFTLTIS SLEPEDFAVY YCQHSRDLPL TFGGGTKVEI K 111
SEQ ID NO:141 pembrolizumab heavy chain CDR1	NYYMY 5
SEQ ID NO:142 pembrolizumab heavy chain CDR2	GINPSNGGTN FNEKFK 16

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:143 pembrolizumab heavy chain CDR3	RDYRFDMGFD Y 11
SEQ ID NO:144 pembrolizumab light chain CDR1	RASKGVSTSG YSYLH 15
SEQ ID NO:145 pembrolizumab light chain CDR2	LASYLES 7
SEQ ID NO:146 pembrolizumab light chain CDR3	QHSRDLPLT 9

[0018] In an embodiment, the PD-1 inhibitor is a commercially-available anti-PD-1 monoclonal antibody, such as anti-m-PD-1 clones J43 (Cat # BE0033-2) and RMP1-14 (Cat # BE0146) (Bio X Cell, Inc., West Lebanon, NH, USA). A number of commercially-available anti-PD-1 antibodies are known to one of ordinary skill in the art.

[0019] In an embodiment, the PD-1 inhibitor is an antibody disclosed in U.S. Patent No. 8,354,509 or U.S. Patent Application Publication Nos. 2010/0266617 A1, 2013/0108651 A1, 2013/0109843 A2, the disclosures of which are incorporated by reference herein. In an embodiment, the PD-1 inhibitor is an anti-PD-1 antibody described in U.S. Patent Nos. 8,287,856, 8,580,247, and 8,168,757 and U.S. Patent Application Publication Nos. 2009/0028857 A1, 2010/0285013 A1, 2013/0022600 A1, and 2011/0008369 A1, the teachings of which are hereby incorporated by reference. In another embodiment, the PD-1 inhibitor is an anti-PD-1 antibody disclosed in U.S. Patent No. 8,735,553 B1, the disclosure of which is incorporated herein by reference. In an embodiment, the PD-1 inhibitor is pidilizumab, also known as CT-011, which is described in U.S. Patent No. 8,686,119, the disclosure of which is incorporated by reference herein.

[0020] In an embodiment, the PD-1 inhibitor may be a small molecule or a peptide, or a peptide derivative, such as those described in U.S. Patent Nos. 8,907,053; 9,096,642; and 9,044,442 and U.S. Patent Application Publication No. US 2015/0087581; 1,2,4-oxadiazole compounds and derivatives such as those described in U.S. Patent Application Publication No. 2015/0073024; cyclic peptidomimetic compounds and derivatives such as those described in U.S. Patent Application Publication No. US 2015/0073042; cyclic compounds and derivatives such as those described in U.S. Patent Application Publication No. US 2015/0125491; 1,3,4-oxadiazole and 1,3,4-thiadiazole compounds and derivatives such as

those described in International Patent Application Publication No. WO 2015/033301; peptide-based compounds and derivatives such as those described in International Patent Application Publication Nos. WO 2015/036927 and WO 2015/04490, or a macrocyclic peptide-based compounds and derivatives such as those described in U.S. Patent Application Publication No. US 2014/0294898; the disclosures of each of which are hereby incorporated by reference in their entirety.

[0021] In an embodiment, the PD-L1 or PD-L2 inhibitor may be any PD-L1 or PD-L2 inhibitor, antagonist, or blocker known in the art. In particular, it is one of the PD-L1 or PD-L2 inhibitors, antagonist, or blockers described in more detail in the following paragraphs. The terms “inhibitor,” “antagonist,” and “blocker” are used interchangeably herein in reference to PD-L1 and PD-L2 inhibitors. For avoidance of doubt, references herein to a PD-L1 or PD-L2 inhibitor that is an antibody may refer to a compound or antigen-binding fragments, variants, conjugates, or biosimilars thereof. For avoidance of doubt, references herein to a PD-L1 or PD-L2 inhibitor may refer to a compound or a pharmaceutically acceptable salt, ester, solvate, hydrate, cocrystal, or prodrug thereof.

[0022] In some embodiments, the compositions, processes and methods described herein include a PD-L1 or PD-L2 inhibitor. In some embodiments, the PD-L1 or PD-L2 inhibitor is a small molecule. In a preferred embodiment, the PD-L1 or PD-L2 inhibitor is an antibody (*i.e.*, an anti-PD-1 antibody), a fragment thereof, including Fab fragments, or a single-chain variable fragment (scFv) thereof. In some embodiments the PD-L1 or PD-L2 inhibitor is a polyclonal antibody. In a preferred embodiment, the PD-L1 or PD-L2 inhibitor is a monoclonal antibody. In some embodiments, the PD-L1 or PD-L2 inhibitor competes for binding with PD-L1 or PD-L2, and/or binds to an epitope on PD-L1 or PD-L2. In an embodiment, the antibody competes for binding with PD-L1 or PD-L2, and/or binds to an epitope on PD-L1 or PD-L2.

[0023] In some embodiments, the PD-L1 inhibitors provided herein are selective for PD-L1, in that the compounds bind or interact with PD-L1 at substantially lower concentrations than they bind or interact with other receptors, including the PD-L2 receptor. In certain embodiments, the compounds bind to the PD-L1 receptor at a binding constant that is at least about a 2-fold higher concentration, about a 3-fold higher concentration, about a 5-fold higher concentration, about a 10-fold higher concentration, about a 20-fold higher concentration, about a 30-fold higher concentration, about a 50-fold higher concentration, about a 100-fold higher concentration, about a 200-fold higher concentration, about a 300-

fold higher concentration, or about a 500-fold higher concentration than to the PD-L2 receptor.

[0024] In some embodiments, the PD-L2 inhibitors provided herein are selective for PD-L2, in that the compounds bind or interact with PD-L2 at substantially lower concentrations than they bind or interact with other receptors, including the PD-L1 receptor. In certain embodiments, the compounds bind to the PD-L2 receptor at a binding constant that is at least about a 2-fold higher concentration, about a 3-fold higher concentration, about a 5-fold higher concentration, about a 10-fold higher concentration, about a 20-fold higher concentration, about a 30-fold higher concentration, about a 50-fold higher concentration, about a 100-fold higher concentration, about a 200-fold higher concentration, about a 300-fold higher concentration, or about a 500-fold higher concentration than to the PD-L1 receptor.

[0025] Without being bound by any theory, it is believed that tumor cells express PD-L1, and that T cells express PD-1. However, PD-L1 expression by tumor cells is not required for efficacy of PD-1 or PD-L1 inhibitors or blockers. In an embodiment, the tumor cells express PD-L1. In another embodiment, the tumor cells do not express PD-L1. In some embodiments, the methods can include a combination of a PD-1 and a PD-L1 antibody, such as those described herein, in combination with a TIL. The administration of a combination of a PD-1 and a PD-L1 antibody and a TIL may be simultaneous or sequential.

[0026] In some embodiments, the PD-L1 and/or PD-L2 inhibitor is one that binds human PD-L1 and/or PD-L2 with a K_D of about 100 pM or lower, binds human PD-L1 and/or PD-L2 with a K_D of about 90 pM or lower, binds human PD-L1 and/or PD-L2 with a K_D of about 80 pM or lower, binds human PD-L1 and/or PD-L2 with a K_D of about 70 pM or lower, binds human PD-L1 and/or PD-L2 with a K_D of about 60 pM or lower, a K_D of about 50 pM or lower, binds human PD-L1 and/or PD-L2 with a K_D of about 40 pM or lower, or binds human PD-L1 and/or PD-L2 with a K_D of about 30 pM or lower,

[0027] In some embodiments, the PD-L1 and/or PD-L2 inhibitor is one that binds to human PD-L1 and/or PD-L2 with a k_{assoc} of about 7.5×10^5 1/M·s or faster, binds to human PD-L1 and/or PD-L2 with a k_{assoc} of about 8×10^5 1/M·s or faster, binds to human PD-L1 and/or PD-L2 with a k_{assoc} of about 8.5×10^5 1/M·s or faster, binds to human PD-L1 and/or PD-L2 with a k_{assoc} of about 9×10^5 1/M·s or faster, binds to human PD-L1 and/or PD-L2 with a k_{assoc} of about 9.5×10^5 1/M·s and/or faster, or binds to human PD-L1 and/or PD-L2

with a k_{assoc} of about 1×10^6 1/M·s or faster.

[0028] In some embodiments, the PD-L1 and/or PD-L2 inhibitor is one that binds to human PD-L1 or PD-L2 with a k_{dissoc} of about 2×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.1×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.2×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.3×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.4×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.5×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.6×10^{-5} 1/s or slower, binds to human PD-L1 or PD-L2 with a k_{dissoc} of about 2.7×10^{-5} 1/s or slower, or binds to human PD-L1 or PD-L2 with a k_{dissoc} of about 3×10^{-5} 1/s or slower.

[0029] In some embodiments, the PD-L1 and/or PD-L2 inhibitor is one that blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 10 nM or lower; blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 9 nM or lower; blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 8 nM or lower; blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 7 nM or lower; blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 6 nM or lower; blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 5 nM or lower; blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 4 nM or lower; blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 3 nM or lower; blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 2 nM or lower; or blocks human PD-1, or blocks binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 1 nM or lower.

[0030] In an embodiment, the PD-L1 inhibitor is durvalumab, also known as MEDI4736 (which is commercially available from Medimmune, LLC, Gaithersburg, Maryland, a subsidiary of AstraZeneca plc.), or antigen-binding fragments, conjugates, or variants thereof. In an embodiment, the PD-L1 inhibitor is an antibody disclosed in U.S. Patent No. 8,779,108 or U.S. Patent Application Publication No. 2013/0034559, the disclosures of which are incorporated by reference herein. The clinical efficacy of durvalumab has been described in Page, *et al.*, *Ann. Rev. Med.*, **2014**, 65, 185-202; Brahmer, *et al.*, *J. Clin. Oncol.* **2014**, 32, 5s (supplement, abstract 8021); and McDermott, *et al.*, *Cancer Treatment Rev.*, **2014**, 40, 1056-64. The preparation and properties of durvalumab are described in U.S. Patent No. 8,779,108, the disclosure of which is incorporated by reference herein. The amino acid

sequences of durvalumab are set forth in Table 50. The durvalumab monoclonal antibody includes disulfide linkages at 22-96, 22"-96", 23'-89', 23'''-89''', 135'-195', 135'''-195''', 148-204, 148"-204", 215'-224, 215'''-224'', 230-230", 233-233", 265-325, 265"-325", 371-429, and 371"-429'; and N-glycosylation sites at Asn-301 and Asn-301".

[0031] In an embodiment, a PD-L1 inhibitor comprises a heavy chain given by SEQ ID NO:147 and a light chain given by SEQ ID NO:148. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains having the sequences shown in SEQ ID NO:147 and SEQ ID NO:148, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:147 and SEQ ID NO:148, respectively. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:147 and SEQ ID NO:148, respectively. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:147 and SEQ ID NO:148, respectively. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:147 and SEQ ID NO:148, respectively. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:147 and SEQ ID NO:148, respectively.

[0032] In an embodiment, the PD-L1 inhibitor comprises the heavy and light chain CDRs or variable regions (VRs) of durvalumab. In an embodiment, the PD-L1 inhibitor heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:149, and the PD-L1 inhibitor light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:150, and conservative amino acid substitutions thereof. In an embodiment, a PD-L1 inhibitor comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:149 and SEQ ID NO:150, respectively. In an embodiment, a PD-L1 inhibitor comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:149 and SEQ ID NO:150, respectively. In an embodiment, a PD-L1 inhibitor comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:149 and SEQ ID NO:150, respectively. In an embodiment, a PD-L1 inhibitor comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:149 and SEQ ID NO:150, respectively. In an embodiment, a PD-L1 inhibitor comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in

SEQ ID NO:149 and SEQ ID NO:150, respectively.

[0033] In an embodiment, a PD-L1 inhibitor comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:151, SEQ ID NO:152, and SEQ ID NO:153, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:154, SEQ ID NO:155, and SEQ ID NO:156, respectively, and conservative amino acid substitutions thereof. In an embodiment, the antibody competes for binding with, and/or binds to the same epitope on PD-L1 as any of the aforementioned antibodies.

[0034] In an embodiment, the PD-L1 inhibitor is an anti-PD-L1 biosimilar monoclonal antibody approved by drug regulatory authorities with reference to durvalumab. In an embodiment, the biosimilar comprises an anti-PD-L1 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is durvalumab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is an anti-PD-L1 antibody authorized or submitted for authorization, wherein the anti-PD-L1 antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is durvalumab. The anti-PD-L1 antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is durvalumab. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is durvalumab.

TABLE 21. Amino acid sequences for PD-L1 inhibitors related to durvalumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)						
SEQ ID NO:147 durvalumab heavy chain	EVQLVESGGG	LVQPGGSLRL	SCAASGFTFS	RYWMSWVRQA	PGKGLEWVAN	IKQDGSEKYY	60
	VDSVKGRFTI	SRDNAKNSLY	LQMNSLRAED	TAVYYCAREG	GWFGELAFDY	WGQGTILVTVS	120
	SASTKGPSVF	PLAPSSKSTS	GGTAALGCLV	KDYFPEPVTV	SWNSGALTSG	VHTFPAVLQS	180
	SGLYSLSSVV	TVPSSSLGTQ	TYICNVNHKP	SNTKVDKRVE	PKSCDKTHTC	PPCPAPEFEG	240
	GPSVFLFPPK	PKDTLMISRT	PEVTCVVVDV	SHEDPEVKFN	WYVDGVEVHN	AKTKPREEQY	300
	NSTYRVVSVL	TVLHQDWLNG	KEYKCKVSNK	ALPASIEKTI	SKAKGQPREP	QVYTLPPSRE	360
	EMTKNQVSLT	CLVKGFYPSD	IAVEWESNGQ	PENNYKTPPP	VLDSDGSFFL	YSKLTVDKSR	420
	WQQGNVFSCS	VMHEALHNHY	TQKSLSLSPG	K			451
SEQ ID NO:148 durvalumab light chain	EVQLVESGGG	LVQPGGSLRL	SCAASGFTFS	RYWMSWVRQA	PGKGLEWVAN	EIVLTQSPGT	60
	LSLSPGERAT	LSCRASQRVS	SSYLAWYQQK	PGQAPRLLIY	DASSRATGIP	DRFSGSGSGT	120
	DFTLTISRLE	PEDFAVYYCQ	QYGSLPWTFG	QGTKVEIKRT	VAAPSVFIFP	PSDEQLKSGT	180
	ASVVCLLNNE	YPREAKVQWK	VDNALQSGNS	QESVTEQDSK	DSTYLSSTSL	TLSKADYEKH	240
	KVYACEVTHQ	GLSSPVTKSF	NRGEC				265
SEQ ID NO:149 durvalumab variable heavy chain	EVQLVESGGG	LVQPGGSLRL	SCAASGFTFS	RYWMSWVRQA	PGKGLEWVAN	IKQDGSEKYY	60
	VDSVKGRFTI	SRDNAKNSLY	LQMNSLRAED	TAVYYCAREG	GWFGELAFDY	WGQGTILVTVS	120
	S						121
SEQ ID NO:150 durvalumab variable light chain	EIVLTQSPGT	LSLSPGERAT	LSCRASQRVS	SSYLAWYQQK	PGQAPRLLIY	DASSRATGIP	60
	DRFSGSGSGT	DFTLTISRLE	PEDFAVYYCQ	QYGSLPWTFG	QGTKVEIK		108
SEQ ID NO:151 durvalumab heavy chain CDR1	RYWMS						5
SEQ ID NO:152 durvalumab heavy chain CDR2	NIKQDGSEKY	YVDSVKG					17
SEQ ID NO:153 durvalumab heavy chain CDR3	EGGWFGELAF	DY					12
SEQ ID NO:154 durvalumab light chain CDR1	RASQRVSSSY	LA					12
SEQ ID NO:155 durvalumab light chain CDR2	DASSRAT						7
SEQ ID NO:156 durvalumab light chain CDR3	QQYGSLPWT						9

[0035] In an embodiment, the PD-L1 inhibitor is avelumab, also known as MSB0010718C (commercially available from Merck KGaA/EMD Serono), or antigen-binding fragments, conjugates, or variants thereof. The preparation and properties of avelumab are described in U.S. Patent Application Publication No. US 2014/0341917 A1, the disclosure of which is specifically incorporated by reference herein. The amino acid sequences of avelumab are set forth in Table 51. Avelumab has intra-heavy chain disulfide linkages (C23-C104) at 22-96, 147-203, 264-324, 370-428, 22"-96", 147"-203", 264"-324", and 370"-428"; intra-light chain disulfide linkages (C23-C104) at 22'-90', 138'-197', 22'''-90''', and 138'''-197'''; intra-heavy-light chain disulfide linkages (h 5-CL 126) at 223-215' and 223"-215''; intra-heavy-heavy chain disulfide linkages (h 11, h 14) at 229-229" and 232-232"; N-

glycosylation sites (H CH₂ N84.4) at 300, 300"; fucosylated complex bi-antennary CHO-type glycans; and H CHS K2 C-terminal lysine clipping at 450 and 450'.

[0036] In an embodiment, a PD-L1 inhibitor comprises a heavy chain given by SEQ ID NO:157 and a light chain given by SEQ ID NO:158. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains having the sequences shown in SEQ ID NO:157 and SEQ ID NO:158, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:157 and SEQ ID NO:158, respectively. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:157 and SEQ ID NO:158, respectively. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:157 and SEQ ID NO:158, respectively. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:157 and SEQ ID NO:158, respectively. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:157 and SEQ ID NO:158, respectively.

[0037] In an embodiment, the PD-L1 inhibitor comprises the heavy and light chain CDRs or variable regions (VRs) of avelumab. In an embodiment, the PD-L1 inhibitor heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:159, and the PD-L1 inhibitor light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:160, and conservative amino acid substitutions thereof. In an embodiment, a PD-L1 inhibitor comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:159 and SEQ ID NO:160, respectively. In an embodiment, a PD-L1 inhibitor comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:159 and SEQ ID NO:160, respectively. In an embodiment, a PD-L1 inhibitor comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:159 and SEQ ID NO:160, respectively. In an embodiment, a PD-L1 inhibitor comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:159 and SEQ ID NO:160, respectively. In an embodiment, a PD-L1 inhibitor comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:159 and SEQ ID NO:160, respectively.

[0038] In an embodiment, a PD-L1 inhibitor comprises heavy chain CDR1, CDR2 and

CDR3 domains having the sequences set forth in SEQ ID NO:161, SEQ ID NO:162, and SEQ ID NO:163, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:164, SEQ ID NO:165, and SEQ ID NO:166, respectively, and conservative amino acid substitutions thereof. In an embodiment, the antibody competes for binding with, and/or binds to the same epitope on PD-L1 as any of the aforementioned antibodies.

[0039] In an embodiment, the PD-L1 inhibitor is an anti-PD-L1 biosimilar monoclonal antibody approved by drug regulatory authorities with reference to avelumab. In an embodiment, the biosimilar comprises an anti-PD-L1 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is avelumab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is an anti-PD-L1 antibody authorized or submitted for authorization, wherein the anti-PD-L1 antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is avelumab. The anti-PD-L1 antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is avelumab. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is avelumab.

TABLE 22. Amino acid sequences for PD-L1 inhibitors related to avelumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:157 avelumab	EVQLLES GGG LVQPGGSLRL SCAASGFTFS SYIMMWVRQA PGKGLEWVSS IYPSGGITFY 60 ADTVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARIK LGTVTTVDYW GQGTLVTVSS 120

Identifier	Sequence (One-Letter Amino Acid Symbols)
heavy chain	ASTKGPSVFPLAPSSKSTSGGTAALGLCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCTPCPAPPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVVFSCSV MHEALHNHYTQKSLSLSPGK 180 240 300 360 420 450
SEQ ID NO:158 avelumab light chain	QSALTQPASVSGSPGQSITI SCTGTSSDVG GYNYVSWYQQ HPGKAPKLMI YDVSNRPSGV SNRFGSGSKGNTASLTISGLQAEDADYYC SSYTSSSTRV FGTGTVKTVL GQPKANPTVT LFPPSSEELQANKATLVCLISDFYPGAVTV AWKADGSPVK AGVETTKPSK QSNKNYAASS YLSLTPEQWKSHRSYSCQVT HEGSTVEKTVAPTECS 60 120 180 216
SEQ ID NO:159 avelumab variable heavy chain	EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYIMMWVRQA PGKGLEWVSS IYPSGGITFY ADTVKGRFTISRDN SKNTLY LQMNSLRAEDTAVYYCARIK LGTVTTVDYW GQGTLVTVSS 60 120
SEQ ID NO:160 avelumab variable light chain	QSALTQPASVSGSPGQSITI SCTGTSSDVG GYNYVSWYQQ HPGKAPKLMI YDVSNRPSGV SNRFGSGSKGNTASLTISGLQAEDADYYC SSYTSSSTRV FGTGTVKTVL 60 110
SEQ ID NO:161 avelumab heavy chain CDR1	SYIMM 5
SEQ ID NO:162 avelumab heavy chain CDR2	SIYPSGGITFYADTVKG 17
SEQ ID NO:163 avelumab heavy chain CDR3	IKLGTVTTVDDY 11
SEQ ID NO:164 avelumab light chain CDR1	TGTSSDVGGYNYVS 14
SEQ ID NO:165 avelumab light chain CDR2	DVSNRPS 7
SEQ ID NO:166 avelumab light chain CDR3	SSYTSSSTRV 10

[0040] In an embodiment, the PD-L1 inhibitor is atezolizumab, also known as MPDL3280A or RG7446 (commercially available as TECENTRIQ from Genentech, Inc., a subsidiary of Roche Holding AG, Basel, Switzerland), or antigen-binding fragments, conjugates, or variants thereof. In an embodiment, the PD-L1 inhibitor is an antibody disclosed in U.S. Patent No. 8,217,149, the disclosure of which is specifically incorporated by reference herein. In an embodiment, the PD-L1 inhibitor is an antibody disclosed in U.S. Patent Application Publication Nos. 2010/0203056 A1, 2013/0045200 A1, 2013/0045201 A1, 2013/0045202 A1, or 2014/0065135 A1, the disclosures of which are specifically incorporated by reference herein. The preparation and properties of atezolizumab are described in U.S. Patent No. 8,217,149, the disclosure of which is incorporated by reference herein. The amino acid sequences of atezolizumab are set forth in Table 52. Atezolizumab has intra-heavy chain disulfide linkages (C23-C104) at 22-96, 145-201, 262-322, 368-426,

22"-96", 145"-201", 262"-322", and 368"-426"; intra-light chain disulfide linkages (C23-C104) at 23'-88', 134'-194', 23'''-88''', and 134'''-194'''; intra-heavy-light chain disulfide linkages (h 5-CL 126) at 221-214' and 221"-214'''; intra-heavy-heavy chain disulfide linkages (h 11, h 14) at 227-227" and 230-230"; and N-glycosylation sites (H CH₂ N84.4>A) at 298 and 298'.

[0041] In an embodiment, a PD-L1 inhibitor comprises a heavy chain given by SEQ ID NO:167 and a light chain given by SEQ ID NO:168. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains having the sequences shown in SEQ ID NO:167 and SEQ ID NO:168, respectively, or antigen binding fragments, Fab fragments, single-chain variable fragments (scFv), variants, or conjugates thereof. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains that are each at least 99% identical to the sequences shown in SEQ ID NO:167 and SEQ ID NO:168, respectively. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains that are each at least 98% identical to the sequences shown in SEQ ID NO:167 and SEQ ID NO:168, respectively. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains that are each at least 97% identical to the sequences shown in SEQ ID NO:167 and SEQ ID NO:168, respectively. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains that are each at least 96% identical to the sequences shown in SEQ ID NO:167 and SEQ ID NO:168, respectively. In an embodiment, a PD-L1 inhibitor comprises heavy and light chains that are each at least 95% identical to the sequences shown in SEQ ID NO:167 and SEQ ID NO:168, respectively.

[0042] In an embodiment, the PD-L1 inhibitor comprises the heavy and light chain CDRs or variable regions (VRs) of atezolizumab. In an embodiment, the PD-L1 inhibitor heavy chain variable region (V_H) comprises the sequence shown in SEQ ID NO:169, and the PD-L1 inhibitor light chain variable region (V_L) comprises the sequence shown in SEQ ID NO:170, and conservative amino acid substitutions thereof. In an embodiment, a PD-L1 inhibitor comprises V_H and V_L regions that are each at least 99% identical to the sequences shown in SEQ ID NO:169 and SEQ ID NO:170, respectively. In an embodiment, a PD-L1 inhibitor comprises V_H and V_L regions that are each at least 98% identical to the sequences shown in SEQ ID NO:169 and SEQ ID NO:170, respectively. In an embodiment, a PD-L1 inhibitor comprises V_H and V_L regions that are each at least 97% identical to the sequences shown in SEQ ID NO:169 and SEQ ID NO:170, respectively. In an embodiment, a PD-L1 inhibitor comprises V_H and V_L regions that are each at least 96% identical to the sequences shown in SEQ ID NO:169 and SEQ ID NO:170, respectively. In an embodiment, a PD-L1 inhibitor

comprises V_H and V_L regions that are each at least 95% identical to the sequences shown in SEQ ID NO:169 and SEQ ID NO:170, respectively.

[0043] In an embodiment, a PD-L1 inhibitor comprises heavy chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:171, SEQ ID NO:172, and SEQ ID NO:173, respectively, and conservative amino acid substitutions thereof, and light chain CDR1, CDR2 and CDR3 domains having the sequences set forth in SEQ ID NO:174, SEQ ID NO:175, and SEQ ID NO:176, respectively, and conservative amino acid substitutions thereof. In an embodiment, the antibody competes for binding with, and/or binds to the same epitope on PD-L1 as any of the aforementioned antibodies.

[0044] In an embodiment, the anti-PD-L1 antibody is an anti-PD-L1 biosimilar monoclonal antibody approved by drug regulatory authorities with reference to atezolizumab. In an embodiment, the biosimilar comprises an anti-PD-L1 antibody comprising an amino acid sequence which has at least 97% sequence identity, *e.g.*, 97%, 98%, 99% or 100% sequence identity, to the amino acid sequence of a reference medicinal product or reference biological product and which comprises one or more post-translational modifications as compared to the reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is atezolizumab. In some embodiments, the one or more post-translational modifications are selected from one or more of: glycosylation, oxidation, deamidation, and truncation. In some embodiments, the biosimilar is an anti-PD-L1 antibody authorized or submitted for authorization, wherein the anti-PD-L1 antibody is provided in a formulation which differs from the formulations of a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is atezolizumab. The anti-PD-L1 antibody may be authorized by a drug regulatory authority such as the U.S. FDA and/or the European Union's EMA. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is atezolizumab. In some embodiments, the biosimilar is provided as a composition which further comprises one or more excipients, wherein the one or more excipients are the same or different to the excipients comprised in a reference medicinal product or reference biological product, wherein the reference medicinal product or reference biological product is atezolizumab.

TABLE 23. Amino acid sequences for PD-L1 inhibitors related to atezolizumab.

Identifier	Sequence (One-Letter Amino Acid Symbols)
SEQ ID NO:167 atezolizumab heavy chain	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DSWIHWRQA PGKGLEWVAV ISPYGGSTYY 60 ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARRH WPGGFDYWGQ GTLTVVSSAS 120 TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL 180 YSLSSVTVTP SSSLGTQTYI CNVNHKPSNT KVDKKVEPKS CDKTHTCPPC PAPELLGGPS 240 VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYAST 300 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSREEMT 360 KNQVSLTCLV KGFYPSTIAV EWESNGQFEN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ 420 GNVFSCSVMH EALHNHYTQK SLSLSPGK 448
SEQ ID NO:168 atezolizumab light chain	DIQMTQSPSS LSASVGDRVT ITCRASQDVS TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS 60 RFGSGSGSTD FTLTISSLQP EDFATYYCQQ YLYHPATFGQ GTKVEIKRTV AAPSVFIFPP 120 SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYLSLSTLT 180 LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEK 214
SEQ ID NO:169 atezolizumab variable heavy chain	EVQLVESGGG LVQPGGSLRL SCAASGFTFS DSWIHWRQA PGKGLEWVAV ISPYGGSTYY 60 ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARRH WPGGFDYWGQ GTLTVVSA 118
SEQ ID NO:170 atezolizumab variable light chain	DIQMTQSPSS LSASVGDRVT ITCRASQDVS TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS 60 RFGSGSGSTD FTLTISSLQP EDFATYYCQQ YLYHPATFGQ GTKVEIKR 108
SEQ ID NO:171 atezolizumab heavy chain CDR1	GFTFSDSWIH 10
SEQ ID NO:172 atezolizumab heavy chain CDR2	AWISPYGGST YYADSVKG 18
SEQ ID NO:173 atezolizumab heavy chain CDR3	RHWPGGFDY 9
SEQ ID NO:174 atezolizumab light chain CDR1	RASQDVSTAV A 11
SEQ ID NO:175 atezolizumab light chain CDR2	SASFLYS 7
SEQ ID NO:176 atezolizumab light chain CDR3	QQYLYHPAT 9

[0045] In an embodiment, PD-L1 inhibitors include those antibodies described in U.S. Patent Application Publication No. US 2014/0341917 A1, the disclosure of which is incorporated by reference herein. In another embodiment, antibodies that compete with any of these antibodies for binding to PD-L1 are also included. In an embodiment, the anti-PD-L1 antibody is MDX-1105, also known as BMS-935559, which is disclosed in U.S. Patent No. US 7,943,743, the disclosures of which are incorporated by reference herein. In an embodiment, the anti-PD-L1 antibody is selected from the anti-PD-L1 antibodies disclosed in U.S. Patent No. US 7,943,743, which are incorporated by reference herein.

[0046] In an embodiment, the PD-L1 inhibitor is a commercially-available monoclonal antibody, such as INVIVOMAB anti-m-PD-L1 clone 10F.9G2 (Catalog # BE0101, Bio X

Cell, Inc., West Lebanon, NH, USA). In an embodiment, the anti-PD-L1 antibody is a commercially-available monoclonal antibody, such as AFFYMETRIX EBIOSCIENCE (MIH1). A number of commercially-available anti-PD-L1 antibodies are known to one of ordinary skill in the art.

[0047] In an embodiment, the PD-L2 inhibitor is a commercially-available monoclonal antibody, such as BIOLEGEND 24F.10C12 Mouse IgG2a, κ isotype (catalog # 329602 Biologend, Inc., San Diego, CA), SIGMA anti-PD-L2 antibody (catalog # SAB3500395, Sigma-Aldrich Co., St. Louis, MO), or other commercially-available anti-PD-L2 antibodies known to one of ordinary skill in the art.

2. Optional Lymphodepletion Preconditioning of Patients

[00607] In an embodiment, the invention includes a method of treating a cancer with a population of TILs, wherein a patient is pre-treated with non-myeloablative chemotherapy prior to an infusion of TILs according to the present disclosure. In an embodiment, the invention includes a population of TILs for use in the treatment of cancer in a patient which has been pre-treated with non-myeloablative chemotherapy. In an embodiment, the population of TILs is for administration by infusion. In an embodiment, 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 an embodiment, after non-myeloablative chemotherapy and TIL infusion (at day 0) according to the present disclosure, the patient receives an intravenous infusion of IL-2 (aldesleukin, commercially available as PROLEUKIN) intravenously at 720,000 IU/kg every 8 hours to physiologic tolerance. In certain embodiments, the population of TILs is for use in treating cancer in combination with IL-2, wherein the IL-2 is administered after the population of TILs.

[00608] 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.

[00609] In general, lymphodepletion is achieved using administration of fludarabine or cyclophosphamide (the active form being referred to as mafosfamide) and combinations thereof. Such methods are described in Gassner, *et al.*, *Cancer Immunol. Immunother.* **2011**, 60, 75–85, Muranski, *et al.*, *Nat. Clin. Pract. Oncol.*, **2006**, 3, 668–681, Dudley, *et al.*, *J. Clin. Oncol.* **2008**, 26, 5233–5239, and Dudley, *et al.*, *J. Clin. Oncol.* **2005**, 23, 2346–2357, all of which are incorporated by reference herein in their entireties.

[00610] In some embodiments, the fludarabine is administered at a concentration of 0.5 µg/mL -10 µg/mL fludarabine. In some embodiments, the fludarabine is administered at a concentration of 1 µg/mL fludarabine. In some embodiments, the fludarabine treatment is administered for 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days or more. In some embodiments, the fludarabine is administered at a dosage of 10 mg/kg/day, 15 mg/kg/day, 20 mg/kg/day, 25 mg/kg/day, 30 mg/kg/day, 35 mg/kg/day, 40 mg/kg/day, or 45 mg/kg/day. In some embodiments, the fludarabine treatment is administered for 2-7 days at 35 mg/kg/day. In some embodiments, the fludarabine treatment is administered for 4-5 days at 35 mg/kg/day. In some embodiments, the fludarabine treatment is administered for 4-5 days at 25 mg/kg/day.

[00611] In some embodiments, the mafosfamide, the active form of cyclophosphamide, is obtained at a concentration of 0.5 µg/mL -10 µg/mL by administration of cyclophosphamide. In some embodiments, mafosfamide, the active form of cyclophosphamide, is obtained at a concentration of 1 µg/mL by administration of cyclophosphamide. In some embodiments, the cyclophosphamide treatment is administered for 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days or more. In some embodiments, the cyclophosphamide is administered at a dosage of 100 mg/m²/day, 150 mg/m²/day, 175 mg/m²/day, 200 mg/m²/day, 225 mg/m²/day, 250 mg/m²/day, 275 mg/m²/day, or 300 mg/m²/day. In some embodiments, the cyclophosphamide is administered intravenously (*i.e.*, i.v.) In some embodiments, the cyclophosphamide treatment is administered for 2-7 days at 35 mg/kg/day. In some embodiments, the cyclophosphamide treatment is administered for 4-5 days at 250 mg/m²/day i.v. In some embodiments, the cyclophosphamide treatment is administered for 4 days at 250 mg/m²/day i.v.

[00612] In some embodiments, lymphodepletion is performed by administering the fludarabine and the cyclophosphamide together to a patient. In some embodiments, fludarabine is administered at 25 mg/m²/day i.v. and cyclophosphamide is administered at 250 mg/m²/day i.v. over 4 days.

[00613] In an embodiment, the lymphodepletion is performed by administration of cyclophosphamide at a dose of 60 mg/m²/day for two days followed by administration of fludarabine at a dose of 25 mg/m²/day for five days.

3. IL-2 Regimens

[00614] In an embodiment, the IL-2 regimen comprises a high-dose IL-2 regimen, wherein the high-dose IL-2 regimen comprises aldesleukin, or a biosimilar or variant thereof, administered intravenously starting on the day after administering a therapeutically effective portion of therapeutic population of TILs, wherein the aldesleukin or a biosimilar or variant thereof is administered at a dose of 0.037 mg/kg or 0.044 mg/kg IU/kg (patient body mass) using 15-minute bolus intravenous infusions every eight hours until tolerance, for a maximum of 14 doses. Following 9 days of rest, this schedule may be repeated for another 14 doses, for a maximum of 28 doses in total. In some embodiments, IL-2 is administered in 1, 2, 3, 4, 5, or 6 doses. In some embodiments, IL-2 is administered at a maximum dosage of up to 6 doses.

[00615] In an embodiment, the IL-2 regimen comprises a decrescendo IL-2 regimen. Decrescendo IL-2 regimens have been described in O'Day, *et al.*, *J. Clin. Oncol.* **1999**, *17*, 2752-61 and Eton, *et al.*, *Cancer* **2000**, *88*, 1703-9, the disclosures of which are incorporated herein by reference. In an embodiment, a decrescendo IL-2 regimen comprises 18×10^6 IU/m² administered intravenously over 6 hours, followed by 18×10^6 IU/m² administered intravenously over 12 hours, followed by 18×10^6 IU/m² administered intravenously over 24 hours, followed by 4.5×10^6 IU/m² administered intravenously over 72 hours. This treatment cycle may be repeated every 28 days for a maximum of four cycles. In an embodiment, a decrescendo IL-2 regimen comprises 18,000,000 IU/m² on day 1, 9,000,000 IU/m² on day 2, and 4,500,000 IU/m² on days 3 and 4.

[00616] In an embodiment, the IL-2 regimen comprises administration of pegylated IL-2 every 1, 2, 4, 6, 7, 14 or 21 days at a dose of 0.10 mg/day to 50 mg/day.

EXAMPLES

[00617] The embodiments encompassed herein are now described with reference to the following examples. These examples are provided for the purpose of illustration only and the disclosure encompassed herein should in no way be construed as being limited to these

examples, but rather should be construed to encompass any and all variations which become evident as a result of the teachings provided herein.

EXAMPLE 1: PREPARATION OF MEDIA FOR PRE-REP AND REP PROCESSES

[00618] This Example describes the procedure for the preparation of tissue culture media for use in protocols involving the culture of tumor infiltrating lymphocytes (TIL) derived from various tumor types including non-small cell lung carcinoma (NSCLC). This media can be used for preparation of any of the TILs described in the present application and Examples.

Preparation of CM1

[00619] Removed the following reagents from cold storage and warmed them in a 37°C water bath: (RPMI1640, Human AB serum, 200mM L-glutamine). Prepared CM1 medium according to Table 18 below by adding each of the ingredients into the top section of a 0.2 µm filter unit appropriate to the volume to be filtered. Store at 4°C.

TABLE 24: Preparation of CM1

Ingredient	Final concentration	Final Volume 500 ml	Final Volume 1L
RPMI1640	NA	450 ml	900 ml
Human AB serum, heat-inactivated 10%	50 ml	100 ml	
200mM L-glutamine	2 mM	5 ml	10 ml
55mM BME	55 µM	0.5 ml	1 ml
50mg/ml gentamicin sulfate	50 µg/ml	0.5 ml	1 ml

[00620] On the day of use, prewarmed required amount of CM1 in 37°C water bath and add 6000 IU/ml IL-2.

[00621] Additional supplementation - as needed according to Table 25.

TABLE 25: Additional supplementation of CM1, as needed.

Supplement	Stock concentration	Dilution	Final concentration
GlutaMAX™	200mM	1:100	2mM

Penicillin/streptomycin	10,000 U/ml penicillin 10,000 µg/ml streptomycin	1:100	100 U/ml penicillin 100 µg/ml streptomycin
Amphotericin B	250 µg/ml	1:100	2.5 µg/ml

Preparation of CM2

[00622] Removed prepared CM1 from refrigerator or prepare fresh CM1 as per Section 7.3 above. Removed AIM-V® from refrigerator and prepared the amount of CM2 needed by mixing prepared CM1 with an equal volume of AIM-V® in a sterile media bottle. Added 3000 IU/ml IL-2 to CM2 medium on the day of usage. Made sufficient amount of CM2 with 3000 IU/ml IL-2 on the day of usage. Labeled the CM2 media bottle with its name, the initials of the preparer, the date it was filtered/prepared, the two-week expiration date and store at 4°C until needed for tissue culture.

Preparation of CM3

[00623] Prepared CM3 on the day it was required for use. CM3 was the same as AIM-V® medium, supplemented with 3000 IU/ml IL-2 on the day of use. Prepared an amount of CM3 sufficient to experimental needs by adding IL-2 stock solution directly to the bottle or bag of AIM-V. Mixed well by gentle shaking. Label bottle with "3000 IU/ml IL-2" immediately after adding to the AIM-V. If there was excess CM3, stored it in bottles at 4°C labeled with the media name, the initials of the preparer, the date the media was prepared, and its expiration date (7 days after preparation). Discarded media supplemented with IL-2 after 7 days storage at 4°C.

Preparation of CM4

[00624] CM4 was the same as CM3, with the additional supplement of 2mM GlutaMAX™ (final concentration). For every 1L of CM3, added 10ml of 200mM GlutaMAX™. Prepared an amount of CM4 sufficient to experimental needs by adding IL-2 stock solution and GlutaMAX™ stock solution directly to the bottle or bag of AIM-V. Mixed well by gentle shaking. Labeled bottle with "3000 IL-nil IL-2 and GlutaMAX" immediately after adding to the AIM-V. If there was excess CM4, stored it in bottles at 4°C labeled with the media name, "GlutaMAX", and its expiration date (7 days after preparation). Discarded media supplemented with IL-2 after 7-days storage at 4°C.

EXAMPLE 2: USE OF IL-2, IL-15, AND IL-21 CYTOKINE COCKTAIL

[00625] This example describes the use of IL-2, IL-15, and IL-21 cytokines, which serve as additional T cell growth factors, in combination with the TIL process of Examples A to G.

[00626] Using the processes described herein, TILs can be grown from non-small cell lung carcinoma (NSCLC) tumors in presence of IL-2 in one arm of the experiment and, in place of IL-2, a combination of IL-2, IL-15, and IL-21 in another arm at the initiation of culture. At the completion of the pre-REP, cultures were assessed for expansion, phenotype, function (CD107a⁺ and IFN- γ) and TCR V β repertoire. IL-15 and IL-21 are described elsewhere herein and in Grujil, *et al.*, IL-21 promotes the expansion of CD27⁺CD28⁺ tumor infiltrating lymphocytes with high cytotoxic potential and low collateral expansion of regulatory T cells, *Santegoets, S. J.*, J Transl Med., **2013**, 11:37 (located on the World Wide Web at ncbi.nlm.nih.gov/pmc/articles/PMC3626797/).

[00627] The results can show that enhanced TIL expansion (>20%), in both CD4⁺ and CD8⁺ cells in the IL-2, IL-15, and IL-21 treated conditions can observed relative to the IL-2 only conditions. There was a skewing towards a predominantly CD8⁺ population with a skewed TCR V β repertoire in the TILs obtained from the IL-2, IL-15, and IL-21 treated cultures relative to the IL-2 only cultures. IFN- γ and CD107a were elevated in the IL-2, IL-15, and IL-21 treated TILs, in comparison to TILs treated only IL-2.

EXAMPLE 3: QUALIFYING INDIVIDUAL LOTS OF GAMMA-IRRADIATED PERIPHERAL MONONUCLEAR CELLS

[00628] This Example describes a novel abbreviated procedure for qualifying individual lots of gamma-irradiated peripheral mononuclear cells (PBMCs, also known as MNC) for use as allogeneic feeder cells in the exemplary methods described herein.

[00629] Each irradiated MNC feeder lot was prepared from an individual donor. Each lot or donor was screened individually for its ability to expand TIL in the REP in the presence of purified anti-CD3 (clone OKT3) antibody and interleukin-2 (IL-2). In addition, each lot of feeder cells was tested without the addition of TIL to verify that the received dose of gamma radiation was sufficient to render them replication incompetent.

Background

[00630] Gamma-irradiated, growth-arrested MNC feeder cells were required for REP of TIL. Membrane receptors on the feeder MNCs bind to anti-CD3 (clone OKT3) antibody and crosslink to TIL in the REP flask, stimulating the TIL to expand. Feeder lots were prepared from the leukapheresis of whole blood taken from individual donors. The leukapheresis product was subjected to centrifugation over Ficoll-Hypaque, washed, irradiated, and cryopreserved under GMP conditions.

[00631] It is important that patients who received TIL therapy not be infused with viable feeder cells as this can result in Graft-Versus-Host Disease (GVHD). Feeder cells are therefore growth-arrested by dosing the cells with gamma-irradiation, resulting in double strand DNA breaks and the loss of cell viability of the MNC cells upon reculture.

Evaluation Criteria and Experimental Set-Up

[00632] Feeder lots were evaluated on two criteria: 1) their ability to expand TIL in co-culture >100-fold and 2) their replication incompetency.

[00633] Feeder lots were tested in mini-REP format utilizing two primary pre-REP TIL lines grown in upright T25 tissue culture flasks. Feeder lots were tested against two distinct TIL lines, as each TIL line is unique in its ability to proliferate in response to activation in a REP. As a control, a lot of irradiated MNC feeder cells which has historically been shown to meet the criteria above was run alongside the test lots.

[00634] To ensure that all lots tested in a single experiment receive equivalent testing, sufficient stocks of the same pre-REP TIL lines were available to test all conditions and all feeder lots.

[00635] For each lot of feeder cells tested, there was a total of six T25 flasks: Pre-REP TIL line #1 (2 flasks); Pre-REP TIL line #2 (2 flasks); and Feeder control (2 flasks). Flasks containing TIL lines #1 and #2 evaluated the ability of the feeder lot to expand TIL. The feeder control flasks evaluated the replication incompetence of the feeder lot.

EXPERIMENTAL PROTOCOL

Day -2/3, Thaw of TIL lines

Prepared CM2 medium. Warmed CM2 in 37°C water bath. Prepared 40 ml of CM2 supplemented with 3000IU/ml IL-2. Keep warm until use. Placed 20 ml of pre-warmed CM2 without IL-2 into each of two 50ml conical tubes labeled with names of the TIL lines used.

Removed the two designated pre-REP TIL lines from LN2 storage and transferred the vials to the tissue culture room. Thawed vials by placing them inside a sealed zipper storage bag in a 37°C water bath until a small amount of ice remains.

[00636] Using a sterile transfer pipet, immediately transferred the contents of vial into the 20ml of CM2 in the prepared, labeled 50ml conical tube. QS to 40ml using CM2 without IL-2 to wash cells. Centrifuged at 400 x CF for 5 minutes. Aspirated the supernatant and resuspend in 5ml warm CM2 supplemented with 3000 IU/ml IL-2.

[00637] Removed small aliquot (20µl) in duplicate for cell counting using an automated cell counter. Record the counts. While counting, placed the 50ml conical tube with TIL cells into a humidified 37°C, 5% CO₂ incubator, with the cap loosened to allow for gas exchange. Determined cell concentration and diluted TIL to 1×10^6 cells/ml in CM2 supplemented with IL-2 at 3000 IU/ml.

[00638] Cultured in 2ml/well of a 24-well tissue culture plate in as many wells as needed in a humidified 37°C incubator until Day 0 of the mini-REP. Cultured the different TIL lines in separate 24-well tissue culture plates to avoid confusion and potential cross-contamination.

Day 0, initiate Mini-REP

[00639] Prepared enough CM2 medium for the number of feeder lots to be tested. (e.g., for testing 4 feeder lots at one time, prepared 800ml of CM2 medium). Aliquoted a portion of the CM2 prepared above and supplemented it with 3000 IU/ml IL-2 for the culturing of the cells. (e.g., for testing 4 feeder lots at one time, prepare 500ml of CM2 medium with 3000 IU/ml IL-2).

[00640] Working with each TIL line separately to prevent cross-contamination, removed the 24-well plate with TIL culture from the incubator and transferred to the BSC.

[00641] Using a sterile transfer pipet or 100-1000µl Pipettor and tip, removed about 1ml of medium from each well of TIL to be used and place in an unused well of the 24-well tissue culture plate.

[00642] Using a fresh sterile transfer pipet or 100-1000µl Pipettor and tip, mixed remaining medium with TIL in wells to resuspend the cells and then transferred the cell suspension to a 50ml conical tube labeled with the TIL name and recorded the volume.

[00643] Washed the wells with the reserved media and transferred that volume to the same 50ml conical tube. Spun the cells at 400 x CF to collect the cell pellet. Aspirated off the

media supernatant and resuspend the cell pellet in 2-5ml of CM2 medium containing 3000 IU/ml IL-2, volume to be used based on the number of wells harvested and the size of the pellet – volume should be sufficient to ensure a concentration of $>1.3 \times 10^6$ cells/ml.

[00644] Using a serological pipet, mixed the cell suspension thoroughly and recorded the volume. Removed 200 μ l for a cell count using an automated cell counter. While counting, placed the 50ml conical tube with TIL cells into a humidified, 5% CO₂, 37°C incubator, with the cap loosened to allow gas exchange. Recorded the counts.

[00645] Removed the 50ml conical tube containing the TIL cells from the incubator and resuspend them cells at a concentration of 1.3×10^6 cells/ml in warm CM2 supplemented with 3000IU/ml IL-2. Returned the 50ml conical tube to the incubator with a loosened cap.

[00646] Repeated steps above for the second TIL line.

[00647] Just prior to plating the TIL into the T25 flasks for the experiment, TIL were diluted 1:10 for a final concentration of 1.3×10^5 cells/ml as per below.

Prepare MACS GMP CD3 pure (OKT3) working solution

[00648] Took out stock solution of OKT3 (1mg/ml) from 4°C refrigerator and placed in BSC. A final concentration of 30ng/ml OKT3 was used in the media of the mini-REP.

[00649] 600ng of OKT3 were needed for 20ml in each T25 flask of the experiment; this was the equivalent of 60 μ l of a 10 μ g/ml solution for each 20ml, or 360 μ l for all 6 flasks tested for each feeder lot.

[00650] For each feeder lot tested, made 400 μ l of a 1:100 dilution of 1mg/ml OKT3 for a working concentration of 10 μ g/ml (e.g., for testing 4 feeder lots at one time, make 1600 μ l of a 1:100 dilution of 1mg/ml OKT3: 16 μ l of 1mg/ml OKT3 + 1.584ml of CM2 medium with 3000IU/ml IL-2.)

Prepare T25 flasks

[00651] Labeled each flask and filled flask with the CM2 medium prior to preparing the feeder cells. Placed flasks into 37°C humidified 5% CO₂ incubator to keep media warm while waiting to add the remaining components. Once feeder cells were prepared, the components will be added to the CM2 in each flask.

TABLE 26: Solutions

Component	Volume in co-culture flasks	Volume in control (feeder only) flasks
CM2 + 3000 IU/ml IL-2	18ml	18ml
MNC: 1.3×10^7 /ml in CM2 + 3000IU IL-2 (final concentration 1.3×10^7 /flask)	1ml	1ml
OKT3: 10 μ g/ml in CM2 + 3000IU IL-2	50 μ l	50 μ l
TIL: 1.3×10^7 /ml in CM2 with 3000IU of IL-2 (final concentration 1.3×10^7 /flask)	1ml	0

Prepare Feeder Cells

[00652] A minimum of 78×10^6 feeder cells were needed per lot tested for this protocol. Each 1ml vial frozen by SDBB had 100×10^6 viable cells upon freezing. Assuming a 50% recovery upon thaw from LN2 storage, it was recommended to thaw at least two 1ml vials of feeder cells per lot giving an estimated 100×10^6 viable cells for each REP. Alternately, if supplied in 1.8ml vials, only one vial provided enough feeder cells.

[00653] Before thawing feeder cells, pre-warmed approximately 50ml of CM2 without IL-2 for each feeder lot to be tested. Removed the designated feeder lot vials from LN2 storage, placed in zipper storage bag, and place on ice. Thawed vials inside closed zipper storage bag by immersing in a 37°C water bath. Removed vials from zipper bag, spray or wipe with 70% EtOH and transferred vials to BSC.

[00654] Using a transfer pipet immediately transferred the contents of feeder vials into 30ml of warm CM2 in a 50ml conical tube. Washed vial with a small volume of CM2 to remove any residual cells in the vial. Centrifuged at 400 x CF for 5 minutes. Aspirated the supernatant and resuspended in 4ml warm CM2 plus 3000 IU/ml IL-2. Removed 200 μ l for cell counting using the Automated Cell Counter. Recorded the counts.

[00655] Resuspended cells at 1.3×10^7 cells/ml in warm CM2 plus 3000 IU/ml IL-2. Diluted TIL cells from 1.3×10^6 cells/ml to 1.3×10^5 cells/ml.

Setup Co-Culture

[00656] Diluted TIL cells from 1.3×10^6 cells/ml to 1.3×10^5 cells/ml. Added 4.5ml of CM2 medium to a 15ml conical tube. Removed TIL cells from incubator and resuspended well using a 10ml serological pipet. Removed 0.5ml of cells from the 1.3×10^6 cells/ml TIL suspension and added to the 4.5ml of medium in the 15ml conical tube. Returned TIL stock vial to incubator. Mixed well. Repeated for the second TIL line.

Transferred flasks with pre-warmed media for a single feeder lot from the incubator to the BSC. Mixed feeder cells by pipetting up and down several times with a 1ml pipet tip and transferred 1 ml (1.3×10^7 cells) to each flask for that feeder lot. Added 60µl of OKT3 working stock (10µg/ml) to each flask. Returned the two control flasks to the incubator.

[00657] Transferred 1 ml (1.3×10^5) of each TIL lot to the correspondingly labeled T25 flask. Returned flasks to the incubator and incubate upright. Did not disturb until Day 5.

[00658] Repeated for all feeder lots tested.

Day 5, Media change

Prepared CM2 with 3000 IU/ml IL-2. 10ml is needed for each flask. With a 10ml pipette, transferred 10ml warm CM2 with 3000 IU/ml IL-2 to each flask. Returned flasks to the incubator and incubated upright until Day 7. Repeated for all feeder lots tested.

Day 7, Harvest

[00659] Removed flasks from the incubator and transfer to the BSC, care as taken not to disturb the cell layer on the bottom of the flask. Without disturbing the cells growing on the bottom of the flasks, removed 10ml of medium from each test flask and 15ml of medium from each of the control flasks.

[00660] Using a 10ml serological pipet, resuspended the cells in the remaining medium and mix well to break up any clumps of cells. After thoroughly mixing cell suspension by pipetting, removed 200µl for cell counting. Counted the TIL using the appropriate standard operating procedure in conjunction with the automatic cell counter equipment. Recorded counts in Day 7.

[00661] Repeated for all feeder lots tested.

[00662] Feeder control flasks were evaluated for replication incompetence and flasks containing TIL were evaluated for fold expansion from Day 0 according to Table TT below.

Day 7, Continuation of Feeder Control Flasks to Day 14

[00663] After completing the Day 7 counts of the feeder control flasks, added 15ml of fresh CM2 medium containing 3000 IU/ml IL-2 to each of the control flasks. Returned the control flasks to the incubator and incubated in an upright position until Day 14.

Day 14, Extended Non-proliferation of Feeder Control Flasks

[00664] Removed flasks from the incubator and transfer to the BSC, care was taken not to disturb the cell layer on the bottom of the flask. Without disturbing the cells growing on the bottom of the flasks, removed approximately 17ml of medium from each control flasks. Using a 5ml serological pipet, resuspended the cells in the remaining medium and mixed well to break up any clumps of cells. Recorded the volumes for each flask.

[00665] After thoroughly mixing cell suspension by pipetting, removed 200 μ l for cell counting. Counted the TIL using the appropriate standard operating procedure in conjunction with the automatic cell counter equipment. Recorded counts.

[00666] Repeated for all feeder lots tested.

RESULTS AND ACCEPTANCE CRITERIA

Results

[00667] The dose of gamma irradiation was sufficient to render the feeder cells replication incompetent. All lots were expected to meet the evaluation criteria and also demonstrated a reduction in the total viable number of feeder cells remaining on Day 7 of the REP culture compared to Day 0.

[00668] All feeder lots were expected to meet the evaluation criteria of 100-fold expansion of TIL growth by Day 7 of the REP culture.

[00669] Day 14 counts of Feeder Control flasks were expected to continue the non-proliferative trend seen on Day 7.

Acceptance Criteria

[00670] The following acceptance criteria were met for each replicate TIL line tested for each lot of feeder cells

[00671] Acceptance was two-fold, as follows (outlined in Table 27 below).

TABLE 27: Acceptance Criteria

Test	Acceptance criteria
Irradiation of MNC / Replication Incompetence	No growth observed at 7 and 14 days
TIL expansion	At least a 100-fold expansion of each TIL (minimum of 1.3×10^7 viable cells)

[00672] Evaluated whether the dose of radiation was sufficient to render the MNC feeder cells replication incompetent when cultured in the presence of 30ng/ml OKT3 antibody and

3000 IU/ml IL-2. Replication incompetence was evaluated by total viable cell count (TVC) as determined by automated cell counting on Day 7 and Day 14 of the REP.

[00673] Acceptance criteria was “No Growth,” meaning the total viable cell number has not increased on Day 7 and Day 14 from the initial viable cell number put into culture on Day 0 of the REP.

[00674] Evaluated the ability of the feeder cells to support TIL expansion. TIL growth was measured in terms of fold expansion of viable cells from the onset of culture on Day 0 of the REP to Day 7 of the REP. On Day 7, TIL cultures achieved a minimum of 100-fold expansion, (i.e., greater than 100 times the number of total viable TIL cells put into culture on REP Day 0), as evaluated by automated cell counting.

Contingency Testing of MNC Feeder Lots that do not meet acceptance criteria

[00675] In the event that an MNC feeder lot did not meet the either of the acceptance criteria outlined above, the following steps will be taken to retest the lot to rule out simple experimenter error as its cause.

[00676] If there are two or more remaining satellite testing vials of the lot, then the lot was retested. If there were one or no remaining satellite testing vials of the lot, then the lot was failed according to the acceptance criteria listed above.

[00677] In order to be qualified, the lot in question and the control lot had to achieve the acceptance criteria above. Upon meeting these criteria, the lot was then released for use.

EXAMPLE 4: QUALIFYING INDIVIDUAL LOTS OF GAMMA-IRRADIATED PERIPHERAL BLOOD MONONUCLEAR CELLS

[00678] This Example describes a novel abbreviated procedure for qualifying individual lots of gamma-irradiated peripheral blood mononuclear cells (PBMC) for use as allogeneic feeder cells in the exemplary methods described herein. This example provides a protocol for the evaluation of irradiated PBMC cell lots for use in the production of clinical lots of TIL. Each irradiated PBMC lot was prepared from an individual donor. Over the course of more than 100 qualification protocols, it was been shown that, in all cases, irradiated PBMC lots from SDBB (San Diego Blood Bank) expand TIL >100-fold on Day 7 of a REP. This modified qualification protocol was intended to apply to irradiated donor PBMC lots from SDBB which were then further tested to verify that the received dose of gamma radiation was

sufficient to render them replication incompetent. Once demonstrated that they maintained replication incompetence over the course of 14 days, donor PBMC lots were considered “qualified” for usage to produce clinical lots of TIL.

Background

[00679] Gamma-irradiated, growth-arrested PBMC were required for current standard REP of TIL. Membrane receptors on the PBMCs bind to anti-CD3 (clone OKT3) antibody and crosslink to TIL in culture, stimulating the TIL to expand. PBMC lots were prepared from the leukapheresis of whole blood taken from individual donors. The leukapheresis product was subjected to centrifugation over Ficoll-Hypaque, washed, irradiated, and cryopreserved under GMP conditions.

[00680] It is important that patients who received TIL therapy not be infused with viable PBMCs as this could result in Graft-Versus-Host Disease (GVHD). Donor PBMCs are therefore growth-arrested by dosing the cells with gamma-irradiation, resulting in double strand DNA breaks and the loss of cell viability of the PBMCs upon reculture.

Evaluation Criteria

[00681] 7.2.1 Evaluation criterion for irradiated PBMC lots was their replication incompetency.

Experimental Set-up

[00682] Feeder lots were tested in mini-REP format as if they were to be co-cultured with TIL, using upright T25 tissue culture flasks. Control lot: One lot of irradiated PBMCs, which had historically been shown to meet the criterion of 7.2.1, was run alongside the experimental lots as a control. For each lot of irradiated donor PBMC tested, duplicate flasks were run.

Experimental Protocol

Day 0

[00683] Prepared ~90ml of CM2 medium for each lot of donor PBMC to be tested. Kept CM2 warm in 37°C water bath. Thawed an aliquot of 6×10^6 IU/ml IL-2. Returned the CM2 medium to the BSC, wiping with 70% EtOH prior to placing in hood. For each lot of PBMC tested, removed about 60ml of CM2 to a separate sterile bottle. Added IL-2 from the thawed 6×10^6 IU/ml stock solution to this medium for a final concentration of 3000 IU/ml. Labeled this bottle as “CM2/IL2” (or similar) to distinguish it from the unsupplemented CM2.

Prepare OKT3

[00684] Took out the stock solution of anti-CD3 (OKT3) from the 4°C refrigerator and placed in the BSC. A final concentration of 30ng/ml OKT3 was used in the media of the mini-REP. Prepared a 10µg/ml working solution of anti-CD3 (OKT3) from the 1mg/ml stock solution. Placed in refrigerator until needed.

[00685] For each PBMC lot tested, prepare 150µl of a 1:100 dilution of the anti-CD3 (OKT3) stock. For example, for testing 4 PBMC lots at one time, prepare 600µl of 10µg/ml anti-CD3 (OKT3) by adding 6µl of the 1mg/ml stock solution to 594µl of CM2 supplemented with 3000 IU/ml IL-2.

Prepare Flasks

[00686] Added 19ml per flask of CM2/IL-2 to the labeled T25 flasks and placed flasks into 37°C, humidified, 5% CO₂ incubator while preparing cells.

Prepare Irradiate PBMC

[00687] Retrieved vials of PBMC lots to be tested from LN2 storage. These were placed at -80°C or kept on dry ice prior to thawing. Placed 30ml of CM2 (without IL-2 supplement) into 50ml conical tubes for each lot to be thawed. Labeled each tube with the different lot numbers of the PBMC to be thawed. Capped tubes tightly and place in 37°C water bath prior to use. As needed, returned 50ml conical tubes to the BSC, wiping with 70% EtOH prior to placing in the hood.

[00688] Removed a vial PBMC from cold storage and place in a floating tube rack in a 37°C water bath to thaw. Allowed thaw to proceed until a small amount of ice remains in the vial. Using a sterile transfer pipet, immediately transferred the contents of the vial into the 30ml of CM2 in the 50ml conical tube. Removed about 1ml of medium from the tube to rinse the vial; returned rinse to the 50ml conical tube. Capped tightly and swirl gently to wash cells.

[00689] Centrifuged at 400 x g for 5 minutes at room temperature. Aspirated the supernatant and resuspend the cell pellet in 1ml of warm CM2/IL-2 using a 1000µl pipet tip. Alternately, prior to adding medium, resuspended cell pellet by dragging capped tube along an empty tube rack. After resuspending the cell pellet, brought volume to 4ml using CM2/IL-2 medium. Recorded volume.

[00690] Removed a small aliquot (*e.g.*, 100µl) for cell counting using an automated cell counter. Performed counts in duplicate according to the particular automated cell counter

SOP. It most likely was necessary to perform a dilution of the PBMC prior to performing the cell counts. A recommended starting dilution was 1:10, but this varied depending on the type of cell counter used. Recorded the counts.

[00691] Adjusted concentration of PBMC to 1.3×10^7 cells/ml using CM2/IL-2 medium. Mixed well by gentle swirling or by gently aspirating up-and-down using a serological pipet.

Set Up Culture Flasks

[00692] Returned two labeled T25 flasks to the BSC from the tissue culture incubator. Returned the 10µg/ml vial of anti-CD3/OKT3 to the BSC. Added 1ml of the 1.3×10^7 PBMC cell suspension to each flask. Added 60µl of the 10µg/ml anti-CD3/OKT3 to each flask. Returned capped flasks to the tissue culture incubators for 14 days of growth without disturbance. Placed anti-CD3/OKT3 vial back into the refrigerator until needed for the next lot. Repeated for each lot of PBMC to be evaluated.

Day 14, Measurement of Non-proliferation of PBMC

[00693] Returned the duplicate T25 flasks to the BSC. For each flask, using a fresh 10ml serological pipet, removed ~17ml from each of the flasks, then carefully pulled up the remaining media to measure the volume remaining in the flasks. Recorded volume.

[00694] Mixed sample well by pipetting up and down using the same serological pipet.

[00695] Removed a 200µl sample from each flask for counting. Counted cells using an automated cell counter. Repeated steps 7.4.26 – 7.4.31 for each lot of PBMC being evaluated.

RESULTS AND ACCEPTANCE CRITERION

Results

[00696] The dose of gamma irradiation was expected to be sufficient to render the feeder cells replication incompetent. All lots were expected to meet the evaluation criterion, demonstrating a reduction in the total viable number of feeder cells remaining on Day 14 of the REP culture compared to Day 0.

Acceptance Criterion

[00697] The following acceptance criterion were met for each irradiated donor PBMC lot tested: “No growth” – meant that the total number of viable cells on Day 14 was less than the initial viable cell number put into culture on Day 0 of the REP.

Contingency Testing of PBMC lots which do not meet acceptance criterion.

[00698] In the event than an irradiated donor PBMC lot did not meet the acceptance criterion above, the following steps were taken to retest the lot to rule out simple experimenter error as the cause of its failure. If there were two or more remaining satellite vials of the lot, then the lot was retested. If there are one or no remaining satellite vials of the lot, then the lot was failed according to the acceptance criterion above.

[00699] To be qualified, a PBMC lot going through contingency testing had both the control lot and both replicates of the lot in question achieve the acceptance criterion. Upon meeting this criterion, the lot was then released for use.

EXAMPLE 5: PREPARATION OF IL-2 STOCK SOLUTION (CELLGENIX)

[00700] This Example describes the process of dissolving purified, lyophilized recombinant human interleukin-2 into stock samples suitable for use in further tissue culture protocols, including all of those described in the present application and Examples, including those that involve using rhIL-2.

Procedure

[00701] Prepared 0.2% Acetic Acid solution (HAc). Transferred 29mL sterile water to a 50mL conical tube. Added 1mL 1N acetic acid to the 50mL conical tube. Mixed well by inverting tube 2-3 times. Sterilized the HAc solution by filtration using a Steriflip filter

[00702] Prepare 1% HSA in PBS. Added 4mL of 25% HSA stock solution to 96mL PBS in a 150mL sterile filter unit. Filtered solution. Stored at 4°C. For each vial of rhIL-2 prepared, fill out forms.

[00703] Prepared rhIL-2 stock solution (6×10^6 IU/mL final concentration). Each lot of rhIL-2 was different and required information found in the manufacturer's Certificate of Analysis (COA), such as: 1) Mass of rhIL-2 per vial (mg), 2) Specific activity of rhIL-2 (IU/mg) and 3) Recommended 0.2% HAc reconstitution volume (mL).

[00704] Calculated the volume of 1% HSA required for rhIL-2 lot by using the equation below:

$$\left(\frac{\text{Vial Mass (mg)} \times \text{Biological Activity } \left(\frac{\text{IU}}{\text{mg}} \right)}{6 \times 10^6 \frac{\text{IU}}{\text{mL}}} \right) - \text{HAc vol (mL)} = 1\% \text{ HSA vol (mL)}$$

[00705] For example, according to CellGenix's rhIL-2 lot 10200121 COA, the specific activity for the 1mg vial is 25×10^6 IU/mg. It recommends reconstituting the rhIL-2 in 2mL 0.2% HAc.

$$\left(\frac{1\text{mg} \times 25 \times 10^6 \frac{\text{IU}}{\text{mg}}}{6 \times 10^6 \frac{\text{IU}}{\text{mL}}} \right) - 2\text{mL} = 2.167\text{mL HSA}$$

[00706] Wiped rubber stopper of IL-2 vial with alcohol wipe. Using a 16G needle attached to a 3mL syringe, injected recommended volume of 0.2% HAc into vial. Took care to not dislodge the stopper as the needle is withdrawn. Inverted vial 3 times and swirled until all powder is dissolved. Carefully removed the stopper and set aside on an alcohol wipe. Added the calculated volume of 1% HSA to the vial.

[00707] Storage of rhIL-2 solution. For short-term storage (<72hrs), stored vial at 4°C. For long-term storage (>72hrs), aliquoted vial into smaller volumes and stored in cryovials at -20°C until ready to use. Avoided freeze/thaw cycles. Expired 6 months after date of preparation. Rh-IL-2 labels included vendor and catalog number, lot number, expiration date, operator initials, concentration and volume of aliquot.

EXAMPLE 6: CRYOPRESERVATION PROCESS

[00708] This example describes the cryopreservation process method for TILs prepared with the abbreviated, closed procedure described in Example G using the CryoMed Controlled Rate Freezer, Model 7454 (Thermo Scientific).

[00709] The equipment used was as follows: aluminum cassette holder rack (compatible with CS750 freezer bags), cryostorage cassettes for 750 mL bags, low pressure (22 psi) liquid nitrogen tank, refrigerator, thermocouple sensor (ribbon type for bags), and CryoStore CS750 Freezing bags (OriGen Scientific).

[00710] The freezing process provides for a 0.5 °C rate from nucleation to -20 °C and 1 °C per minute cooling rate to -80 °C end temperature. The program parameters are as

follows: Step 1 - wait at 4 °C; Step 2: 1.0 °C/min (sample temperature) to -4 °C; Step 3: 20.0 °C/min (chamber temperature) to -45 °C; Step 4: 10.0 °C/min (chamber temperature) to -10.0 °C; Step 5: 0.5 °C/min (chamber temperature) to -20 °C; and Step 6: 1.0 °C/min (sample temperature) to -80 °C.

EXAMPLE 7: NSCLC TREATMENT WITH ANTI-PD-1 ANTIBODIES

Patient population:

[00711] Treatment naïve NSCLC or post chemotherapy but anti-PD-1/PD-L1 naïve

Treatment schedules:

[00712] Tumor fragment, treat with up to 4 doses of anti-PD-1/PD-L1, treat the primary refractory patients with TIL product which is cryo-preserved and ready for use upon immediate progression. Primary refractory patients may have progressed after 2 doses.

[00713] Relapse patients can also be treated upon progression (the timing may vary from months to years later).

[00714] Full strength IL-2 up to 6 doses.

[00715] Patient populations to further consider with the same manufacturing permutations noted earlier:

- Treatment naïve NSCLC or post chemotherapy but anti-PD-1/PD-L1 naïve.
- Treatment naïve NSCLC or post chemotherapy but anti-PD-1/PD-L1 naïve who have low expression of PD-L1.
- Treatment naïve NSCLC or post chemotherapy but anti-PD-1/PD-L1 naïve who have low expression of PD-L1 and/or have bulky disease at baseline (for example, bulky disease is indicated where the maximal tumor diameter is greater than 7 cm measured in either the transverse or coronal plane or swollen lymph nodes with a short-axis diameter of 20 mm or greater on CT were defined as bulky; see for example, Samejima, J., *Japanese Journal of Clinical Oncology*, 45(11): 1050–10541 2015, incorporated herein by reference).

EXAMPLE 8: A PHASE 2, MULTICENTER STUDY OF AUTOLOGOUS TUMOR INFILTRATING LYMPHOCYTES IN PATIENTS WITH SOLID TUMORS

STUDY DESIGN

Overview

[00716] This example describes a prospective, open-label, multi-cohort, non-randomized, multicenter Phase 2 study evaluating ACT using TIL in combination with pembrolizumab or TIL as a single therapy, using TILs prepared as described in the present application as well as in this example.

Objectives:

Primary:

[00717] To evaluate the efficacy of autologous TIL in combination with pembrolizumab in MM, HNSCC, or NSCLC patients or TIL as a single therapy in relapsed or refractory (r/r) NSCLC patients, who had previously progressed on or after treatment with CPIs, as determined by objective response rate (ORR), using the Response Evaluation Criteria in Solid Tumors (RECIST 1.1), as assessed by Investigator.

[00718] To characterize the safety profile of TIL in combination with pembrolizumab in MM, HNSCC, and NSCLC patients or TIL as a single therapy in r/r NSCLC patients as measured by the incidence of Grade ≥ 3 treatment-emergent adverse events (TEAEs).

Secondary:

[00719] To further evaluate the efficacy of autologous TIL in combination with pembrolizumab in MM, HNSCC, and NSCLC patients or TIL as a single therapy in r/r NSCLC patients using complete response (CR) rate, duration of response (DOR), disease control rate (DCR), progression-free survival (PFS) using RECIST 1.1, as assessed by Investigator, and overall survival (OS).

Cohorts:

[00720] Cohort 1A: TIL therapy in combination with pembrolizumab in patients with Stage IIIC or Stage IV unresectable or MM with ≤ 3 prior lines of systemic therapy excluding immunotherapy. If previously treated, patients must have had radiographically documented progression on or after most recent therapy.

[00721] Cohort 2A: TIL therapy in combination with pembrolizumab in patients with advanced, recurrent or metastatic HNSCC (*e.g.*, Stages T1N1-N2B, T2-4N0-N2b) with ≤ 3 prior lines of systemic therapy, excluding immunotherapy. If previously treated, patients must have had radiographically documented progression on or after most recent therapy.

[00722] Cohort 3A: TIL therapy in combination with pembrolizumab in patients with locally advanced or metastatic (Stage III–IV) NSCLC with ≤ 3 prior lines of systemic therapy, excluding immunotherapy. If previously treated, patients must have had radiographically documented progression on or after most recent therapy.

[00723] Cohort 3B: TIL therapy as a single agent in patients Stage III or Stage IV NSCLC who have previously received systemic therapy with CPIs (*e.g.*, anti-PD-1/anti-PD-L1) as part of ≤ 3 prior lines of systemic therapy. If previously treated, patients must have had radiographically documented progression on or after most recent therapy.

[00724] Patients in Cohorts 3A and 3B (NSCLC) with oncogene-driven tumors with available effective targeted therapy must have received at least one line of targeted therapy.

[00725] All patients received autologous cryopreserved TIL therapy (with or without pembrolizumab, depending on cohort assignment), preceded by a nonmyeloablative lymphodepletion (NMA-LD) preconditioning regimen consisting of cyclophosphamide and fludarabine. Following TIL infusion, up to 6 IV interleukin-2 (IL-2) doses maximum were administered.

[00726] The following general study periods took place in all 4 cohorts, unless specified otherwise.

[00727] Screening and Tumor Resection: Up to 4 weeks (28 days) from study entry; manufacturing of the TIL Product: approximately ≤ 22 days from tumor resection; and treatment period, as discussed below.

[00728] Treatment Period (Cohorts 1A, 2A, and 3A): up to 2 years, including NMA-LD (7 days), TIL infusion (1 day) followed by IL-2 administrations (1 to 4 days). Patients receive a single infusion of pembrolizumab after the completion of their tumor resection for TIL production and baseline scans but before the initiation of the NMA-LD regimen. The next dose of pembrolizumab will be no earlier than following the completion of IL-2 and continue Q3W ± 3 days thereafter for ≤ 2 years (24 months) or until disease progression or unacceptable toxicity, whichever occurs first. The end-of-treatment (EOT) visit occurred within 30 days after the last dose of pembrolizumab. The visit could be combined with end-

of-assessment (EOA) visit if applicable (*e.g.*, pembrolizumab discontinuation occurred at disease progression or at the start of new anticancer therapy).

[00729] Treatment Period (Cohort 3B): up to 12 days, including NMA-LD (7 days), TIL, infusion (1 day) followed by IL-2 administrations (1 to 4 days). The EOT visit occurred once a patient received the last dose of IL-2. The EOT visit was performed within 30 days after treatment discontinuation and it may be combined with any scheduled visit occurring within this interval during the assessment period.

[00730] Assessment Period: began after TIL infusion on Day 0 and ends upon disease progression, with the start of a new anticancer therapy, partial withdrawal of consent to study assessments, or 5 years (Month 60), whichever occurred first. An end-of assessment (EOA) visit occurred once a patient reached disease progression or started a new anticancer therapy.

[00731] The TIL autologous therapy with the TILs prepared as described herein was comprised of the following steps:

1. Tumor resection to provide the autologous tissue that serves as the source of the TIL cellular product;
2. TIL product produced at a central Good Manufacturing Practice (GMP) facility;
3. A 7-day NMA-LD preconditioning regimen;
4. Cohorts 1A, 2A, and 3A: Patients receive a single infusion of pembrolizumab after the completion of their tumor resection for TIL production and baseline scans but before the initiation of NMA-LD regimen. The next dose of pembrolizumab will be no earlier than following the completion of IL-2 and continue Q3W $\pm$ 3 days thereafter.
5. Infusion of the autologous TIL product (Day 0); and
6. IV IL-2 administrations for up to 6 doses maximum.

[00732] In Cohorts 1A, 2A, and 3A, the next dose of pembrolizumab was no earlier than following the completion of IL-2 and continue Q3W $\pm$ 3 days thereafter for $\leq$ 2 years (24 months), or until disease progression or unacceptable toxicity, whichever occurred first.

[00733] Flowcharts for Cohorts 1A, 2A, and 3A can be found in Figure 7. The Flowchart for Cohort 3B can be found in Figure 8. Patients were assigned to the appropriate cohort by tumor indication.

TIL Therapy + Pembrolizumab (Cohorts 1A, 2A, and 3A)

[00734] Patients were screened and scheduled for surgery for tumor resection. Patients then had one or more tumor lesions resected, which were sent to a central manufacturing facility for TIL production.

[00735] Next, the NMA-LD regimen was imitated and consisted of 2 days of IV cyclophosphamide (60 mg/kg) with mesna (per site standard of care or USPI/SmPC) on Days -7 and Day -6 followed by 5 days of IV fludarabine (25 mg/m²: Day -5 through Day -1).

[00736] Patients in Cohorts 1A, 2A, and 3A received a single infusion of pembrolizumab after the completion of their tumor resection for TIL production and baseline scans and before the initiation of NMA-LD regimen. IL-2 administrations at a dose of 600,000 IU/kg IV begun as soon as 3 hours after, but no later than 24 hours after, completion of the TIL infusion on Day 0. Additional IL-2 administrations will be given approximately every 8 to 12 hours for up to 6 doses maximum. The second dose of pembrolizumab was no earlier than following the completion of IL-2. Patients should have recovered from all IL-2-related toxicities (Grade ≤ 2), prior to the second pembrolizumab administration. Pembrolizumab will continue Q3W $\pm$ 3 days thereafter for ≤ 2 years (24 months) or until disease progression or unacceptable toxicity, whichever occurred first.

TIL Therapy as a Single Agent (Cohort 3B)

[00737] Patients were screened and scheduled for surgery for tumor resection. Patients then had one or more tumor lesions resected, which were sent to a central manufacturing facility for TIL production.

[00738] Next, the NMA-LD regimen consisted of 2 days of IV cyclophosphamide (60 mg/kg) with mesna (per site standard of care or USPI/SmPC) on Day -7 and Day -6 followed by 5 days of IV fludarabine (25 mg/m²: Day -5 through Day -1).

Infusion of the tumor-derived autologous TIL product occurred no sooner than 24 hours after last dose of fludarabine. IL-2 administrations at a dose of 600,000 IU/kg IV may have begun as soon as 3 hours after, but no later than 24 hours after, completion of the TIL infusion.

[00739] Additional IL-2 administrations were given approximately every 8 to 12 hours for up to 6 doses maximum.

Production and Expansion of Tumor Infiltrating Lymphocytes

[00740] The TIL autologous cellular product was composed of viable cytotoxic T lymphocytes derived from a patient's tumor/lesion, which are manufactured ex vivo at a

central GMP facility. An exemplary flow diagram depicting the TIL production process is provided in Figure 9, for example.

[00741] The TIL manufacturing process begun at the clinical site after surgical excision of a primary or secondary metastatic tumor lesion(s) of ≥ 1.5 cm in diameter in each individual patient. Multiple tumor lesions from various anatomical locations can be excised to compile a total aggregate of tumor tissue; however, the aggregate should not exceed 4.0 cm in diameter, or 10 g in weight, due to the limited quantity of the biopreservation media present in the transport bottle.

[00742] Once the tumor lesion(s) was placed in the biopreservation transport bottle, it is shipped at 2°C to 8°C using an express courier to a central GMP manufacturing facility. Upon arrival, the tumor specimen(s) were dissected into fragments, which were then cultured in a pre-rapid expansion protocol (Pre-REP) with human recombinant IL-2 for ~11 days.

[00743] These pre-REP cells were then further expanded using a rapid expansion protocol (REP) for 11 days in the presence of IL-2, OKT3 (a murine monoclonal antibody to human CD3, also known as [muromonab-CD3]) and irradiated allogenic peripheral blood mononuclear cells (PBMC) as feeder cells.

[00744] The expanded cells were then harvested, washed, formulated, cryopreserved, and shipped to the clinical site via an express courier. The dosage form of the TIL cellular product was a cryopreserved autologous “live-cell suspension” that was ready for infusion into the patient from whom the TIL were derived. Patients were to receive the full dose of product that was manufactured and released, which contained between 1×10^9 and 150×10^9 viable cells per the product specification. Clinical experience indicated that objective tumor responses were achieved across this dose range, which has also been shown to be safe (Radvanyi L.G., *et al.*, Clin Cancer Res. **2012**;18(24):6758-70). The full dose of product was provided in up to four infusion bags.

Preparation of Patients to Receive the TIL Cellular Product

[00745] The NMA-LD preconditioning regimen used in this study (i.e., 2 days of cyclophosphamide plus mesna, followed by 5 days of fludarabine) was based on the method developed and tested by the National Cancer Institute (Rosenberg S.A., *et al.*, Clin Cancer Res. **2011**;17(13):4550-7; Radvanyi L.G., *et al.*, Clin Cancer Res. **2012**;18(24):6758-70; Dudley M.E., *et al.*, J Clin Oncol. **2008**;26(32):5233-9; Pilon-Thomas S, *et al.*, J Immunother. **2012**;35(8):615-20; Dudley M.E., *et al.*, J Clin Oncol. **2005**;23(10):2346-57; and Dudley

M.E., et al., Science. **2002**;298(5594):850-4). Following the 7-day preconditioning regimen, the patient was infused with the TIL cellular product.

[00746] The TIL infusion was followed by the administration of IV IL-2 (600,000 IU/kg) every 8 to 12 hours, with the first dose administered between 3 and 24 hours after the completion of the TIL infusion and continuing for up to 6 doses maximum. Per institutional standards, the doses of IL-2 can be calculated on the basis of actual weight.

SELECTION OF PATIENT POPULATIONS

Cohort 1A:

[00747] Patients had a confirmed diagnosis of unresectable MM (Stage IIIC or Stage IV, histologically confirmed as per American Joint Committee on Cancer [AJCC] staging system). Ocular melanoma patients were excluded. Patients must not have received prior immuno-oncology targeted agents. If BRAF-mutation positive, patient could have received prior BRAF/MEKtargeted therapy.

Cohort 2A:

[00748] Patients had advanced, recurrent and/or metastatic HNSCC and can be treatment naïve; histologic diagnosis of the primary tumor is required via the pathology report. Patients must not have received prior immunotherapy regimens.

Cohort 3A:

[00749] Patients had a confirmed diagnosis of Stage III or Stage IV NSCLC (squamous, adenocarcinoma, large cell carcinoma). Patients with oncogene-driven tumors with available effective targeted therapy had received at least one line of targeted therapy.

Cohort 3B:

[00750] Patients had a confirmed diagnosis of Stage III or Stage IV NSCLC (squamous, adenocarcinoma, large cell carcinoma) and had previously received systemic therapy with CPIs (*e.g.*, anti-PD-1/anti-PD-L1). Patients with oncogene-driven tumors with available effective targeted therapy had received at least one line of targeted therapy.

[00751] All patients had received up to 3 prior systemic anticancer therapies (see, inclusion criteria below), excluding immunotherapy for Cohorts 1A, 2A, and 3A. If previously treated, patients had radiographically confirmed progression on or after most recent therapy.

Inclusion Criteria

[00752] Patients must have met ALL of the following inclusion criteria for participation in the study:

1. All patients had a histologically or pathologically confirmed diagnosis of malignancy of their respective histologies:
 - Unresectable or metastatic melanoma (Cohort 1A)
 - Advanced, recurrent or metastatic squamous cell carcinoma of the head and neck (Cohort 2A)
 - Stage III or Stage IV NSCLC (squamous, nonsquamous, adenocarcinoma, large cell carcinoma) (Cohorts 3A and 3B).
2. Cohorts 1A, 2A, and 3A only: Patients were immunotherapy naïve. If previously treated, patients had progressed on or after most recent therapy. Cohorts 1A, 2A, and 3A may have received up to 3 prior systemic anticancer therapies, specifically:
 - In Cohort 1A: Patients with unresectable or metastatic melanoma (Stage IIIC or Stage IV); if BRAF mutation-positive, patients could have received a BRAF inhibitor.
 - In Cohort 2A: Patients with unresectable or metastatic HNSCC. Those who had received initial chemo-radiotherapy were allowed.
 - In Cohort 3A: Patients with Stage III or Stage IV NSCLC (squamous, nonsquamous, adenocarcinoma, or large cell carcinoma) and who were immunotherapy naïve and progressed after ≤ 3 lines of prior systemic therapy in the locally advanced or metastatic setting. Patients who received systemic therapy in the adjuvant or neoadjuvant setting, or as part of definitive chemoradiotherapy, were eligible and were considered to have had one line of therapy if the disease has progressed within 12 months of completion of prior systemic therapy. Patients with known oncogene drivers (*e.g.*, EGFR, ALK, ROS) who had mutations that were sensitive to targeted therapies must have progressed after at least 1 line of targeted therapy.
3. Cohort 3B only: Patients with Stage III or Stage IV NSCLC (squamous, nonsquamous, adenocarcinoma, or large cell carcinoma) who had previously received systemic therapy with CPIs (*e.g.*, anti-PD-1/anti-PD-L1) as part of ≤ 3 prior lines of systemic therapy.
 - Patients had radiographically confirmed progression on or after most recent therapy.
 - Patients who received systemic therapy in the adjuvant or neoadjuvant setting, or as part of definitive chemoradiotherapy, were eligible and were considered to have had 1 line of therapy if the disease had progressed within 12 months of completion of prior systemic therapy.

- Patients with known oncogene drivers (*e.g.*, EGFR, ALK, ROS) who had mutations that are sensitive to targeted therapies must have progressed after at least 1 line of targeted therapy.
4. Patients had at least 1 resectable lesion (or aggregate lesions) of a minimum 1.5 cm in diameter post-resection for TIL investigational product production. It was encouraged that tumor tissue be obtained from multiple and diverse metastatic lesions, as long as the surgical resection did not pose additional risks to the patient.
- If the lesion considered for resection for TIL generation is within a previously irradiated field, the lesion must have demonstrated radiographic progression prior to resection.
 - Patients must have an adequate histopathology specimen for protocol-required testing.
5. Patients had remaining measurable disease as defined by the standard and well known RECIST 1.1 guidelines (*see*, for example, Eisenhauer, European Journal of Cancer 45:228–247 (2009), also available on the World Wide Web at project.eortc.org/recist/wp-content/uploads/sites/4/2015/03/RECISTGuidelines.pdf) following tumor resection for TIL manufacturing:
- Lesions in previously irradiated areas were not be selected as target lesions unless there had been demonstrated progression of disease in those lesions;
 - Lesions that were partially resected for TIL generation that were still measurable per RECIST may be selected as nontarget lesions but could not serve as a target lesion for response assessment.
6. Patients were ≥ 18 years at the time of consent.
7. Patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and an estimated life expectancy of ≥ 3 months.
8. Patients of childbearing potential or those with partners of childbearing potential had to be willing to practice an approved method of highly effective birth control during treatment and continue for 12 months after receiving all protocol-related therapy (Note: Females of reproductive potential were to use effective contraception during treatment and for 12 months after their last dose of IL-2, or 4 months after their last dose of pembrolizumab whichever occurred later). Males could not donate sperm during the study or for 12 months after treatment discontinuation, whichever occurred later.
9. Patients had the following hematologic parameters:
- Absolute neutrophil count (ANC) $\geq 1000/\text{mm}^3$;

- Hemoglobin ≥ 9.0 g/dL;
- Platelet count $\geq 100,000/\text{mm}^3$.

10. Patients had adequate organ function:

- Serum alanine aminotransferase (ALT)/serum glutamic-pyruvic transaminase (SGPT) and aspartate aminotransferase (AST)/SGOT ≤ 3 times the upper limit of normal (ULN), patients with liver metastasis ≤ 5 times ULN.
- An estimated creatinine clearance ≥ 40 mL/min using the Cockcroft Gault formula at Screening.
- Total bilirubin ≤ 2 mg/dL.
- Patients with Gilbert's Syndrome must have a total bilirubin ≤ 3 mg/dL.

11. Patients were seronegative for the human immunodeficiency virus (HIV1 and HIV2). Patients with positive serology for hepatitis B virus surface antigen (HBsAg), hepatitis B core antibody (anti HBc), or hepatitis C virus (anti-HCV) indicating acute or chronic infection were enrolled depending on the viral load based on polymerase chain reaction (PCR) and the local prevalence of certain viral exposures.

12. Patients had a washout period from prior anticancer therapy(ies) of a minimum duration, as detailed below prior to the first study treatment (*i.e.*, start of NMA-LD or pembrolizumab):

- Targeted therapy: prior targeted therapy with an epidermal growth factor receptor (EGFR), MEK, BRAF, ALK, ROS1 or other-targeted agents (*e.g.*, erlotinib, afatinib, dacomitinib, osimertinib, crizotinib, ceritinib, lorlatinib) was allowed provided the washout is a minimum of 14 days prior to the start of treatment.
- Chemotherapy: adjuvant, neoadjuvant or definitive chemotherapy/chemoradiation was allowed provided the washout is a minimum of 21 days prior to the start of treatment.
- Immunotherapy for Cohort 3B only, prior checkpoint-targeted therapy with an anti-PD-1, other mAbs, or vaccines were allowed with a washout period of ≥ 21 days before the start of NMA-LD.
- Palliative radiation therapy: prior external beam radiation was allowed provided all radiation-related toxicities were resolved to Grade 1 or baseline, excluding alopecia, skin pigmentation change, or other clinically insignificant events, *e.g.*, small area radiation dermatitis or rectal or urinary urgency
- The tumor lesion(s) being assessed as target for response via RECIST 1.1 were outside of the radiation portal; however, if within the portal, they must have demonstrated progression (see Inclusion Criterion above).
- Surgery/pre-planned procedure: previous surgical procedure(s) was permitted provided that wound healing had occurred, all complications had resolved, and

at least 14 days have elapsed (for major operative procedures) prior to the tumor resection.

13. Patients had recovered from all prior anticancer treatment-related adverse events (TRAEs) to Grade ≤ 1 (per Common Terminology Criteria for Adverse Events [CTCAE]), except for alopecia or vitiligo, prior to cohort assignment.

14. Patients with stable Grade ≥ 2 toxicity from prior anticancer therapy were considered on a case by case basis after consultation with the Medical Monitor.

15. Cohorts 1A, 2A, and 3A patients with irreversible toxicity not reasonably expected to be exacerbated by treatment with pembrolizumab were included only after consultation with the Medical Monitor. For patients in Cohort 3B only, patients with documented Grade ≥ 2 or higher diarrhea or colitis as a result of a previous treatment with immune checkpoint inhibitor CPI(s) must have been asymptomatic for at least 6 months or had a normal by visual assessment colonoscopy post-treatment prior to tumor resection.

16. Patients must have provided written authorization for use and disclosure of protected health information.

Exclusion Criteria

[00753] Patients who meet ANY of the following criteria were excluded from the study:

1. Patients with melanoma of uveal/ocular origin
2. Patients who had received an organ allograft or prior cell transfer therapy that included a nonmyeloablative or myeloablative chemotherapy regimen within the past 20 years. (Note: This criterion was applicable for patients undergoing retreatment with TIL, with the exception that they had a prior NMA-LD regimen with their prior TIL treatment.)
3. Patients with symptomatic and/or untreated brain metastases.
 - • Patients with definitively-treated brain metastases will be considered for enrollment after discussion with Medical Monitor; if, prior to the start of treatment the patient is clinically stable for ≥ 2 weeks, there are no new brain lesions via magnetic resonance imaging (MRI) post-treatment, and the patient does not require ongoing

corticosteroid treatment.

4. Patients who are on a systemic steroid therapy within 21 days of enrollment.

5. Patients who are pregnant or breastfeeding.

6. Patients who had an active medical illness(es), which in the opinion of the Investigator, posed increased risks for study participation; such as systemic infections (*e.g.*, syphilis or any other infection requiring antibiotics), coagulation disorders, or other active major medical illnesses of the cardiovascular, respiratory, or immune systems.

7. Patients may not have active or prior documented autoimmune or inflammatory disorders (including pneumonitis, inflammatory bowel disease [*e.g.*, colitis or Crohn's disease], diverticulitis [with the exception of diverticulosis], systemic lupus erythematosus, sarcoidosis syndrome, or Wegener syndrome [granulomatosis with polyangiitis, Graves' disease, rheumatoid arthritis, hypophysitis, uveitis, etc.]). The following were exceptions to this criterion:

- Patients with vitiligo or alopecia.
- Patients with hypothyroidism (*e.g.*, following Hashimoto syndrome) stable on hormone replacement.
- Any chronic skin condition that did not require systemic therapy.
- Patients with celiac disease controlled by diet alone.

8. Patients who had received a live or attenuated vaccination within 28 days prior to the start of treatment.

9. Patients who had any form of primary immunodeficiency (such as severe combined immunodeficiency disease [SCID] and acquired immune deficiency syndrome [AIDS]).

10. Patients with a history of hypersensitivity to any component of the study drugs. TILs were not administered to patients with a known hypersensitivity to any component of TIL product formulation including, but not limited to any of the following:

- • NMA-LD (cyclophosphamide, mesna, and fludarabine)
- • Proleukin®, aldesleukin, IL-2
- • Antibiotics of the aminoglycoside group (*i.e.*, streptomycin, gentamicin [excluding those who are skin-test negative for gentamicin hypersensitivity])

- • Any component of the TIL product formulation including dimethyl sulfoxide
- [DMSO], HSA, IL-2, and dextran-40
- • Pembrolizumab

11. Patients who had a left ventricular ejection fraction (LVEF) <45% or who are New York Heart Association Class II or higher. A cardiac stress test demonstrating any irreversible wall movement abnormality in any patients ≥ 60 years of age or in patients who have a history of ischemic heart disease, chest pain, or clinically significant atrial and/or ventricular arrhythmias.

- Patients with an abnormal cardiac stress test could be enrolled if they had adequate ejection fraction and cardiology clearance with approval of the Sponsor's Medical Monitor.

12. Patients who had obstructive or restrictive pulmonary disease and have a documented FEV1 (forced expiratory volume in 1 second) of $\leq 60\%$ of predicted normal.

- • If a patient was not able to perform reliable spirometry due to abnormal upper airway anatomy (*i.e.*, tracheostomy), a 6-minute walk test was used to assess pulmonary function. Patients who were unable to walk a distance of at least 80% predicted for age and sex or demonstrates evidence of hypoxia at any point during the test ($SpO_2 < 90\%$) are excluded.

13. Patients who had another primary malignancy within the previous 3 years (except for those which did not require treatment or had been curatively treated greater than 1 year ago, and in the judgment of the Investigator, did not pose a significant risk of recurrence including, but not limited to, non-melanoma skin cancer, DCIS, LCIS, prostate cancer Gleason score ≤ 6 or bladder cancer).

14. Participation in another clinical study with an investigational product within 21 days of the initiation of treatment.

Study Endpoints and Planned Analyses

[00754] The primary and secondary endpoints were analyzed separately by cohort.

Primary Endpoints:

[00755] The ORR was defined as the proportion of patients who achieved either a confirmed PR or CR as best response as assessed by Investigators per RECIST 1.1 among the efficacy analysis set.

[00756] Objective response was evaluated per each disease assessment and the ORR was expressed as a binomial proportion with the corresponding 2-sided 90% CI. The primary analysis for each cohort occurred when all treated patients per cohort have an opportunity to be followed for 12 months, unless progressed/expired or discontinued early from the assessment period.

[00757] The safety primary endpoint was measured by any Grade 3 or higher TEAE incidence rate within each cohort expressed as binomial proportions with the corresponding 2-sided 90% CI.

Secondary Endpoints:

Efficacy:

[00758] The secondary efficacy endpoints were defined as follows:

[00759] CR rate as based on responders who achieved confirmed CR as assessed by Investigators. DCR was derived as the sum of the number of patients who achieved confirmed PR/CR or sustained SD (at least 6 weeks) divided by the number of patients in the efficacy analysis set $\times 100\%$. The CR rate and DCR was summarized using a point estimate and its 2-sided 90% CI.

[00760] DOR was defined among patients who achieved objective response. It was measured from the first-time response (PR/CR) criteria are met until the first date that recurrent or progressive disease was objectively documented, or receipt of subsequent anticancer therapy or the patient dies (whichever is first recorded). Patients not experiencing PD or have not died prior to the time of data cut or the final database lock will have their event times censored on the last date that an adequate assessment of tumor status is made.

[00761] PFS was defined as the time (in months) from the time of lymphodepletion to PD, or death due to any cause, whichever event is earlier. Patients not experiencing PD or not having expired at the time of the data cut or the final database lock had their event times censored on the last date that an adequate assessment of tumor status is made.

[00762] OS was defined as the time (in months) from the time of lymphodepletion to death due to any cause. Patients not having expired by the time of data cut or the final database lock had their event times censored on the last date of their known survival status.

[00763] DOR, PFS, and OS was subjected to right censoring. The Kaplan-Meier method will be used to summarize the time-to-event efficacy endpoints. The baseline data for the tumor assessment was the last scan before the lymphodepletion for all cohorts.

[00764] The above efficacy parameters will be estimated for applicable cohort for subsets defined by baseline disease characteristics; BRAF status (Cohort 1A only), HPV status (Cohort 2A only), squamous or non-squamous lung disease (Cohorts 3A and 3B only), and anti-PD-L1 status.

WHAT IS CLAIMED IS:

1. A method of treating non-small cell lung carcinoma (NSCLC) with a population of tumor infiltrating lymphocytes (TILs) 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 NSCLC tumor in a patient, including from multiple tumor fragments or biopsies;
 - (c) contacting the tumor fragments with a first cell culture medium;
 - (d) performing an initial expansion of the first population of TILs in the first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, wherein the first cell culture medium comprises IL-2;
 - (e) performing a rapid expansion of the second population of TILs in a second cell culture medium to obtain a third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; wherein the second cell culture medium comprises IL-2, OKT-3 (anti-CD3 antibody), and optionally irradiated allogeneic peripheral blood mononuclear cells (PBMCs); and wherein the rapid expansion is performed over a period of 14 days or less;
 - (f) harvesting the third population of TILs; and
 - (g) administering a therapeutically effective portion of the third population of TILs to a patient with the NSCLC;wherein the NSCLC is refractory to treatment with an anti-PD-1 antibody.
2. The method of Claim 1, wherein the obtaining the first population of TILs comprises a multilesional sampling method.
3. The method of Claim 1, wherein the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 and/or anti-PD-L2 antibody.
4. The method of Claim 1, wherein the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody.
5. The method of Claim 1, wherein the refractory NSCLC has been treated with a

chemotherapeutic agent.

6. The method of Claim 1, wherein the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has been previously treated a chemotherapeutic agent.
7. The method of Claim 1, wherein the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has been previously treated a chemotherapeutic agent.
8. The method of Claims 5 to 7, wherein the refractory NSCLC has been treated with a chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent.
9. The method of Claim 1, wherein the refractory NSCLC has low expression of PD-L1.
10. The method of Claim 1, wherein the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has low expression of PD-L1.
11. The method of Claim 1, wherein the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has low expression of PD-L1.
12. The method of Claim 1, wherein the refractory NSCLC has been treated with a chemotherapeutic agent and has low expression of PD-L1.
13. The method of Claim 1, wherein the refractory NSCLC has been treated with a chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent and has low expression of PD-L1
14. The method of Claim 1, wherein the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has bulky disease at baseline.
15. The method of Claim 1, wherein the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has bulky disease at baseline.
16. The method of Claim 1, wherein the refractory NSCLC has been treated with a chemotherapeutic agent and has bulky disease at baseline.
17. The method of Claim 1, wherein the refractory NSCLC has been treated with a chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent and has bulky disease at baseline.
18. The method of Claims 14 to 17, wherein bulky disease is indicated where the maximal

tumor diameter is greater than 7 cm measured in either the transverse or coronal plane or swollen lymph nodes with a short-axis diameter of 20 mm or greater.

19. The method of Claim 1, wherein the refractory NSCLC is refractory to at least two prior systemic treatment courses, not including neo-adjuvant or adjuvant therapies.
20. The method of Claim 1, wherein the refractory NSCLC is refractory to an anti-PD-1 antibody selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, JS001, TSR-042, pidilizumab, (BGB-A317, SHR-1210, REGN2810, MDX-1106, PDR001, anti-PD-1 from clone: RMP1-14; and an anti-PD-1 antibodies disclosed in U.S. Patent No. 8,008,449, durvalumab, atezolizumab, avelumab, and fragments, derivatives, variants, as well as biosimilars thereof.
21. The method of Claim 1, wherein the refractory NSCLC is refractory to pembrolizumab or a biosimilar thereof.
22. The method of Claim 1, wherein the refractory NSCLC is refractory to nivolumab or a biosimilar thereof.
23. The method of Claim 1, wherein the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof.
24. The method of Claim 1, wherein the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof and pembrolizumab or a biosimilar thereof.
25. The method of Claim 1, wherein the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof and nivolumab or a biosimilar thereof.
26. The method of Claim 1, wherein the refractory NSCLC is refractory to durvalumab or a biosimilar thereof.
27. The method of Claim 1, wherein the refractory NSCLC is refractory to atezolizumab or a biosimilar thereof.
28. The method of Claim 1, wherein the refractory NSCLC is refractory to avelumab or a biosimilar thereof.
29. The method of any one of Claims 1 to 28, wherein the initial expansion is performed over a period of 21 days or less.
30. The method of any one of Claims 1 to 29, wherein the initial expansion is performed over a period of 14 days or less.

31. The method of any one of Claims 1 to 30, wherein the initial expansion is performed over a period of about 11 days and the rapid expansion is performed over a period of about 11 days.
32. The method of any one of Claims 1 to 31, wherein the IL-2 is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL in the first cell culture medium.
33. The method of any one of Claims 1 to 32, wherein 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 in the second cell culture medium.
34. The method of any one of Claims 1 to 33, wherein the initial expansion is performed using a gas permeable container.
35. The method of any one of Claims 1 to 34, wherein the rapid expansion is performed using a gas permeable container.
36. The method of any one of Claims 1 to 35, wherein the first cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.
37. The method of any one of Claims 1 to 36, wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.
38. The method of any one of Claims 1 to 37, further comprising the step of treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the third population of TILs to the patient.
39. The method of Claim 38, wherein the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m²/day for two days followed by administration of fludarabine at a dose of 25 mg/m²/day for five days.
40. The method of any one of Claims 1 to 39, further comprising the step of treating the patient with an IL-2 regimen starting on the day after administration of the third population of TILs to the patient.
41. The method of Claim 40, 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.

42. A method of treating non-small cell lung carcinoma (NSCLC) with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:
- (a) resecting one or more tumors from a patient, the one or more tumors comprising a first population of TILs;
 - (b) fragmenting the one or more tumor into tumor fragments;
 - (c) contacting the tumor fragments with a first cell culture medium;
 - (d) performing an initial expansion of the first population of TILs in the first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, wherein the first cell culture medium comprises IL-2;
 - (e) performing a rapid expansion of the second population of TILs in a second cell culture medium to obtain a third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; wherein the second cell culture medium comprises IL-2, OKT-3 (anti-CD3 antibody), and optionally irradiated allogeneic peripheral blood mononuclear cells (PBMCs); and wherein the rapid expansion is performed over a period of 14 days or less;
 - (f) harvesting the third population of TILs; and
 - (g) administering a therapeutically effective portion of the third population of TILs to a patient with the NSCLC;
- wherein the NSCLC is refractory to treatment with an anti-PD-1 antibody.
43. The method of Claim 42, wherein the tumor is resected from one or more tumor cites.
44. The method of Claim 42, wherein the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 and/or anti-PD-L2 antibody.
45. The method of Claim 42, wherein the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody.
46. The method of Claim 42, wherein the refractory NSCLC has been treated with a chemotherapeutic agent.
47. The method of Claim 42, wherein the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has been previously treated a

chemotherapeutic agent.

48. The method of Claim 42, wherein the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has been previously treated a chemotherapeutic agent.
49. The method of Claims 46 to 48, wherein the refractory NSCLC has been treated with a chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent.
50. The method of Claim 42, wherein the refractory NSCLC has low expression of PD-L1.
51. The method of Claim 42, wherein the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has low expression of PD-L1.
52. The method of Claim 42, wherein the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has low expression of PD-L1.
53. The method of Claim 42, wherein the refractory NSCLC has been treated with a chemotherapeutic agent and has low expression of PD-L1.
54. The method of Claim 42, wherein the refractory NSCLC has been treated with a chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent and has low expression of PD-L1.
55. The method of Claim 42, wherein the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has bulky disease at baseline.
56. The method of Claim 42, wherein the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has bulky disease at baseline.
57. The method of Claim 42, wherein the refractory NSCLC has been treated with a chemotherapeutic agent and has bulky disease at baseline.
58. The method of Claim 42, wherein the refractory NSCLC has been treated with a chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent and has bulky disease at baseline.
59. The method of Claims 55 to 58, wherein said bulky disease is indicated where the maximal tumor diameter is greater than 7 cm measured in either the transverse or coronal plane or swollen lymph nodes with a short-axis diameter of 20 mm or greater.
60. The method of Claim 42, wherein the refractory NSCLC is refractory to at least two prior

systemic treatment courses, not including neo-adjuvant or adjuvant therapies.

61. The method of Claim 42, wherein the refractory NSCLC is refractory to an anti-PD-1 antibody selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, JS001, TSR-042, pidilizumab, (BGB-A317, SHR-1210, REGN2810, MDX-1106, PDR001, anti-PD-1 from clone: RMP1-14; and an anti-PD-1 antibodies disclosed in U.S. Patent No. 8,008,449, durvalumab, atezolizumab, avelumab, and fragments, derivatives, variants, as well as biosimilars thereof.
62. The method of Claim 42, wherein the refractory NSCLC is refractory to pembrolizumab or a biosimilar thereof.
63. The method of Claim 42, wherein the refractory NSCLC is refractory to nivolumab or a biosimilar thereof.
64. The method of Claim 42, wherein the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof.
65. The method of Claim 42, wherein the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof and pembrolizumab or a biosimilar thereof.
66. The method of Claim 42, wherein the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof and nivolumab or a biosimilar thereof.
67. The method of Claim 42, wherein the refractory NSCLC is refractory to durvalumab or a biosimilar thereof.
68. The method of Claim 42, wherein the refractory NSCLC is refractory to atezolizumab or a biosimilar thereof.
69. The method of Claim 42, wherein the refractory NSCLC is refractory to avelumab or a biosimilar thereof.
70. The method of any one of Claims 42 to 69, wherein the initial expansion is performed over a period of 21 days or less.
71. The method of any one of Claims 42 to 70, wherein the initial expansion is performed over a period of 14 days or less.
72. The method of any one of Claims 42 to 71, wherein the initial expansion is performed over a period of about 11 days and the rapid expansion is performed over a period of about 11 days.

73. The method of any one of Claims 42 to 72, wherein the IL-2 is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL in the first cell culture medium.
74. The method of any one of Claims 42 to 73, wherein 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 in the second cell culture medium.
75. The method of any one of Claims 42 to 74, wherein the initial expansion is performed using a gas permeable container.
76. The method of any one of Claims 42 to 75, wherein the rapid expansion is performed using a gas permeable container.
77. The method of any one of Claims 42 to 76, wherein the first cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.
78. The method of any one of Claims 42 to 77, wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.
79. The method of any one of Claims 42 to 78, further comprising the step of treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the third population of TILs to the patient.
80. The method of Claim 79, wherein the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m²/day for two days followed by administration of fludarabine at a dose of 25 mg/m²/day for five days.
81. The method of any one of Claims 42 to 80, further comprising the step of treating the patient with an IL-2 regimen starting on the day after administration of the third population of TILs to the patient.
82. The method of Claim 81, 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.
83. A method for treating a subject with non-small cell lung carcinoma (NSCLC), wherein the cancer is refractory to treatment with an anti-PD-1 antibody, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

- (a) obtaining and/or receiving a first population of TILs from one or more tumors resected from a subject by processing the one or more tumors 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 3-14 days to obtain the second population of TILs, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs, 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 which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second 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; and
- (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; and
- (h) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (g) to the subject.

84. The method of Claim 83, wherein the tumor sample is derived from a multilesional sampling method.

85. The method of Claim 83, wherein the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody.

86. The method of Claim 83, wherein the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody.
87. The method of Claim 83, wherein the refractory NSCLC has been treated with a chemotherapeutic agent.
88. The method of Claim 83, wherein the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has been previously treated a chemotherapeutic agent.
89. The method of Claim 83, wherein the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has been previously treated a chemotherapeutic agent.
90. The method of Claims 87 to 89, wherein the refractory NSCLC has been treated with a chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent.
91. The method of Claim 83, wherein the refractory NSCLC has low expression of PD-L1.
92. The method of Claim 83, wherein the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has low expression of PD-L1.
93. The method of Claim 83, wherein the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has low expression of PD-L1.
94. The method of Claim 83, wherein the refractory NSCLC has been treated with a chemotherapeutic agent and has low expression of PD-L1.
95. The method of Claim 83, wherein the refractory NSCLC has been treated with a chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent and has low expression of PD-L1.
96. The method of Claim 83, wherein the refractory NSCLC has not been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has bulky disease at baseline.
97. The method of Claim 83, wherein the refractory NSCLC has been previously treated with an anti-PD-1 and/or anti-PD-L1 antibody and has bulky disease at baseline.
98. The method of Claim 83, wherein the refractory NSCLC has been treated with a chemotherapeutic agent and has bulky disease at baseline.
99. The method of Claim 83, wherein the refractory NSCLC has been treated with a

chemotherapeutic agent but is not being currently treated with a chemotherapeutic agent and has bulky disease at baseline.

100. The method of Claims 96 to 99, wherein bulky disease is indicated where the maximal tumor diameter is greater than 7 cm measured in either the transverse or coronal plane or swollen lymph nodes with a short-axis diameter of 20 mm or greater.
101. The method of Claim 83, wherein the refractory NSCLC is refractory to at least two prior systemic treatment courses, not including neo-adjuvant or adjuvant therapies.
102. The method of Claim 83, wherein the refractory NSCLC is refractory to an anti-PD-1 antibody selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, JS001, TSR-042, pidilizumab, (BGB-A317, SHR-1210, REGN2810, MDX-1106, PDR001, anti-PD-1 from clone: RMP1-14; and an anti-PD-1 antibodies disclosed in U.S. Patent No. 8,008,449, durvalumab, atezolizumab, avelumab, and fragments, derivatives, variants, as well as biosimilars thereof.
103. The method of Claim 83, wherein the refractory NSCLC is refractory to pembrolizumab or a biosimilar thereof.
104. The method of Claim 83, wherein the refractory NSCLC is refractory to nivolumab or a biosimilar thereof.
105. The method of Claim 83, wherein the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof.
106. The method of Claim 83, wherein the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof and pembrolizumab or a biosimilar thereof.
107. The method of Claim 83, wherein the refractory NSCLC is refractory to ipilimumab or a biosimilar thereof and nivolumab or a biosimilar thereof.
108. The method of Claim 83, wherein the refractory NSCLC is refractory to durvalumab or a biosimilar thereof.
109. The method of Claim 83, wherein the refractory NSCLC is refractory to atezolizumab or a biosimilar thereof.
110. The method of Claim 83, wherein the refractory NSCLC is refractory to avelumab or a biosimilar thereof.
111. The method of any one of Claims 83 to 110, wherein the initial expansion is

- performed over a period of 21 days or less.
112. The method of any one of Claims 83 to 111, wherein the initial expansion is performed over a period of 14 days or less.
113. The method of any one of Claims 83 to 112, wherein the initial expansion is performed over a period of about 3-11 days and the second expansion is performed over a period of about 7-11 days.
114. The method of any one of Claims 83 to 113, wherein the initial expansion is performed over a period of about 11 days and the rapid expansion is performed over a period of about 11 days.
115. The method of any one of Claims 83 to 114, wherein the IL-2 is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL in the first cell culture medium.
116. The method of any one of Claims 83 to 115, wherein 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 in the second cell culture medium.
117. The method of any one of Claims 83 to 116, wherein the initial expansion is performed using a gas permeable container.
118. The method of any one of Claims 83 to 117, wherein the rapid expansion is performed using a gas permeable container.
119. The method of any one of Claims 83 to 118, wherein the first cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.
120. The method of any one of Claims 83 to 119, wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.
121. The method of any one of Claims 83 to 120, further comprising the step of treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the third population of TILs to the patient.
122. The method of Claim 121, wherein the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m²/day for two days followed by administration of fludarabine at a dose of 25 mg/m²/day for

five days.

123. The method of any one of Claims 83 to 122, further comprising the step of treating the patient with an IL-2 regimen starting on the day after administration of the third population of TILs to the patient.
124. The method of Claim 123, 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.

Figure 1

Process 2A: about 22 days from Steps A - E

1. STEP A

Obtain Patient Tumor Sample

2. STEP B

Fragmentation and First Expansion

3 days to 14 days

3. STEP C

First Expansion to Second Expansion Transition

No Storage and Closed System

4. STEP D

Second Expansion

IL-2, OKT-3, and antigen-presenting feeder cells

Closed System

5. STEP E

Harvest TILS from Step D

Closed System

6. STEP F

Final Formulation and/or Transfer to Infusion Bag
(optionally cryopreserve)

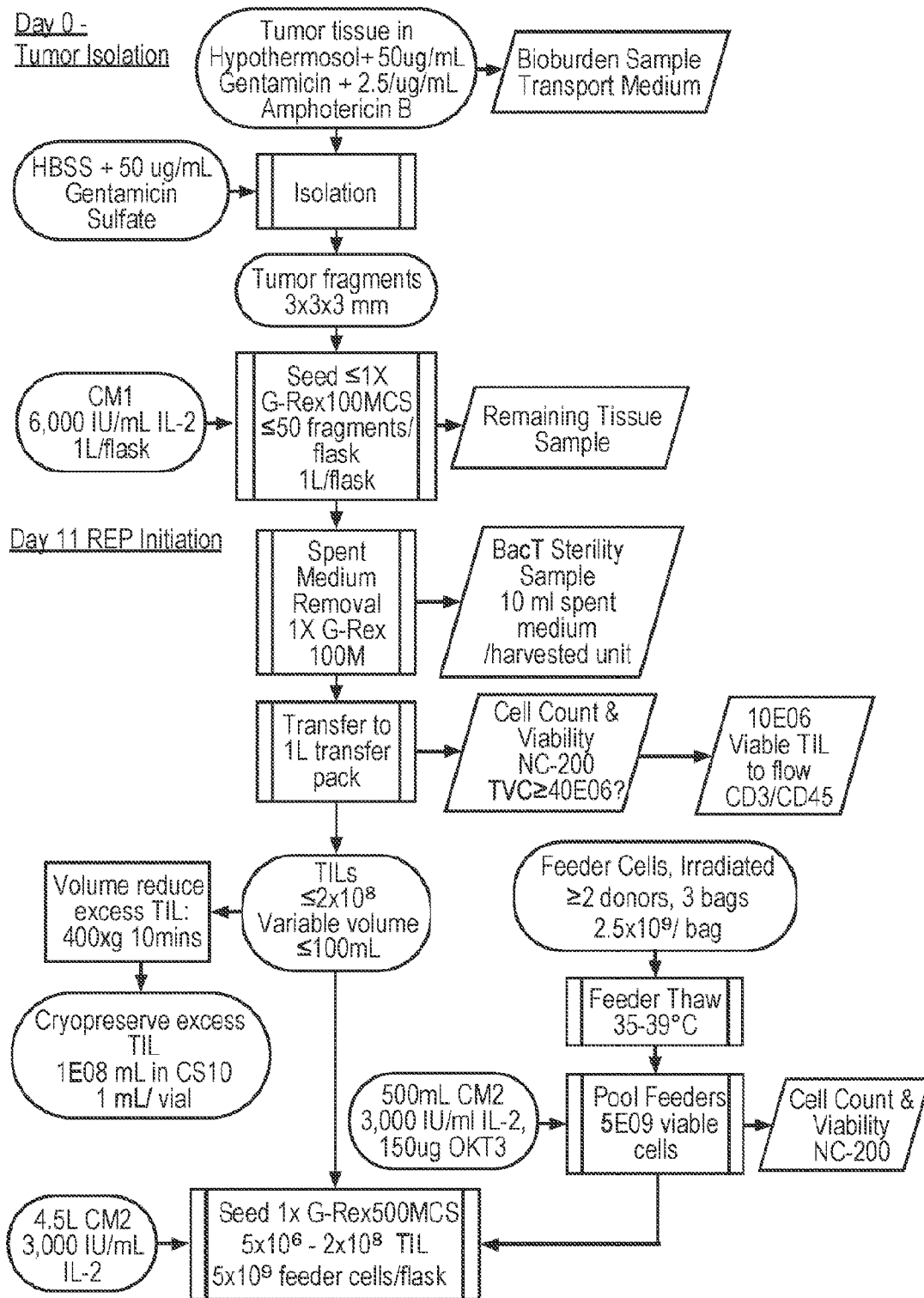
Figure 2A**Figure 2B**

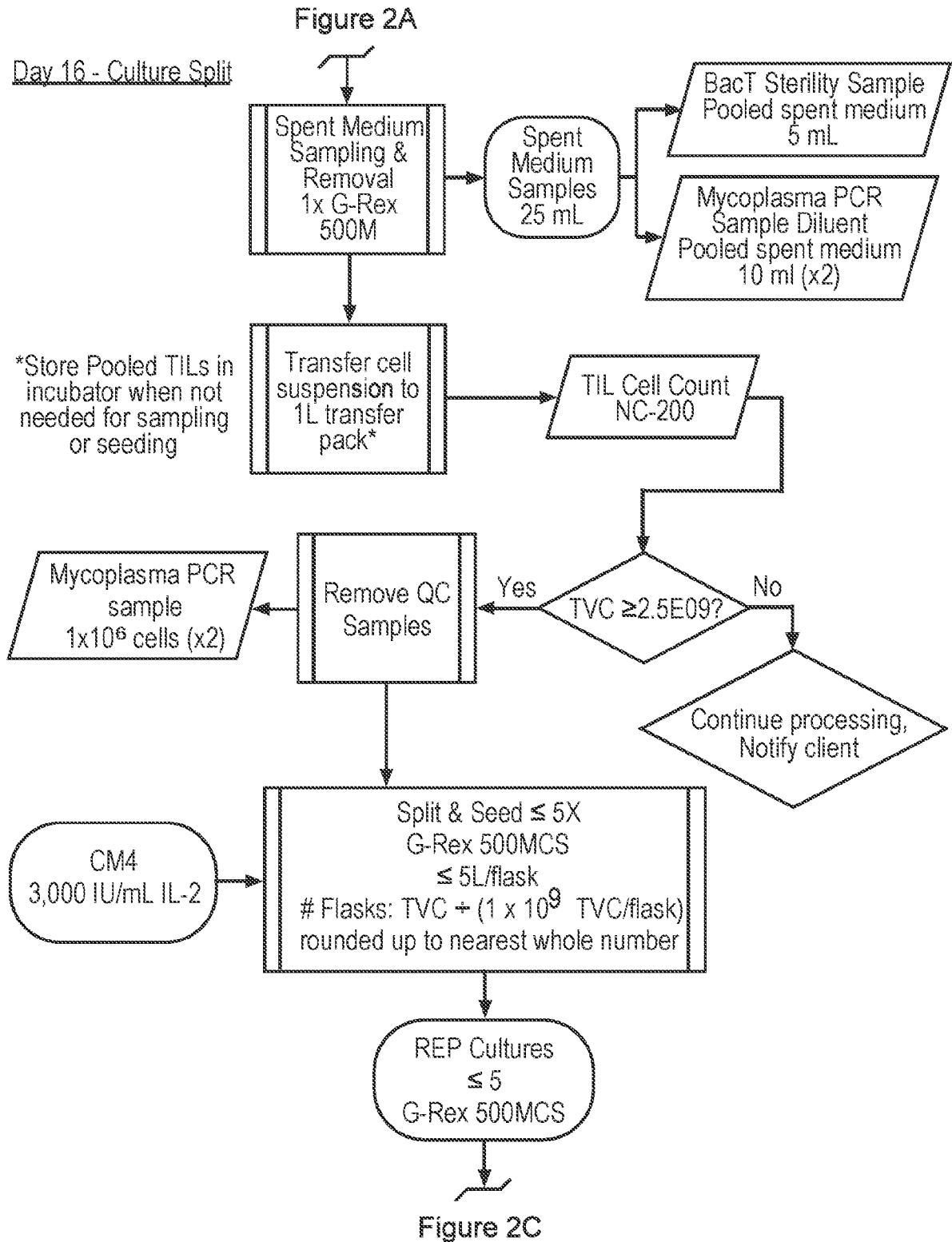
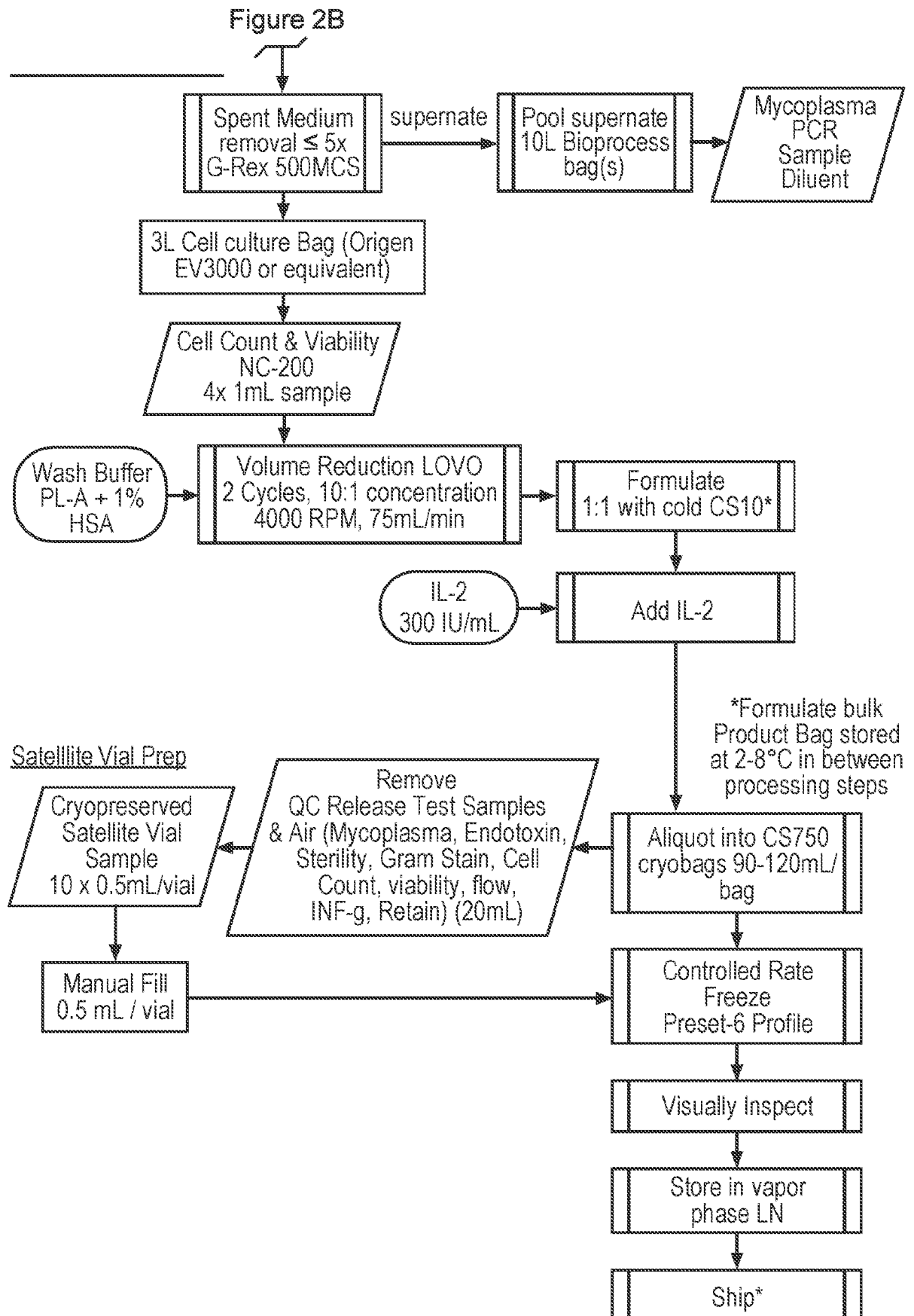
Figure 2B

Figure 2C

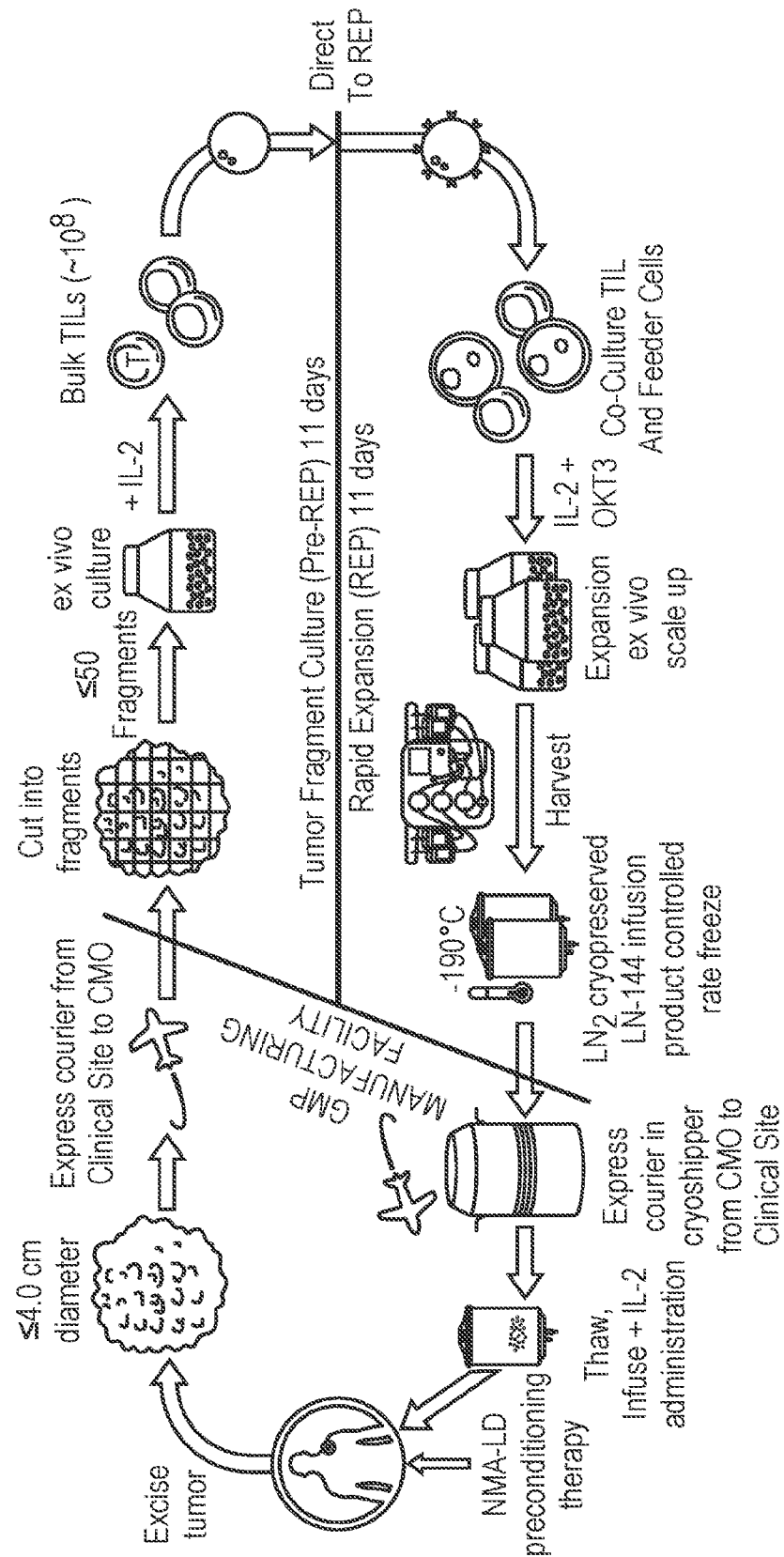


Figure 3

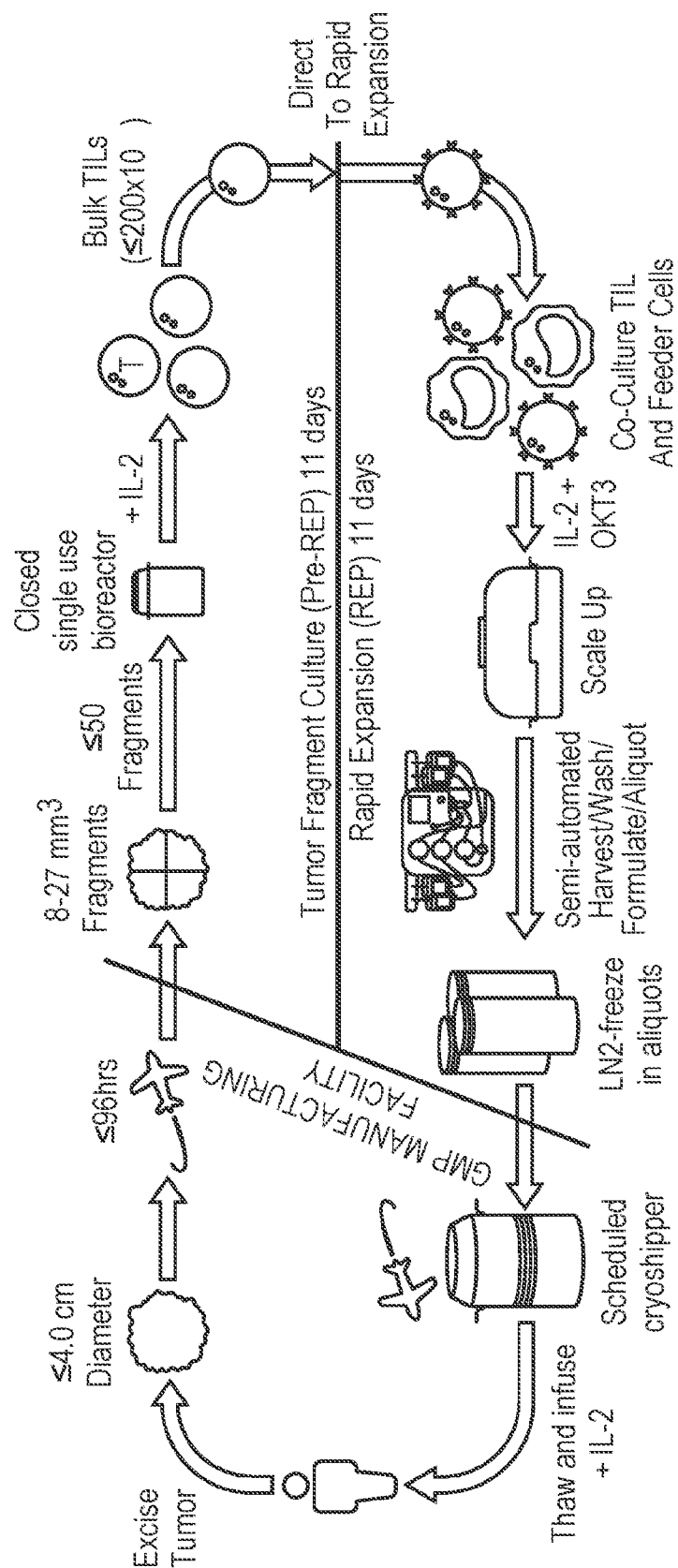


Figure 4

Figure 5

Process 1C: 43-55 Days for Steps A - E	Process 2A: about 22 days from Steps A - E
1. <u>STEP A</u> Obtain Patient Tumor Sample	1. <u>STEP A</u> Obtain Patient Tumor Sample
2. <u>STEP B</u> Fragmentation and First Expansion 11 days to 21 days	2. <u>STEP B</u> Fragmentation and First Expansion 3 days to 14 days
3. <u>STEP C</u> First Expansion to Second Expansion Transition Optional Storage until Selection	3. <u>STEP C</u> First Expansion to Second Expansion Transition No Storage and Closed System
4. <u>STEP D</u> Second Expansion IL-2, OKT-3, antigen-presenting feeder cells Optionally repeat one or more times	4. <u>STEP D</u> Second Expansion IL-2, OKT-3, and antigen-presenting feeder cells Closed System
5. <u>STEP E</u> Harvest TILS from Step D	5. <u>STEP E</u> Harvest TILS from Step D Closed System
6. <u>STEP F</u> Final Formulation and/or Transfer to Infusion Bag	6. <u>STEP F</u> 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 $>40 \times 10^6$ TIL	- Pre-REP TIL directly move to REP on day 11 - REP requires 25-200 $\times 10^6$ 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 $\times 10^6$ TIL and 5×10^9 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 LN₂ - 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

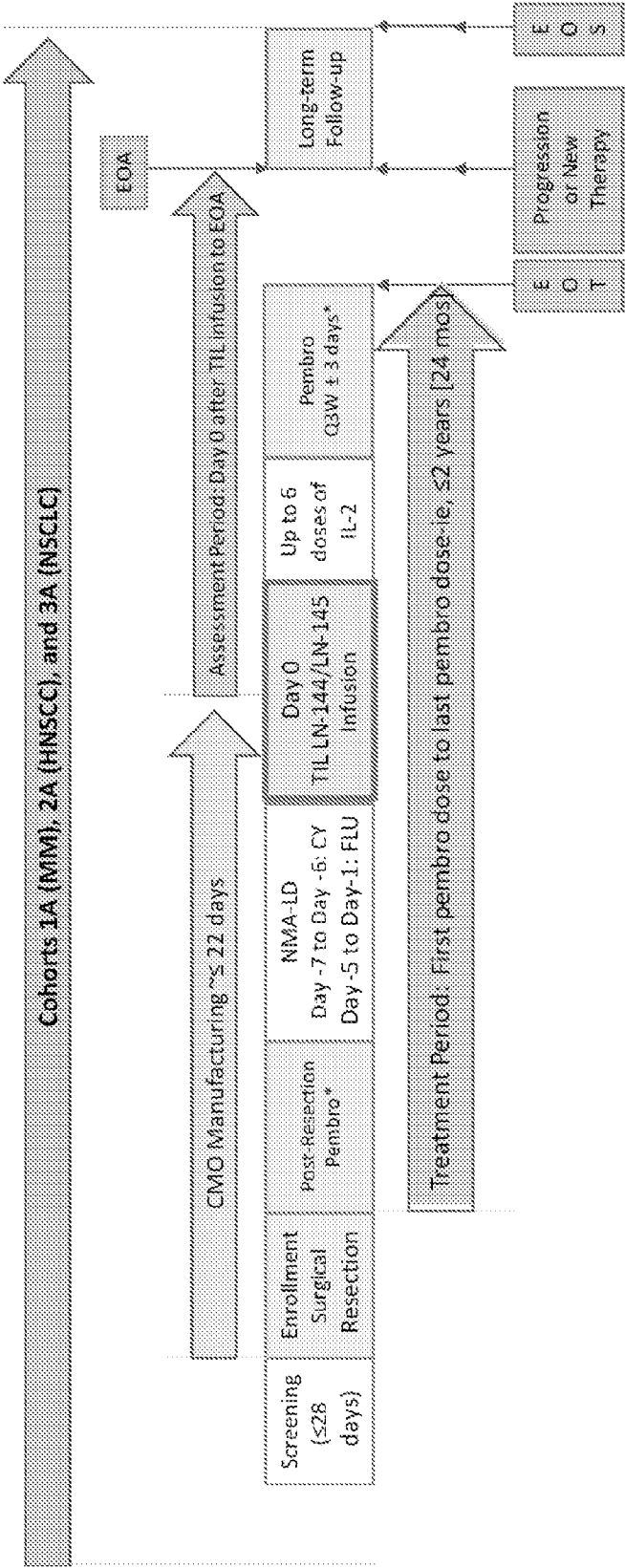


Figure 7

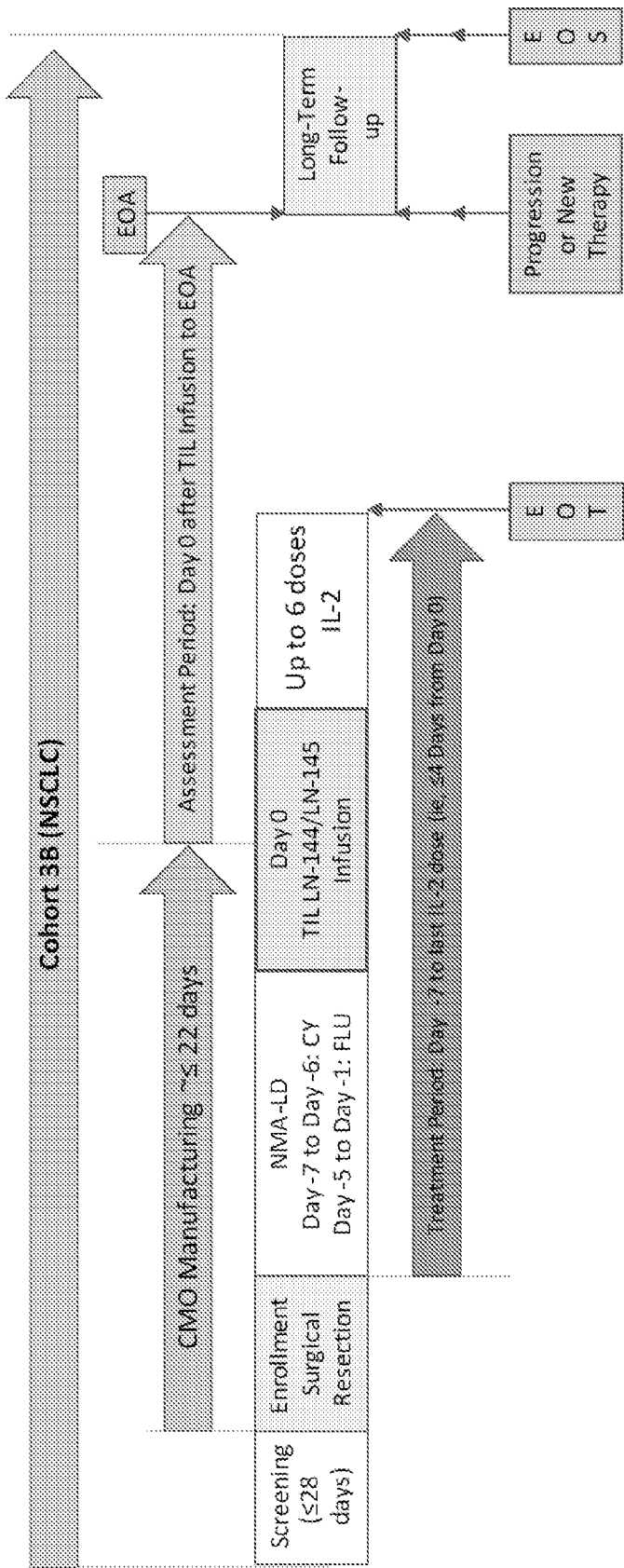


Figure 8

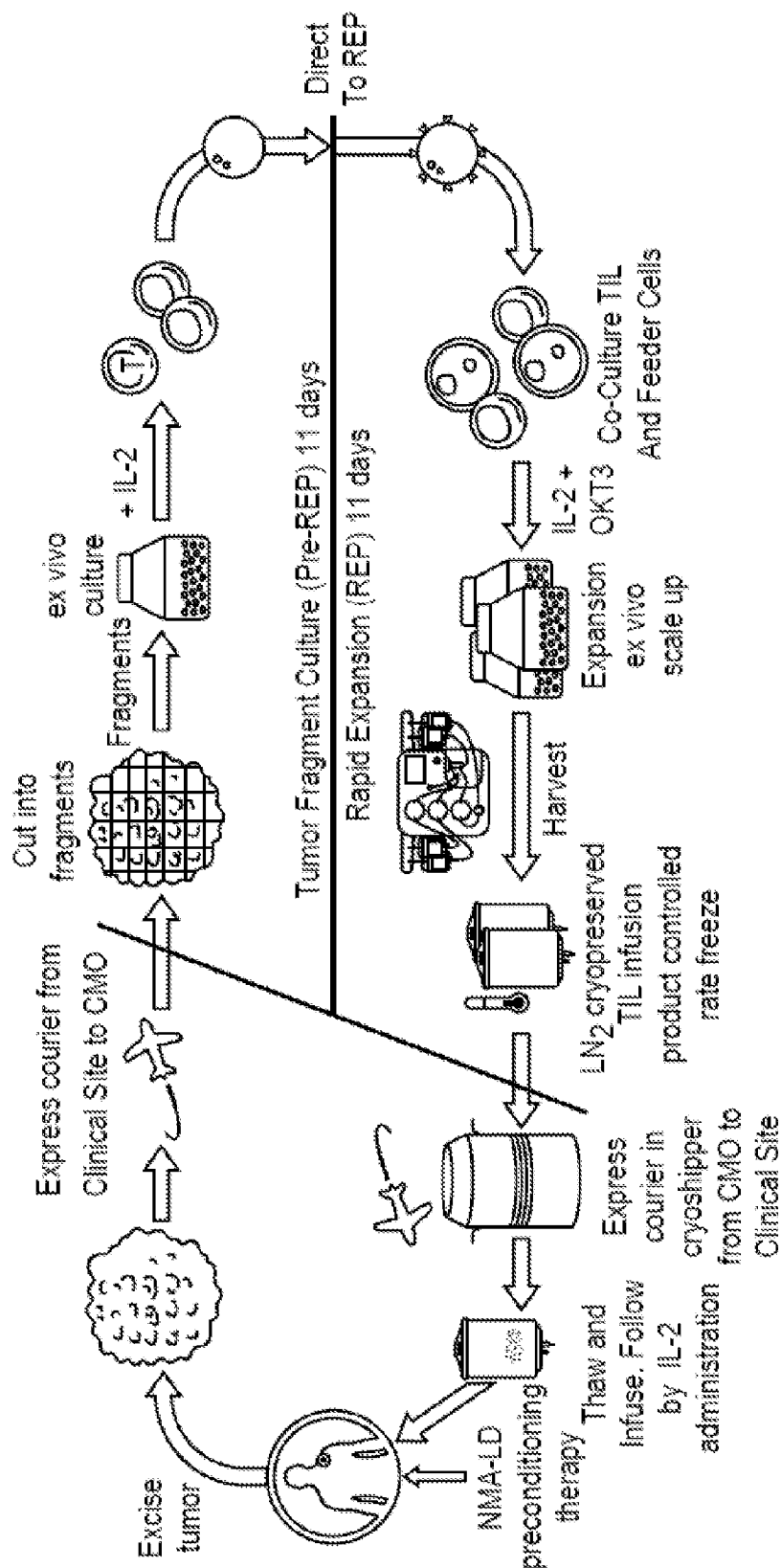
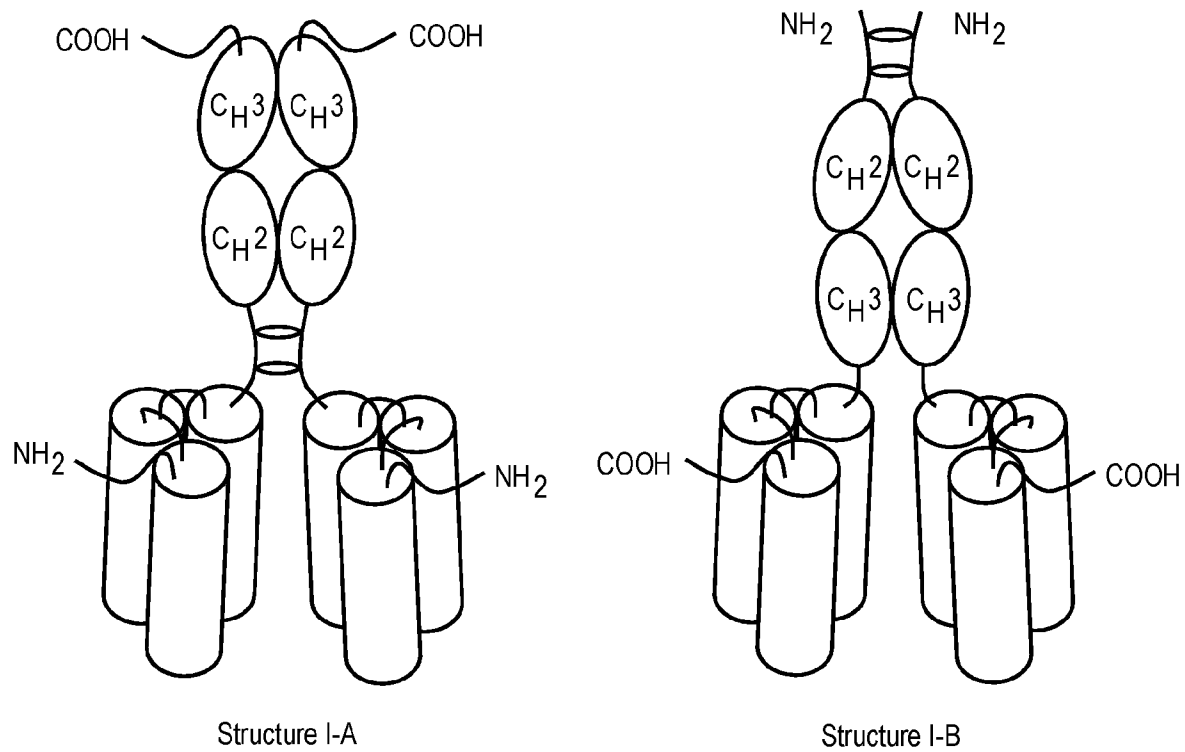


Figure 9

**Figure 10**

SEQUENCE LISTING

<110> Iovance Biotherapeutics, Inc.
Fardis, Maria
Natarajan, Arvind

<120> TREATMENT OF NSCLC PATIENTS REFRACTORY FOR ANTI-PD-1 ANTIBODY

<130> 116983-5043-WO

<150> US 62/725,976

<151> 2018-08-31

<150> US 62/726,919

<151> 2018-09-04

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			20					25					30		

Thr	Met	His	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
		35					40					45			

Gly	Tyr	Ile	Asn	Pro	Ser	Arg	Gly	Tyr	Thr	Asn	Tyr	Asn	Gln	Lys	Phe
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Lys	Asp	Lys	Ala	Thr	Leu	Thr	Thr	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr
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Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr
115 120 125

Pro Leu Ala Pro Val Cys Gly Gly Thr Thr Gly Ser Ser Val Thr Leu
130 135 140

Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu Thr Trp
145 150 155 160

Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Asp Leu Tyr Thr Leu Ser Ser Val Thr Val Thr Ser Ser
180 185 190

Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro Ala Ser
195 200 205

Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Pro Lys Ser Cys Asp
210 215 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225 230 235 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
 290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
 305 310 315 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
 325 330 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
 340 345 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
 355 360 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
 370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
 385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
 405 410 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
 420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
 435 440 445

Gly Lys
 450

<210> 2
 <211> 213
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Muromonab light chain

<400> 2

Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala His Phe Arg Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Gly Met Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
85 90 95

Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn Arg Ala Asp Thr Ala Pro
100 105 110

Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly Gly
115 120 125

Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile Asn
130 135 140

Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val Leu Asn
145 150 155 160

Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser Ser
165 170 175

Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr Thr
180 185 190

Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val Lys Ser Phe
195 200 205

Asn Arg Asn Glu Cys
210

<210> 3
<211> 134
<212> PRT
<213> Artificial Sequence

<220>
<223> recombinant human IL-2 (rhIL-2)

<400> 3

Met Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu
1 5 10 15

His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr
20 25 30

Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro
35 40 45

Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu
50 55 60

Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His
65 70 75 80

Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu
85 90 95

Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr
100 105 110

Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser
115 120 125

Ile Ile Ser Thr Leu Thr
130

<210> 4
<211> 132
<212> PRT
<213> Artificial Sequence

<220>
<223> Aldesleukin

<400> 4

Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu
1 5 10 15

Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn
20 25 30

Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys Lys
35 40 45

Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro
50 55 60

Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg
65 70 75 80

Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys
85 90 95

Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr
100 105 110

Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ser Gln Ser Ile Ile
115 120 125

Ser Thr Leu Thr
130

<210> 5
<211> 130
<212> PRT
<213> Artificial Sequence

<220>
<223> recombinant human IL-4 (rhIL-4)

<400> 5

Met His Lys Cys Asp Ile Thr Leu Gln Glu Ile Ile Lys Thr Leu Asn
1 5 10 15

Ser Leu Thr Glu Gln Lys Thr Leu Cys Thr Glu Leu Thr Val Thr Asp
20 25 30

Ile Phe Ala Ala Ser Lys Asn Thr Thr Glu Lys Glu Thr Phe Cys Arg
35 40 45

Ala Ala Thr Val Leu Arg Gln Phe Tyr Ser His His Glu Lys Asp Thr
50 55 60

Arg Cys Leu Gly Ala Thr Ala Gln Gln Phe His Arg His Lys Gln Leu
65 70 75 80

Ile Arg Phe Leu Lys Arg Leu Asp Arg Asn Leu Trp Gly Leu Ala Gly
85 90 95

Leu Asn Ser Cys Pro Val Lys Glu Ala Asn Gln Ser Thr Leu Glu Asn
100 105 110

Phe Leu Glu Arg Leu Lys Thr Ile Met Arg Glu Lys Tyr Ser Lys Cys
115 120 125

Ser Ser
130

<210> 6
 <211> 153
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> recombinant human IL-7 (rhIL-7)

<400> 6

Met Asp Cys Asp Ile Glu Gly Lys Asp Gly Lys Gln Tyr Glu Ser Val
 1 5 10 15

Leu Met Val Ser Ile Asp Gln Leu Leu Asp Ser Met Lys Glu Ile Gly
 20 25 30

Ser Asn Cys Leu Asn Asn Glu Phe Asn Phe Phe Lys Arg His Ile Cys
 35 40 45

Asp Ala Asn Lys Glu Gly Met Phe Leu Phe Arg Ala Ala Arg Lys Leu
 50 55 60

Arg Gln Phe Leu Lys Met Asn Ser Thr Gly Asp Phe Asp Leu His Leu
 65 70 75 80

Leu Lys Val Ser Glu Gly Thr Thr Ile Leu Leu Asn Cys Thr Gly Gln
 85 90 95

Val Lys Gly Arg Lys Pro Ala Ala Leu Gly Glu Ala Gln Pro Thr Lys
 100 105 110

Ser Leu Glu Glu Asn Lys Ser Leu Lys Glu Gln Lys Lys Leu Asn Asp
 115 120 125

Leu Cys Phe Leu Lys Arg Leu Leu Gln Glu Ile Lys Thr Cys Trp Asn
 130 135 140

Lys Ile Leu Met Gly Thr Lys Glu His
 145 150

<210> 7
 <211> 115
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> recombinant human IL-15 (rhIL-15)

<400> 7

Met Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu
 1 5 10 15

Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val
 20 25 30

His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu
 35 40 45

Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val
 50 55 60

Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn
 65 70 75 80

Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn
 85 90 95

Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile
 100 105 110

Asn Thr Ser
 115

<210> 8
 <211> 132
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> recombinant human IL-21 (rhIL-21)

<400> 8

Met Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile Asp Ile Val
1 5 10 15

Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu Phe Leu Pro
20 25 30

Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala Phe Ser Cys
35 40 45

Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn Asn Glu Arg
50 55 60

Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro Ser Thr
65 70 75 80

Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro Ser Cys Asp
85 90 95

Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Phe Lys Ser
100 105 110

Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg Thr His Gly
115 120 125

Ser Glu Asp Ser
130

<210> 9

<211> 255

<212> PRT

<213> Artificial Sequence

<220>

<223> human 4-1BB, Tumor necrosis factor receptor superfamily, member 9
(Homo sapiens)

<400> 9

Met Gly Asn Ser Cys Tyr Asn Ile Val Ala Thr Leu Leu Leu Val Leu

1	5	10	15
Asn Phe Glu Arg Thr Arg Ser Leu Gln Asp Pro Cys Ser Asn Cys Pro	20	25	30
Ala Gly Thr Phe Cys Asp Asn Asn Arg Asn Gln Ile Cys Ser Pro Cys	35	40	45
Pro Pro Asn Ser Phe Ser Ser Ala Gly Gly Gln Arg Thr Cys Asp Ile	50	55	60
Cys Arg Gln Cys Lys Gly Val Phe Arg Thr Arg Lys Glu Cys Ser Ser	65	70	75
Thr Ser Asn Ala Glu Cys Asp Cys Thr Pro Gly Phe His Cys Leu Gly	85	90	95
Ala Gly Cys Ser Met Cys Glu Gln Asp Cys Lys Gln Gly Gln Glu Leu	100	105	110
Thr Lys Lys Gly Cys Lys Asp Cys Cys Phe Gly Thr Phe Asn Asp Gln	115	120	125
Lys Arg Gly Ile Cys Arg Pro Trp Thr Asn Cys Ser Leu Asp Gly Lys	130	135	140
Ser Val Leu Val Asn Gly Thr Lys Glu Arg Asp Val Val Cys Gly Pro	145	150	155
Ser Pro Ala Asp Leu Ser Pro Gly Ala Ser Ser Val Thr Pro Pro Ala	165	170	175
Pro Ala Arg Glu Pro Gly His Ser Pro Gln Ile Ile Ser Phe Phe Leu	180	185	190
Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu	195	200	205

Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe
 210 215 220

Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly
 225 230 235 240

Cys Ser Cys Arg Phe Pro Glu Glu Glu Gly Gly Cys Glu Leu
 245 250 255

<210> 10

<211> 256

<212> PRT

<213> Artificial Sequence

<220>

<223> murine 4-1BB, Tumor necrosis factor receptor superfamily, member
 9 (Mus musculus)

<400> 10

Met Gly Asn Asn Cys Tyr Asn Val Val Val Ile Val Leu Leu Leu Val
 1 5 10 15

Gly Cys Glu Lys Val Gly Ala Val Gln Asn Ser Cys Asp Asn Cys Gln
 20 25 30

Pro Gly Thr Phe Cys Arg Lys Tyr Asn Pro Val Cys Lys Ser Cys Pro
 35 40 45

Pro Ser Thr Phe Ser Ser Ile Gly Gly Gln Pro Asn Cys Asn Ile Cys
 50 55 60

Arg Val Cys Ala Gly Tyr Phe Arg Phe Lys Lys Phe Cys Ser Ser Thr
 65 70 75 80

His Asn Ala Glu Cys Glu Cys Ile Glu Gly Phe His Cys Leu Gly Pro
 85 90 95

Gln Cys Thr Arg Cys Glu Lys Asp Cys Arg Pro Gly Gln Glu Leu Thr
 100 105 110

Lys Gln Gly Cys Lys Thr Cys Ser Leu Gly Thr Phe Asn Asp Gln Asn
115 120 125

Gly Thr Gly Val Cys Arg Pro Trp Thr Asn Cys Ser Leu Asp Gly Arg
130 135 140

Ser Val Leu Lys Thr Gly Thr Thr Glu Lys Asp Val Val Cys Gly Pro
145 150 155 160

Pro Val Val Ser Phe Ser Pro Ser Thr Thr Ile Ser Val Thr Pro Glu
165 170 175

Gly Gly Pro Gly Gly His Ser Leu Gln Val Leu Thr Leu Phe Leu Ala
180 185 190

Leu Thr Ser Ala Leu Leu Leu Ala Leu Ile Phe Ile Thr Leu Leu Phe
195 200 205

Ser Val Leu Lys Trp Ile Arg Lys Lys Phe Pro His Ile Phe Lys Gln
210 215 220

Pro Phe Lys Lys Thr Thr Gly Ala Ala Gln Glu Glu Asp Ala Cys Ser
225 230 235 240

Cys Arg Cys Pro Gln Glu Glu Glu Gly Gly Gly Gly Gly Tyr Glu Leu
245 250 255

<210> 11

<211> 441

<212> PRT

<213> Artificial Sequence

<220>

<223> heavy chain for utomilumab

<400> 11

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1 5 10 15

Ser Leu Arg Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Ser Thr Tyr
20 25 30

Trp Ile Ser Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Lys Ile Tyr Pro Gly Asp Ser Tyr Thr Asn Tyr Ser Pro Ser Phe
50 55 60

Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65 70 75 80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg Gly Tyr Gly Ile Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
115 120 125

Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu
130 135 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145 150 155 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
165 170 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe
180 185 190

Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr
195 200 205

Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro
 210 215 220

Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro
 225 230 235 240

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
 245 250 255

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp
 260 265 270

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
 275 280 285

Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val
 290 295 300

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
 305 310 315 320

Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly
 325 330 335

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
 340 345 350

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
 355 360 365

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
 370 375 380

Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe
 385 390 395 400

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
 405 410 415

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
420 425 430

Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440

<210> 12
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain for utomilumab

<400> 12

Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln
1 5 10 15

Thr Ala Ser Ile Thr Cys Ser Gly Asp Asn Ile Gly Asp Gln Tyr Ala
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Val Leu Val Ile Tyr
35 40 45

Gln Asp Lys Asn Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60

Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Met
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Ala Thr Tyr Thr Gly Phe Gly Ser Leu
85 90 95

Ala Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln Pro Lys
100 105 110

Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu Gln
115 120 125

Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr Pro Gly
 130 135 140

Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys Ala Gly
 145 150 155 160

Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr Ala Ala
 165 170 175

Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His Arg Ser
 180 185 190

Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys Thr Val
 195 200 205

Ala Pro Thr Glu Cys Ser
 210

<210> 13
 <211> 116
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> heavy chain variable region for utomilumab

<400> 13

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
 1 5 10 15

Ser Leu Arg Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Ser Thr Tyr
 20 25 30

Trp Ile Ser Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
 35 40 45

Gly Lys Ile Tyr Pro Gly Asp Ser Tyr Thr Asn Tyr Ser Pro Ser Phe
 50 55 60

Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65 70 75 80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg Gly Tyr Gly Ile Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 14
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain variable region for utomilumab

<400> 14

Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln
1 5 10 15

Thr Ala Ser Ile Thr Cys Ser Gly Asp Asn Ile Gly Asp Gln Tyr Ala
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Val Leu Val Ile Tyr
35 40 45

Gln Asp Lys Asn Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60

Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Met
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Ala Thr Tyr Thr Gly Phe Gly Ser Leu
85 90 95

Ala Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105

<210> 15
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain CDR1 for utomilumab

<400> 15

Ser Thr Tyr Trp Ile Ser
1 5

<210> 16
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain CDR2 for utomilumab

<400> 16

Lys Ile Tyr Pro Gly Asp Ser Tyr Thr Asn Tyr Ser Pro Ser Phe Gln
1 5 10 15

Gly

<210> 17
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain CDR3 for utomilumab

<400> 17

Arg Gly Tyr Gly Ile Phe Asp Tyr
1 5

<210> 18
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain CDR1 for utomilumab

<400> 18

Ser Gly Asp Asn Ile Gly Asp Gln Tyr Ala His
1 5 10

<210> 19
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain CDR2 for utomilumab

<400> 19

Gln Asp Lys Asn Arg Pro Ser
1 5

<210> 20
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain CDR3 for utomilumab

<400> 20

Ala Thr Tyr Thr Gly Phe Gly Ser Leu Ala Val
1 5 10

<210> 21
<211> 448
<212> PRT
<213> Artificial Sequence

<220>

<223> heavy chain for urelumab

<400> 21

Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asn His Gly Gly Tyr Val Thr Tyr Asn Pro Ser Leu Glu
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Asp Tyr Gly Pro Gly Asn Tyr Asp Trp Tyr Phe Asp Leu Trp Gly
100 105 110

Arg Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190

Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly
210 215 220

Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser
225 230 235 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
245 250 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro
260 265 270

Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
275 280 285

Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val
290 295 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305 310 315 320

Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr
325 330 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
340 345 350

Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
355 360 365

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
370 375 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
 385 390 395 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser
 405 410 415

Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
 420 425 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
 435 440 445

<210> 22
 <211> 216
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> light chain for urelumab

<400> 22

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
 20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
 35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
 65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
 85 90 95

Ala Leu Thr Phe Cys Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val
100 105 110

Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys
115 120 125

Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg
130 135 140

Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn
145 150 155 160

Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser
165 170 175

Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys
180 185 190

Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
195 200 205

Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 23

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> variable heavy chain for urelumab

<400> 23

Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
1 5 10 15

Val Leu Ser Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys
20 25 30

Pro Ser Glu Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe
35 40 45

Ser Gly Tyr Tyr Trp Ser Trp Ile Arg Gln Ser Pro Glu Lys Gly Leu
50 55 60

Glu Trp Ile Gly Glu Ile Asn His Gly Gly Tyr Val Thr Tyr Asn Pro
65 70 75 80

Ser Leu Glu Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln
85 90 95

Phe Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr
100 105 110

Tyr Cys Ala Arg Asp Tyr Gly Pro
115 120

<210> 24
<211> 110
<212> PRT
<213> Artificial Sequence

<220>
<223> variable light chain for urelumab

<400> 24

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser
35 40 45

Val Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln
100 105 110

<210> 25
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain CDR1 for urelumab

<400> 25

Gly Tyr Tyr Trp Ser
1 5

<210> 26
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain CDR2 for urelumab

<400> 26

Glu Ile Asn His Gly Gly Tyr Val Thr Tyr Asn Pro Ser Leu Glu Ser
1 5 10 15

<210> 27
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain CDR3 for urelumab

<400> 27

Asp Tyr Gly Pro Gly Asn Tyr Asp Trp Tyr Phe Asp Leu
1 5 10

<210> 28
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain CDR1 for urelumab

<400> 28

Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala
1 5 10

<210> 29
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain CDR2 for urelumab

<400> 29

Asp Ala Ser Asn Arg Ala Thr
1 5

<210> 30
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain CDR3 for urelumab

<400> 30

Gln Gln Arg Ser Asp Trp Pro Pro Ala Leu Thr
1 5 10

<210> 31
<211> 230
<212> PRT
<213> Artificial Sequence

<220>

<223> Fc domain

<400> 31

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
1 5 10 15

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
20 25 30

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
35 40 45

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
50 55 60

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
65 70 75 80

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
85 90 95

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
100 105 110

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
115 120 125

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
130 135 140

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
145 150 155 160

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
165 170 175

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
180 185 190

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
195 200 205

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
210 215 220

Ser Leu Ser Pro Gly Lys
225 230

<210> 32
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 32

Gly Gly Pro Gly Ser Ser Lys Ser Cys Asp Lys Thr His Thr Cys Pro
1 5 10 15

Pro Cys Pro Ala Pro Glu
20

<210> 33
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 33

Gly Gly Ser Gly Ser Ser Lys Ser Cys Asp Lys Thr His Thr Cys Pro
1 5 10 15

Pro Cys Pro Ala Pro Glu
20

<210> 34
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 34

Gly Gly Pro Gly Ser Ser Ser Ser Ser Ser Ser Lys Ser Cys Asp Lys
1 5 10 15

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
20 25

<210> 35
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 35

Gly Gly Ser Gly Ser Ser Ser Ser Ser Ser Ser Lys Ser Cys Asp Lys
1 5 10 15

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
20 25

<210> 36
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 36

Gly Gly Pro Gly Ser Ser Ser Ser Ser Ser Ser Ser Ser Lys Ser Cys
1 5 10 15

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
20 25

<210> 37
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 37

Gly Gly Ser Gly Ser Ser Ser Ser Ser Ser Ser Ser Ser Lys Ser Cys
1 5 10 15

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
20 25

<210> 38
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 38

Gly Gly Pro Gly Ser Ser Gly Ser Gly Ser Ser Asp Lys Thr His Thr
1 5 10 15

Cys Pro Pro Cys Pro Ala Pro Glu
20

<210> 39
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 39

Gly Gly Pro Gly Ser Ser Gly Ser Gly Ser Asp Lys Thr His Thr Cys
1 5 10 15

Pro Pro Cys Pro Ala Pro Glu
20

<210> 40

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> linker

<400> 40

Gly Gly Pro Ser Ser Ser Gly Ser Asp Lys Thr His Thr Cys Pro Pro
1 5 10 15

Cys Pro Ala Pro Glu
20

<210> 41

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> linker

<400> 41

Gly Gly Ser Ser Ser Ser Ser Ser Ser Gly Ser Asp Lys Thr His
1 5 10 15

Thr Cys Pro Pro Cys Pro Ala Pro Glu
20 25

<210> 42

<211> 246

<212> PRT

<213> Artificial Sequence

<220>

<223> Fc domain

<400> 42

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Ala Gly Asn Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
20 25 30

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
35 40 45

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
50 55 60

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
65 70 75 80

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
85 90 95

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
100 105 110

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
115 120 125

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
130 135 140

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
145 150 155 160

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
165 170 175

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
180 185 190

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
195 200 205

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
210 215 220

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
225 230 235 240

Leu Ser Leu Ser Pro Gly
245

<210> 43
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 43

Ser Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser
1 5 10

<210> 44
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 44

Ser Ser Ser Ser Ser Ser Gly Ser Gly Ser Gly Ser
1 5 10

<210> 45
<211> 16

<212> PRT
<213> Artificial Sequence

<220>
<223> linker

<400> 45

Ser Ser Ser Ser Ser Ser Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser
1 5 10 15

<210> 46
<211> 254
<212> PRT
<213> Artificial Sequence

<220>
<223> 4-1BBL

<400> 46

Met Glu Tyr Ala Ser Asp Ala Ser Leu Asp Pro Glu Ala Pro Trp Pro
1 5 10 15

Pro Ala Pro Arg Ala Arg Ala Cys Arg Val Leu Pro Trp Ala Leu Val
20 25 30

Ala Gly Leu Leu Leu Leu Leu Leu Leu Ala Ala Ala Cys Ala Val Phe
35 40 45

Leu Ala Cys Pro Trp Ala Val Ser Gly Ala Arg Ala Ser Pro Gly Ser
50 55 60

Ala Ala Ser Pro Arg Leu Arg Glu Gly Pro Glu Leu Ser Pro Asp Asp
65 70 75 80

Pro Ala Gly Leu Leu Asp Leu Arg Gln Gly Met Phe Ala Gln Leu Val
85 90 95

Ala Gln Asn Val Leu Leu Ile Asp Gly Pro Leu Ser Trp Tyr Ser Asp
100 105 110

Pro Gly Leu Ala Gly Val Ser Leu Thr Gly Gly Leu Ser Tyr Lys Glu
115 120 125

Asp Thr Lys Glu Leu Val Val Ala Lys Ala Gly Val Tyr Tyr Val Phe
130 135 140

Phe Gln Leu Glu Leu Arg Arg Val Val Ala Gly Glu Gly Ser Gly Ser
145 150 155 160

Val Ser Leu Ala Leu His Leu Gln Pro Leu Arg Ser Ala Ala Gly Ala
165 170 175

Ala Ala Leu Ala Leu Thr Val Asp Leu Pro Pro Ala Ser Ser Glu Ala
180 185 190

Arg Asn Ser Ala Phe Gly Phe Gln Gly Arg Leu Leu His Leu Ser Ala
195 200 205

Gly Gln Arg Leu Gly Val His Leu His Thr Glu Ala Arg Ala Arg His
210 215 220

Ala Trp Gln Leu Thr Gln Gly Ala Thr Val Leu Gly Leu Phe Arg Val
225 230 235 240

Thr Pro Glu Ile Pro Ala Gly Leu Pro Ser Pro Arg Ser Glu
245 250

<210> 47
<211> 168
<212> PRT
<213> Artificial Sequence

<220>
<223> 4-1BBL soluble domain

<400> 47

Leu Arg Gln Gly Met Phe Ala Gln Leu Val Ala Gln Asn Val Leu Leu
1 5 10 15

Ile Asp Gly Pro Leu Ser Trp Tyr Ser Asp Pro Gly Leu Ala Gly Val
20 25 30

Ser Leu Thr Gly Gly Leu Ser Tyr Lys Glu Asp Thr Lys Glu Leu Val
35 40 45

Val Ala Lys Ala Gly Val Tyr Tyr Val Phe Phe Gln Leu Glu Leu Arg
50 55 60

Arg Val Val Ala Gly Glu Gly Ser Gly Ser Val Ser Leu Ala Leu His
65 70 75 80

Leu Gln Pro Leu Arg Ser Ala Ala Gly Ala Ala Ala Leu Ala Leu Thr
85 90 95

Val Asp Leu Pro Pro Ala Ser Ser Glu Ala Arg Asn Ser Ala Phe Gly
100 105 110

Phe Gln Gly Arg Leu Leu His Leu Ser Ala Gly Gln Arg Leu Gly Val
115 120 125

His Leu His Thr Glu Ala Arg Ala Arg His Ala Trp Gln Leu Thr Gln
130 135 140

Gly Ala Thr Val Leu Gly Leu Phe Arg Val Thr Pro Glu Ile Pro Ala
145 150 155 160

Gly Leu Pro Ser Pro Arg Ser Glu
165

<210> 48
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> variable heavy chain for 4B4-1-1 version 1

<400> 48

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ser Tyr
20 25 30

Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Val Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asn Pro Gly Asn Gly His Thr Asn Tyr Asn Glu Lys Phe
50 55 60

Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Phe Thr Thr Ala Arg Gly Phe Ala Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser
115

<210> 49

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> variable light chain for 4B4-1-1 version 1

<400> 49

Asp Ile Val Met Thr Gln Ser Pro Ala Thr Gln Ser Val Thr Pro Gly
1 5 10 15

Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Thr Ile Ser Asp Tyr
20 25 30

Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile
35 40 45

Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro
65 70 75 80

Glu Asp Val Gly Val Tyr Tyr Cys Gln Asp Gly His Ser Phe Pro Pro
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 50
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> variable heavy chain for 4B4-1-1 version 2

<400> 50

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ser Tyr
20 25 30

Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Val Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asn Pro Gly Asn Gly His Thr Asn Tyr Asn Glu Lys Phe
50 55 60

Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Phe Thr Thr Ala Arg Gly Phe Ala Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ala
115

<210> 51
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> variable light chain for 4B4-1-1 version 2

<400> 51

Asp Ile Val Met Thr Gln Ser Pro Ala Thr Gln Ser Val Thr Pro Gly
1 5 10 15

Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Thr Ile Ser Asp Tyr
20 25 30

Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile
35 40 45

Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro
65 70 75 80

Glu Asp Val Gly Val Tyr Tyr Cys Gln Asp Gly His Ser Phe Pro Pro
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 52
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> variable heavy chain for H39E3-2

<400> 52

Met Asp Trp Thr Trp Arg Ile Leu Phe Leu Val Ala Ala Ala Thr Gly
 1 5 10 15

Ala His Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
 20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
 35 40 45

Ser Asp Tyr Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
 50 55 60

Glu Trp Val Ala Asp Ile Lys Asn Asp Gly Ser Tyr Thr Asn Tyr Ala
 65 70 75 80

Pro Ser Leu Thr Asn Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
 85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
 100 105 110

Tyr Tyr Cys Ala Arg Glu Leu Thr
 115 120

<210> 53
 <211> 109
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> variable light chain for H39E3-2

<400> 53

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala
20 25 30

Val Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser
35 40 45

Leu Leu Ser Ser Gly Asn Gln Lys Asn Tyr Leu Trp Tyr Gln Gln Lys
50 55 60

Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Tyr Ala Ser Thr Arg Gln
65 70 75 80

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
85 90 95

Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala
100 105

<210> 54

<211> 277

<212> PRT

<213> Artificial Sequence

<220>

<223> human OX40 (Homo sapiens)

<400> 54

Met Cys Val Gly Ala Arg Arg Leu Gly Arg Gly Pro Cys Ala Ala Leu
1 5 10 15

Leu Leu Leu Gly Leu Gly Leu Ser Thr Val Thr Gly Leu His Cys Val
20 25 30

Gly Asp Thr Tyr Pro Ser Asn Asp Arg Cys Cys His Glu Cys Arg Pro
35 40 45

Gly Asn Gly Met Val Ser Arg Cys Ser Arg Ser Gln Asn Thr Val Cys
50 55 60

Arg Pro Cys Gly Pro Gly Phe Tyr Asn Asp Val Val Ser Ser Lys Pro
65 70 75 80

Cys Lys Pro Cys Thr Trp Cys Asn Leu Arg Ser Gly Ser Glu Arg Lys
85 90 95

Gln Leu Cys Thr Ala Thr Gln Asp Thr Val Cys Arg Cys Arg Ala Gly
100 105 110

Thr Gln Pro Leu Asp Ser Tyr Lys Pro Gly Val Asp Cys Ala Pro Cys
115 120 125

Pro Pro Gly His Phe Ser Pro Gly Asp Asn Gln Ala Cys Lys Pro Trp
130 135 140

Thr Asn Cys Thr Leu Ala Gly Lys His Thr Leu Gln Pro Ala Ser Asn
145 150 155 160

Ser Ser Asp Ala Ile Cys Glu Asp Arg Asp Pro Pro Ala Thr Gln Pro
165 170 175

Gln Glu Thr Gln Gly Pro Pro Ala Arg Pro Ile Thr Val Gln Pro Thr
180 185 190

Glu Ala Trp Pro Arg Thr Ser Gln Gly Pro Ser Thr Arg Pro Val Glu
195 200 205

Val Pro Gly Gly Arg Ala Val Ala Ala Ile Leu Gly Leu Gly Leu Val
210 215 220

Leu Gly Leu Leu Gly Pro Leu Ala Ile Leu Leu Ala Leu Tyr Leu Leu
225 230 235 240

Arg Arg Asp Gln Arg Leu Pro Pro Asp Ala His Lys Pro Pro Gly Gly
245 250 255

Gly Ser Phe Arg Thr Pro Ile Gln Glu Glu Gln Ala Asp Ala His Ser
260 265 270

Thr Leu Ala Lys Ile
275

<210> 55
<211> 272
<212> PRT
<213> Artificial Sequence

<220>
<223> murine OX40 (Mus musculus)

<400> 55

Met Tyr Val Trp Val Gln Gln Pro Thr Ala Leu Leu Leu Leu Gly Leu
1 5 10 15

Thr Leu Gly Val Thr Ala Arg Arg Leu Asn Cys Val Lys His Thr Tyr
20 25 30

Pro Ser Gly His Lys Cys Cys Arg Glu Cys Gln Pro Gly His Gly Met
35 40 45

Val Ser Arg Cys Asp His Thr Arg Asp Thr Leu Cys His Pro Cys Glu
50 55 60

Thr Gly Phe Tyr Asn Glu Ala Val Asn Tyr Asp Thr Cys Lys Gln Cys
65 70 75 80

Thr Gln Cys Asn His Arg Ser Gly Ser Glu Leu Lys Gln Asn Cys Thr
85 90 95

Pro Thr Gln Asp Thr Val Cys Arg Cys Arg Pro Gly Thr Gln Pro Arg
100 105 110

Gln Asp Ser Gly Tyr Lys Leu Gly Val Asp Cys Val Pro Cys Pro Pro
115 120 125

Gly His Phe Ser Pro Gly Asn Asn Gln Ala Cys Lys Pro Trp Thr Asn
130 135 140

Cys Thr Leu Ser Gly Lys Gln Thr Arg His Pro Ala Ser Asp Ser Leu
145 150 155 160

Asp Ala Val Cys Glu Asp Arg Ser Leu Leu Ala Thr Leu Leu Trp Glu
165 170 175

Thr Gln Arg Pro Thr Phe Arg Pro Thr Thr Val Gln Ser Thr Thr Val
180 185 190

Trp Pro Arg Thr Ser Glu Leu Pro Ser Pro Pro Thr Leu Val Thr Pro
195 200 205

Glu Gly Pro Ala Phe Ala Val Leu Leu Gly Leu Gly Leu Gly Leu Leu
210 215 220

Ala Pro Leu Thr Val Leu Leu Ala Leu Tyr Leu Leu Arg Lys Ala Trp
225 230 235 240

Arg Leu Pro Asn Thr Pro Lys Pro Cys Trp Gly Asn Ser Phe Arg Thr
245 250 255

Pro Ile Gln Glu Glu His Thr Asp Ala His Phe Thr Leu Ala Lys Ile
260 265 270

<210> 56

<211> 451

<212> PRT

<213> Artificial Sequence

<220>

<223> heavy chain for tavolixizumab

<400> 56

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
 1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Ser Gly
 20 25 30

Tyr Trp Asn Trp Ile Arg Lys His Pro Gly Lys Gly Leu Glu Tyr Ile
 35 40 45

Gly Tyr Ile Ser Tyr Asn Gly Ile Thr Tyr His Asn Pro Ser Leu Lys
 50 55 60

Ser Arg Ile Thr Ile Asn Arg Asp Thr Ser Lys Asn Gln Tyr Ser Leu
 65 70 75 80

Gln Leu Asn Ser Val Thr Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95

Arg Tyr Lys Tyr Asp Tyr Asp Gly Gly His Ala Met Asp Tyr Trp Gly
 100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
 115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
 130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
 145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
 165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
 180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
 195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys
 210 215 220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
 225 230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
 245 250 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
 260 265 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
 275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
 290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
 305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
 325 330 335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
 340 345 350

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
 355 360 365

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
 370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
 385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

Pro Gly Lys
450

<210> 57
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain for tavolixizumab

<400> 57

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Tyr Thr Ser Lys Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Ser Ala Leu Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 58
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain variable region for tavolixizumab

<400> 58

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Ser Gly
20 25 30

Tyr Trp Asn Trp Ile Arg Lys His Pro Gly Lys Gly Leu Glu Tyr Ile
35 40 45

Gly Tyr Ile Ser Tyr Asn Gly Ile Thr Tyr His Asn Pro Ser Leu Lys
50 55 60

Ser Arg Ile Thr Ile Asn Arg Asp Thr Ser Lys Asn Gln Tyr Ser Leu
65 70 75 80

Gln Leu Asn Ser Val Thr Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Tyr Lys Tyr Asp Tyr Asp Gly Gly His Ala Met Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr
115

<210> 59

<211> 108

<212> PRT

<213> Artificial Sequence

<220>

<223> light chain variable region for tavolixizumab

<400> 59

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Tyr Thr Ser Lys Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Ser Ala Leu Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 60
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain CDR1 for tavolixizumab

<400> 60

Gly Ser Phe Ser Ser Gly Tyr Trp Asn
1 5

<210> 61
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain CDR2 for tavolixizumab

<400> 61

Tyr Ile Gly Tyr Ile Ser Tyr Asn Gly Ile Thr Tyr His
1 5 10

<210> 62
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain CDR3 for tavolixizumab

<400> 62

Arg Tyr Lys Tyr Asp Tyr Asp Gly Gly His Ala Met Asp Tyr
1 5 10

<210> 63
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain CDR1 for tavolixizumab

<400> 63

Gln Asp Ile Ser Asn Tyr Leu Asn
1 5

<210> 64
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain CDR2 for tavolixizumab

<400> 64

Leu Leu Ile Tyr Tyr Thr Ser Lys Leu His Ser
1 5 10

<210> 65
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain CDR3 for tavolixizumab

<400> 65

Gln Gln Gly Ser Ala Leu Pro Trp
1 5

<210> 66
<211> 444
<212> PRT
<213> Artificial Sequence

<220>

<223> heavy chain for 11D4

<400> 66

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Ser Ser Thr Ile Asp Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Ser Gly Trp Tyr Leu Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
130 135 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190

Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
195 200 205

Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys
210 215 220

Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe
225 230 235 240

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
245 250 255

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe
260 265 270

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
275 280 285

Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr
290 295 300

Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
305 310 315 320

Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr
325 330 335

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
340 345 350

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
355 360 365

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
370 375 380

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser
 385 390 395 400

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
 405 410 415

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 420 425 430

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 435 440

<210> 67
 <211> 214
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> light chain for 11D4

<400> 67

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
 20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile
 35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr Pro Pro
 85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 68
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain variable region for 11D4

<400> 68

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Ser Thr Ile Asp Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Ser Gly Trp Tyr Leu Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 69

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> light chain variable region for 11D4

<400> 69

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr Pro Pro
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 70
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain CDR1 for 11D4

<400> 70

Ser Tyr Ser Met Asn
1 5

<210> 71
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain CDR2 for 11D4

<400> 71

Tyr Ile Ser Ser Ser Ser Ser Thr Ile Asp Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 72
<211> 9
<212> PRT
<213> Artificial Sequence

<220>

<223> heavy chain CDR3 for 11D4

<400> 72

Glu Ser Gly Trp Tyr Leu Phe Asp Tyr
1 5

<210> 73

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> light chain CDR1 for 11D4

<400> 73

Arg Ala Ser Gln Gly Ile Ser Ser Trp Leu Ala
1 5 10

<210> 74

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> light chain CDR2 for 11D4

<400> 74

Ala Ala Ser Ser Leu Gln Ser
1 5

<210> 75

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> light chain CDR3 for 11D4

<400> 75

Gln Gln Tyr Asn Ser Tyr Pro Pro Thr
1 5

<210> 76
 <211> 450
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> heavy chain for 18D8

<400> 76

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
 20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Gly Ile Ser Trp Asn Ser Gly Ser Ile Gly Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys
 85 90 95

Ala Lys Asp Gln Ser Thr Ala Asp Tyr Tyr Phe Tyr Tyr Gly Met Asp
 100 105 110

Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys
 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
 130 135 140

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
 145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
165 170 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
180 185 190

Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn
195 200 205

Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg
210 215 220

Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
225 230 235 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
290 295 300

Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
325 330 335

Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
 355 360 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
 370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
 385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
 405 410 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
 420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
 435 440 445

Gly Lys
 450

<210> 77
 <211> 213
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> light chain for 18D8

<400> 77

Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
 20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
 35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Thr
85 90 95

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

<210> 78

<211> 124

<212> PRT

<213> Artificial Sequence

<220>

<223> heavy chain variable region for 18D8

<400> 78

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Ser Trp Asn Ser Gly Ser Ile Gly Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Lys Asp Gln Ser Thr Ala Asp Tyr Tyr Phe Tyr Tyr Gly Met Asp
100 105 110

Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 79

<211> 106

<212> PRT

<213> Artificial Sequence

<220>

<223> light chain variable region for 18D8

<400> 79

Glu Ile Val Val Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Thr
85 90 95

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 80

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> heavy chain CDR1 for 18D8

<400> 80

Asp Tyr Ala Met His
1 5

<210> 81

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> heavy chain CDR2 for 18D8

<400> 81

Gly Ile Ser Trp Asn Ser Gly Ser Ile Gly Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 82
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain CDR3 for 18D8

<400> 82

Asp	Gln	Ser	Thr	Ala	Asp	Tyr	Tyr	Phe	Tyr	Tyr	Gly	Met	Asp	Val
1				5					10					15

<210> 83
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain CDR1 for 18D8

<400> 83

Arg	Ala	Ser	Gln	Ser	Val	Ser	Ser	Tyr	Leu	Ala
1				5					10	

<210> 84
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain CDR2 for 18D8

<400> 84

Asp	Ala	Ser	Asn	Arg	Ala	Thr
1				5		

<210> 85
<211> 8

<212> PRT
<213> Artificial Sequence

<220>
<223> light chain CDR3 for 18D8

<400> 85

Gln Gln Arg Ser Asn Trp Pro Thr
1 5

<210> 86
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain variable region for Hu119-122

<400> 86

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Glu Tyr Glu Phe Pro Ser His
20 25 30

Asp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val
35 40 45

Ala Ala Ile Asn Ser Asp Gly Gly Ser Thr Tyr Tyr Pro Asp Thr Met
50 55 60

Glu Arg Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg His Tyr Asp Asp Tyr Tyr Ala Trp Phe Ala Tyr Trp Gly Gln
100 105 110

Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 87
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain variable region for Hu119-122

<400> 87

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Ser
20 25 30

Gly Tyr Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
35 40 45

Arg Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Ser Arg
85 90 95

Glu Leu Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 88
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain CDR1 for Hu119-122

<400> 88

Ser His Asp Met Ser
1 5

<210> 89

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> heavy chain CDR2 for Hu119-122

<400> 89

Ala Ile Asn Ser Asp Gly Gly Ser Thr Tyr Tyr Pro Asp Thr Met Glu
1 5 10 15

Arg

<210> 90

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> heavy chain CDR3 for Hu119-122

<400> 90

His Tyr Asp Asp Tyr Tyr Ala Trp Phe Ala Tyr
1 5 10

<210> 91

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> light chain CDR1 for Hu119-122

<400> 91

Arg Ala Ser Lys Ser Val Ser Thr Ser Gly Tyr Ser Tyr Met His
1 5 10 15

<210> 92
 <211> 7
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> light chain CDR2 for Hu119-122

 <400> 92

Leu Ala Ser Asn Leu Glu Ser
 1 5

<210> 93
 <211> 9
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> light chain CDR3 for Hu119-122

 <400> 93

Gln His Ser Arg Glu Leu Pro Leu Thr
 1 5

<210> 94
 <211> 122
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> heavy chain variable region for Hu106-222

 <400> 94

Gln Val Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
 20 25 30

Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Lys Trp Met
 35 40 45

Gly Trp Ile Asn Thr Glu Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Asn Pro Tyr Tyr Asp Tyr Val Ser Tyr Tyr Ala Met Asp Tyr Trp
100 105 110

Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 95

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> light chain variable region for Hu106-222

<400> 95

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Ser Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Tyr Leu Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln His Tyr Ser Thr Pro Arg
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 96
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain CDR1 for Hu106-222

<400> 96

Asp Tyr Ser Met His
1 5

<210> 97
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain CDR2 for Hu106-222

<400> 97

Trp Ile Asn Thr Glu Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe Lys
1 5 10 15

Gly

<210> 98
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain CDR3 for Hu106-222

<400> 98

Pro Tyr Tyr Asp Tyr Val Ser Tyr Tyr Ala Met Asp Tyr
1 5 10

<210> 99

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> light chain CDR1 for Hu106-222

<400> 99

Lys Ala Ser Gln Asp Val Ser Thr Ala Val Ala
1 5 10

<210> 100

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> light chain CDR2 for Hu106-222

<400> 100

Ser Ala Ser Tyr Leu Tyr Thr
1 5

<210> 101

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> light chain CDR3 for Hu106-222

<400> 101

Gln Gln His Tyr Ser Thr Pro Arg Thr
1 5

<210> 102

<211> 183

<212> PRT
<213> Artificial Sequence

<220>
<223> OX40L

<400> 102

Met Glu Arg Val Gln Pro Leu Glu Glu Asn Val Gly Asn Ala Ala Arg
1 5 10 15

Pro Arg Phe Glu Arg Asn Lys Leu Leu Leu Val Ala Ser Val Ile Gln
20 25 30

Gly Leu Gly Leu Leu Leu Cys Phe Thr Tyr Ile Cys Leu His Phe Ser
35 40 45

Ala Leu Gln Val Ser His Arg Tyr Pro Arg Ile Gln Ser Ile Lys Val
50 55 60

Gln Phe Thr Glu Tyr Lys Lys Glu Lys Gly Phe Ile Leu Thr Ser Gln
65 70 75 80

Lys Glu Asp Glu Ile Met Lys Val Gln Asn Asn Ser Val Ile Ile Asn
85 90 95

Cys Asp Gly Phe Tyr Leu Ile Ser Leu Lys Gly Tyr Phe Ser Gln Glu
100 105 110

Val Asn Ile Ser Leu His Tyr Gln Lys Asp Glu Glu Pro Leu Phe Gln
115 120 125

Leu Lys Lys Val Arg Ser Val Asn Ser Leu Met Val Ala Ser Leu Thr
130 135 140

Tyr Lys Asp Lys Val Tyr Leu Asn Val Thr Thr Asp Asn Thr Ser Leu
145 150 155 160

Asp Asp Phe His Val Asn Gly Gly Glu Leu Ile Leu Ile His Gln Asn
165 170 175

Pro Gly Glu Phe Cys Val Leu
180

<210> 103
<211> 131
<212> PRT
<213> Artificial Sequence

<220>
<223> OX40L soluble domain

<400> 103

Ser His Arg Tyr Pro Arg Ile Gln Ser Ile Lys Val Gln Phe Thr Glu
1 5 10 15

Tyr Lys Lys Glu Lys Gly Phe Ile Leu Thr Ser Gln Lys Glu Asp Glu
20 25 30

Ile Met Lys Val Gln Asn Asn Ser Val Ile Ile Asn Cys Asp Gly Phe
35 40 45

Tyr Leu Ile Ser Leu Lys Gly Tyr Phe Ser Gln Glu Val Asn Ile Ser
50 55 60

Leu His Tyr Gln Lys Asp Glu Glu Pro Leu Phe Gln Leu Lys Lys Val
65 70 75 80

Arg Ser Val Asn Ser Leu Met Val Ala Ser Leu Thr Tyr Lys Asp Lys
85 90 95

Val Tyr Leu Asn Val Thr Thr Asp Asn Thr Ser Leu Asp Asp Phe His
100 105 110

Val Asn Gly Gly Glu Leu Ile Leu Ile His Gln Asn Pro Gly Glu Phe
115 120 125

Cys Val Leu
130

<210> 104
 <211> 128
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> OX40L soluble domain (alternative)

<400> 104

Tyr Pro Arg Ile Gln Ser Ile Lys Val Gln Phe Thr Glu Tyr Lys Lys
 1 5 10 15

Glu Lys Gly Phe Ile Leu Thr Ser Gln Lys Glu Asp Glu Ile Met Lys
 20 25 30

Val Gln Asn Asn Ser Val Ile Ile Asn Cys Asp Gly Phe Tyr Leu Ile
 35 40 45

Ser Leu Lys Gly Tyr Phe Ser Gln Glu Val Asn Ile Ser Leu His Tyr
 50 55 60

Gln Lys Asp Glu Glu Pro Leu Phe Gln Leu Lys Lys Val Arg Ser Val
 65 70 75 80

Asn Ser Leu Met Val Ala Ser Leu Thr Tyr Lys Asp Lys Val Tyr Leu
 85 90 95

Asn Val Thr Thr Asp Asn Thr Ser Leu Asp Asp Phe His Val Asn Gly
 100 105 110

Gly Glu Leu Ile Leu Ile His Gln Asn Pro Gly Glu Phe Cys Val Leu
 115 120 125

<210> 105
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>

<223> variable heavy chain for 008

<400> 105

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30

Thr Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Asp Arg Tyr Ser Gln Val His Tyr Ala Leu Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser
115 120

<210> 106

<211> 108

<212> PRT

<213> Artificial Sequence

<220>

<223> variable light chain for 008

<400> 106

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Ala Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Gln Gln Tyr
85 90 95

Tyr Asn His Pro Thr Thr Phe Gly Gln Gly Thr Lys
100 105

<210> 107

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> variable heavy chain for 011

<400> 107

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Thr Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Arg Lys Gly
50 55 60

Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln
65 70 75 80

Met Asn Asn Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
85 90 95

Asp Arg Tyr Phe Arg Gln Gln Asn Ala Phe Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser Ala
115 120

<210> 108

<211> 108

<212> PRT

<213> Artificial Sequence

<220>

<223> variable light chain for 011

<400> 108

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Ala Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Gln Gln Tyr
85 90 95

Tyr Asn His Pro Thr Thr Phe Gly Gln Gly Thr Lys
100 105

<210> 109
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> variable heavy chain for 021

<400> 109

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Arg Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Asp Arg Tyr Ile Thr Leu Pro Asn Ala Leu Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser
115 120

<210> 110
<211> 108
<212> PRT
<213> Artificial Sequence

<220>

<223> variable light chain for 021

<400> 110

Asp Ile Gln Met Thr Gln Ser Pro Val Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Gln Gln Tyr
85 90 95

Lys Ser Asn Pro Pro Thr Phe Gly Gln Gly Thr Lys
100 105

<210> 111

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> variable heavy chain for 023

<400> 111

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val His Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Gly Thr Gly Gly Gly Thr Tyr Tyr Ala Asp Ser Val Met
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Tyr Asp Asn Val Met Gly Leu Tyr Trp Phe Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 112

<211> 108

<212> PRT

<213> Artificial Sequence

<220>

<223> variable light chain for 023

<400> 112

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
85 90 95

Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 113

<211> 119

<212> PRT

<213> Artificial Sequence

<220>

<223> heavy chain variable region

<400> 113

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Val Met His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Asn Tyr Tyr Gly Ser Ser Leu Ser Met Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Ser Val Thr Val Ser Ser
115

<210> 114
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain variable region

<400> 114

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
1 5 10 15

Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
35 40 45

Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
65 70 75 80

Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 115
<211> 121
<212> PRT
<213> Artificial Sequence

<220>

<223> heavy chain variable region

<400> 115

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr Phe Lys Asp Tyr
20 25 30

Thr Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
35 40 45

Gly Gly Ile Tyr Pro Asn Asn Gly Gly Ser Thr Tyr Asn Gln Asn Phe
50 55 60

Lys Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Phe Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Met Gly Tyr His Gly Pro His Leu Asp Phe Asp Val Trp Gly
100 105 110

Ala Gly Thr Thr Val Thr Val Ser Pro
115 120

<210> 116

<211> 108

<212> PRT

<213> Artificial Sequence

<220>

<223> light chain variable region

<400> 116

Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Leu Gly
1 5 10 15

Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Gly Ala Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
35 40 45

Tyr Trp Ala Ser Thr Arg His Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Gly Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln Ser
65 70 75 80

Glu Asp Leu Thr Asp Tyr Phe Cys Gln Gln Tyr Ile Asn Tyr Pro Leu
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 117

<211> 122

<212> PRT

<213> Artificial Sequence

<220>

<223> heavy chain variable region of humanized antibody

<400> 117

Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
1 5 10 15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Ser Met His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
35 40 45

Gly Trp Ile Asn Thr Glu Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
85 90 95

Ala Asn Pro Tyr Tyr Asp Tyr Val Ser Tyr Tyr Ala Met Asp Tyr Trp
100 105 110

Gly His Gly Thr Ser Val Thr Val Ser Ser
115 120

<210> 118
<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> heavy chain variable region of humanized antibody

<400> 118

Gln Val Gln Leu Val Gln Ser Gly Ser Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Ser Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Lys Trp Met
35 40 45

Gly Trp Ile Asn Thr Glu Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Asn Pro Tyr Tyr Asp Tyr Val Ser Tyr Tyr Ala Met Asp Tyr Trp
100 105 110

Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 119
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain variable region of humanized antibody

<400> 119

Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val Arg
1 5 10 15

Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Ser Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Tyr Leu Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val Gln Ala
65 70 75 80

Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln His Tyr Ser Thr Pro Arg
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 120
<211> 107
<212> PRT
<213> Artificial Sequence

<220>

<223> light chain variable region of humanized antibody

<400> 120

Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val Arg
1 5 10 15

Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Ser Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Tyr Leu Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val Gln Ala
65 70 75 80

Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln His Tyr Ser Thr Pro Arg
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 121

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> heavy chain variable region of humanized antibody

<400> 121

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Glu
1 5 10 15

Ser Leu Lys Leu Ser Cys Glu Ser Asn Glu Tyr Glu Phe Pro Ser His
20 25 30

Asp Met Ser Trp Val Arg Lys Thr Pro Glu Lys Arg Leu Glu Leu Val
 35 40 45

Ala Ala Ile Asn Ser Asp Gly Gly Ser Thr Tyr Tyr Pro Asp Thr Met
 50 55 60

Glu Arg Arg Phe Ile Ile Ser Arg Asp Asn Thr Lys Lys Thr Leu Tyr
 65 70 75 80

Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys
 85 90 95

Ala Arg His Tyr Asp Asp Tyr Tyr Ala Trp Phe Ala Tyr Trp Gly Gln
 100 105 110

Gly Thr Leu Val Thr Val Ser Ala
 115 120

<210> 122

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> heavy chain variable region of humanized antibody

<400> 122

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Glu Tyr Glu Phe Pro Ser His
 20 25 30

Asp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val
 35 40 45

Ala Ala Ile Asn Ser Asp Gly Gly Ser Thr Tyr Tyr Pro Asp Thr Met
 50 55 60

Glu Arg Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg His Tyr Asp Asp Tyr Tyr Ala Trp Phe Ala Tyr Trp Gly Gln
100 105 110

Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 123

<211> 111

<212> PRT

<213> Artificial Sequence

<220>

<223> light chain variable region of humanized antibody

<400> 123

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Ser
20 25 30

Gly Tyr Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ser Arg
85 90 95

Glu Leu Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105 110

<210> 124
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain variable region of humanized antibody

<400> 124

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Ser
20 25 30

Gly Tyr Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
35 40 45

Arg Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Ser Arg
85 90 95

Glu Leu Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 125
<211> 138
<212> PRT
<213> Artificial Sequence

<220>

<223> heavy chain variable region

<400> 125

Met Tyr Leu Gly Leu Asn Tyr Val Phe Ile Val Phe Leu Leu Asn Gly
1 5 10 15

Val Gln Ser Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln
20 25 30

Pro Gly Gly Ser Met Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Asp Ala Trp Met Asp Trp Val Arg Gln Ser Pro Glu Lys Gly Leu
50 55 60

Glu Trp Val Ala Glu Ile Arg Ser Lys Ala Asn Asn His Ala Thr Tyr
65 70 75 80

Tyr Ala Glu Ser Val Asn Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser
85 90 95

Lys Ser Ser Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
100 105 110

Gly Ile Tyr Tyr Cys Thr Trp Gly Glu Val Phe Tyr Phe Asp Tyr Trp
115 120 125

Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
130 135

<210> 126

<211> 126

<212> PRT

<213> Artificial Sequence

<220>

<223> light chain variable region

<400> 126

Met Arg Pro Ser Ile Gln Phe Leu Gly Leu Leu Leu Phe Trp Leu His
 1 5 10 15

Gly Ala Gln Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser
 20 25 30

Ala Ser Leu Gly Gly Lys Val Thr Ile Thr Cys Lys Ser Ser Gln Asp
 35 40 45

Ile Asn Lys Tyr Ile Ala Trp Tyr Gln His Lys Pro Gly Lys Gly Pro
 50 55 60

Arg Leu Leu Ile His Tyr Thr Ser Thr Leu Gln Pro Gly Ile Pro Ser
 65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Arg Asp Tyr Ser Phe Ser Ile Ser
 85 90 95

Asn Leu Glu Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln Tyr Asp
 100 105 110

Asn Leu Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
 115 120 125

<210> 127
 <211> 440
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> nivolumab heavy chain

<400> 127

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
 20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
 100 105 110

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser
 115 120 125

Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
 130 135 140

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
 145 150 155 160

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
 165 170 175

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys
 180 185 190

Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp
 195 200 205

Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala
 210 215 220

Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
 225 230 235 240

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
245 250 255

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
260 265 270

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
275 280 285

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
290 295 300

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
305 310 315 320

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
325 330 335

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
340 345 350

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
355 360 365

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
370 375 380

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
385 390 395 400

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
405 410 415

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
420 425 430

Ser Leu Ser Leu Ser Leu Gly Lys
435 440

<210> 128
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> nivolumab light chain

<400> 128

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Ser Asn Trp Pro Arg
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
 145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
 165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
 180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
 195 200 205

Phe Asn Arg Gly Glu Cys
 210

<210> 129
 <211> 113
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> nivolumab variable heavy chain

<400> 129

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
 20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
100 105 110

Ser

<210> 130
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> nivolumab variable light chain

<400> 130

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Ser Asn Trp Pro Arg
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 131
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> nivolumab heavy chain CDR1

<400> 131

Asn Ser Gly Met His
1 5

<210> 132
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> nivolumab heavy chain CDR2

<400> 132

Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 133
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> nivolumab heavy chain CDR3

<400> 133

Asn Asp Asp Tyr
1

<210> 134
<211> 11
<212> PRT
<213> Artificial Sequence

<220>

<223> nivolumab light chain CDR1

<400> 134

Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala
1 5 10

<210> 135

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> nivolumab light chain CDR2

<400> 135

Asp Ala Ser Asn Arg Ala Thr
1 5

<210> 136

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> nivolumab light chain CDR3

<400> 136

Gln Gln Ser Ser Asn Trp Pro Arg Thr
1 5

<210> 137

<211> 447

<212> PRT

<213> Artificial Sequence

<220>

<223> pembrolizumab heavy chain

<400> 137

Gln Val Gln Leu Val Gln Ser Gly Val Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe
50 55 60

Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr Thr Thr Ala Tyr
65 70 75 80

Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190

Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
 210 215 220

Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val
 225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
 260 265 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
 275 280 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
 290 295 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
 305 310 315 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
 325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
 340 345 350

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
 355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
 405 410 415

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
435 440 445

<210> 138
<211> 218
<212> PRT
<213> Artificial Sequence

<220>
<223> pembrolizumab light chain

<400> 138

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Lys Gly Val Ser Thr Ser
20 25 30

Gly Tyr Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
35 40 45

Arg Leu Leu Ile Tyr Leu Ala Ser Tyr Leu Glu Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Ser Arg
85 90 95

Asp Leu Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 139

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> pembrolizumab variable heavy chain

<400> 139

Gln Val Gln Leu Val Gln Ser Gly Val Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe
50 55 60

Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr Thr Thr Ala Tyr
65 70 75 80

Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 140

<211> 111

<212> PRT

<213> Artificial Sequence

<220>

<223> pembrolizumab variable light chain

<400> 140

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Lys Gly Val Ser Thr Ser
20 25 30

Gly Tyr Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
35 40 45

Arg Leu Leu Ile Tyr Leu Ala Ser Tyr Leu Glu Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Ser Arg
85 90 95

Asp Leu Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 141
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> pembrolizumab heavy chain CDR1

<400> 141

Asn Tyr Tyr Met Tyr
1 5

<210> 142
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> pembrolizumab heavy chain CDR2

<400> 142

Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe Lys
1 5 10 15

<210> 143
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> pembrolizumab heavy chain CDR3

<400> 143

Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr
1 5 10

<210> 144
<211> 15

<212> PRT
<213> Artificial Sequence

<220>
<223> pembrolizumab light chain CDR1

<400> 144

Arg	Ala	Ser	Lys	Gly	Val	Ser	Thr	Ser	Gly	Tyr	Ser	Tyr	Leu	His
1				5					10				15	

<210> 145
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> pembrolizumab light chain CDR2

<400> 145

Leu	Ala	Ser	Tyr	Leu	Glu	Ser
1				5		

<210> 146
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> pembrolizumab light chain CDR3

<400> 146

Gln	His	Ser	Arg	Asp	Leu	Pro	Leu	Thr
1				5				

<210> 147
<211> 451
<212> PRT
<213> Artificial Sequence

<220>
<223> durvalumab heavy chain

<400> 147

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
 20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Glu Gly Gly Trp Phe Gly Glu Leu Ala Phe Asp Tyr Trp Gly
 100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
 115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
 130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
 145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
 165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
 180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
 195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys
 210 215 220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu Gly
 225 230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
 245 250 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
 260 265 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
 275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
 290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
 305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Ser Ile
 325 330 335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
 340 345 350

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
 355 360 365

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
 370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
 385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

Pro Gly Lys
450

<210> 148
<211> 265
<212> PRT
<213> Artificial Sequence

<220>
<223> durvalumab light chain

<400> 148

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Asn Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser
50 55 60

Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Arg Val Ser
65 70 75 80

Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg
85 90 95

Leu Leu Ile Tyr Asp Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg
100 105 110

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg
115 120 125

Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser
130 135 140

Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
145 150 155 160

Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
165 170 175

Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
180 185 190

Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
195 200 205

Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
210 215 220

Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
225 230 235 240

Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
245 250 255

Thr Lys Ser Phe Asn Arg Gly Glu Cys
260 265

<210> 149

<211> 121

<212> PRT

<213> Artificial Sequence

<220>

<223> durvalumab variable heavy chain

<400> 149

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Gly Gly Trp Phe Gly Glu Leu Ala Phe Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 150

<211> 108

<212> PRT

<213> Artificial Sequence

<220>

<223> durvalumab variable light chain

<400> 150

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Arg Val Ser Ser Ser
 20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45

Ile Tyr Asp Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
 65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Leu Pro
 85 90 95

Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 151
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> durvalumab heavy chain CDR1

<400> 151

Arg Tyr Trp Met Ser
 1 5

<210> 152
 <211> 17
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> durvalumab heavy chain CDR2

<400> 152

Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val Lys
 1 5 10 15

Gly

<210> 153
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> durvalumab heavy chain CDR3

<400> 153

Glu	Gly	Gly	Trp	Phe	Gly	Glu	Leu	Ala	Phe	Asp	Tyr
1				5						10	

<210> 154
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> durvalumab light chain CDR1

<400> 154

Arg	Ala	Ser	Gln	Arg	Val	Ser	Ser	Ser	Tyr	Leu	Ala
1				5						10	

<210> 155
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> durvalumab light chain CDR2

<400> 155

Asp	Ala	Ser	Ser	Arg	Ala	Thr
1				5		

<210> 156
<211> 9

<212> PRT
<213> Artificial Sequence

<220>
<223> durvalumab light chain CDR3

<400> 156

Gln Gln Tyr Gly Ser Leu Pro Trp Thr
1 5

<210> 157
<211> 450
<212> PRT
<213> Artificial Sequence

<220>
<223> avelumab heavy chain

<400> 157

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ile Met Met Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Tyr Pro Ser Gly Gly Ile Thr Phe Tyr Ala Asp Thr Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ile Lys Leu Gly Thr Val Thr Thr Val Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
210 215 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225 230 235 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
325 330 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
355 360 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
405 410 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

Gly Lys
450

<210> 158
<211> 216
<212> PRT
<213> Artificial Sequence

<220>
<223> avelumab light chain

<400> 158

Gln Ser Ala Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
1 5 10 15

Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr
20 25 30

Asn Tyr Val Ser Trp Tyr Gln Gln His Pro Gly Lys Ala Pro Lys Leu
35 40 45

Met Ile Tyr Asp Val Ser Asn Arg Pro Ser Gly Val Ser Asn Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
65 70 75 80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ser Ser Tyr Thr Ser Ser
85 90 95

Ser Thr Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Gln
100 105 110

Pro Lys Ala Asn Pro Thr Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
115 120 125

Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
130 135 140

Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Gly Ser Pro Val Lys
145 150 155 160

Ala Gly Val Glu Thr Thr Lys Pro Ser Lys Gln Ser Asn Asn Lys Tyr
165 170 175

Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
180 185 190

Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
195 200 205

Thr Val Ala Pro Thr Glu Cys Ser
210 215

<210> 159
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> avelumab variable heavy chain

<400> 159

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ile Met Met Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Tyr Pro Ser Gly Gly Ile Thr Phe Tyr Ala Asp Thr Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ile Lys Leu Gly Thr Val Thr Thr Val Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 160
<211> 110
<212> PRT
<213> Artificial Sequence

<220>

<223> avelumab variable light chain

<400> 160

Gln Ser Ala Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
1 5 10 15

Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr
20 25 30

Asn Tyr Val Ser Trp Tyr Gln Gln His Pro Gly Lys Ala Pro Lys Leu
35 40 45

Met Ile Tyr Asp Val Ser Asn Arg Pro Ser Gly Val Ser Asn Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
65 70 75 80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ser Ser Tyr Thr Ser Ser
85 90 95

Ser Thr Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu
100 105 110

<210> 161

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> avelumab heavy chain CDR1

<400> 161

Ser Tyr Ile Met Met
1 5

<210> 162

<211> 17

<212> PRT
<213> Artificial Sequence

<220>
<223> avelumab heavy chain CDR2

<400> 162

Ser	Ile	Tyr	Pro	Ser	Gly	Gly	Ile	Thr	Phe	Tyr	Ala	Asp	Thr	Val	Lys
1				5					10					15	

Gly

<210> 163
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> avelumab heavy chain CDR3

<400> 163

Ile	Lys	Leu	Gly	Thr	Val	Thr	Thr	Val	Asp	Tyr
1				5					10	

<210> 164
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> avelumab light chain CDR1

<400> 164

Thr	Gly	Thr	Ser	Ser	Asp	Val	Gly	Gly	Tyr	Asn	Tyr	Val	Ser
1				5					10				

<210> 165
<211> 7
<212> PRT
<213> Artificial Sequence

<220>

<223> avelumab light chain CDR2

<400> 165

Asp Val Ser Asn Arg Pro Ser
1 5

<210> 166

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> avelumab light chain CDR3

<400> 166

Ser Ser Tyr Thr Ser Ser Ser Thr Arg Val
1 5 10

<210> 167

<211> 448

<212> PRT

<213> Artificial Sequence

<220>

<223> atezolizumab heavy chain

<400> 167

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser
20 25 30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
130 135 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
195 200 205

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
210 215 220

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
225 230 235 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
245 250 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
260 265 270

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
275 280 285

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Ala Ser Thr Tyr Arg Val Val
290 295 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305 310 315 320

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
325 330 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
340 345 350

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
355 360 365

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
370 375 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
385 390 395 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
405 410 415

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
420 425 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 168

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<223> atezolizumab light chain

<400> 168

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Leu Tyr His Pro Ala
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 169
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> atezolizumab variable heavy chain

<400> 169

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser
20 25 30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala
115

<210> 170
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> atezolizumab variable light chain

<400> 170

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Leu Tyr His Pro Ala
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 171
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> atezolizumab heavy chain CDR1

<400> 171

Gly Phe Thr Phe Ser Asp Ser Trp Ile His
1 5 10

<210> 172
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> atezolizumab heavy chain CDR2

<400> 172

Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
1 5 10 15

Lys Gly

<210> 173
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> atezolizumab heavy chain CDR3

<400> 173

Arg His Trp Pro Gly Gly Phe Asp Tyr
1 5

<210> 174
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> atezolizumab light chain CDR1

<400> 174

Arg Ala Ser Gln Asp Val Ser Thr Ala Val Ala
1 5 10

<210> 175
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> atezolizumab light chain CDR2

<400> 175

Ser Ala Ser Phe Leu Tyr Ser
1 5

<210> 176
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> atezolizumab light chain CDR3

<400> 176

Gln Gln Tyr Leu Tyr His Pro Ala Thr
1 5