inhibitory B and T lymphocyte attenuator (BTLA) receptor and CD160 in a monomeric manner, providing an inhibitory signal to T cells. Studies of the HVEM-LIGHT interaction suggest that it primarily has a CD28-independent costimulatory effect on CD8+ T cells, but also affects CD4+ T cells. Liu, *et al.*, *Int. Immunol.* **2003**, *15,* 861-70; Scheu, *et al.*, *J. Exp. Med.* **2002,** *195,* 1613-24.

[0012] CD95, also known as Fas, APO-1, and TNFRSF6, is a 45 kDa type-I transmembrane protein which, unlike 4-1BB, OX40, GITR, CD27, and HVEM, contains a death domain. Kischkel, *et al.*, *EMBO J.* **1995,** *14,* 5579-88; Krammer, *Nature* **2000,** *407,* 789-95. The binding of the inducible CD95 ligand (CD95L) to CD95 on activated T cells leads to apoptotic cell death, and thus it is not normally associated with the same costimulatory function as 4-1BB, OX40, GITR, CD27, and HVEM. Strauss, *et al.*, *J. Exp. Med.* **2009,** *206,* 1379-93. However, CD95 also behaves as a dual function receptor that provides for anti-apoptotic and costimulatory effects on T cells under some conditions. Paulsen, *et al.*, *Cell Death Differ.* **2011,** *18,* 619-31. CD95 engagement modulates TCR-driven signal initiation in a dose-dependent manner, wherein high doses of CD95 agonists or cellular CD95L silence T cells, while lower doses of these agonists strongly enhance TCR-driven T cell activation and proliferation.

[0013] The present invention provides the unexpected finding that TNFRSF agonists, such as a 4-1BB agonist, a CD27 agonist, a GITR agonist, an OX40 agonist, a HVEM agonist, or a CD95 agonist, are useful in the expansion of TILs from tumors from which it is known to be difficult to obtain TILs and treat the tumor with TILs, and are further useful in the treatment of patients in combination with TIL therapy.

SUMMARY

[0013a] In one aspect, the present disclosure refers to the use of a first population of tumor infiltrating lymphocytes (TILs) in the manufacture of a pharmaceutical composition for treating cancer, comprising that the first population of TILs is to be obtained from a tumor resected from a patient, an initial expansion of the first population of TILs is performed in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein

the initial expansion is performed over a period of 21 days or less; a rapid expansion of the second population of TILs is performed 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, peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist and a second TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;
wherein the third population of TILs is harvested; and wherein a therapeutically effective portion of the third population of TILs is to be administered to the patient.

[0014] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

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

[0015] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is selected from the group consisting of a 4-1BB agonist, an OX40 agonist, a CD27 agonist, a GITR agonist, a HVEM agonist, a CD95 agonist, and combinations thereof.

[0016] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist,

and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is a 4-1BB agonist, and the 4-1BB agonist is selected from the group consisting of urelumab, utomilumab, EU-101 and fragments, derivatives, variants, biosimilars, and combinations thereof.

[0017] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is a 4-1BB agonist, and the 4-1BB agonist is a 4-1BB agonist fusion protein.

[0018] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is a 4-1BB agonist fusion protein, and the 4-1BB agonist fusion protein comprises (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 comprises a Fc fragment domain and hinge domain, and wherein the fusion protein is a dimeric structure according to structure I-A or structure I-B.

[0019] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is a OX40 agonist, and the OX40 agonist is selected from the group consisting of tavolixizumab, GSK3174998, MEDI6469, MEDI6383, MOXR0916, PF-04518600, Creative Biolabs MOM-18455, and fragments, derivatives, variants, biosimilars, and combinations thereof.

[0020] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is an OX40 agonist, and the OX40 agonist is an OX40 agonist fusion protein.

[0021] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is an OX40 agonist fusion protein, and the OX40 agonist fusion protein comprises (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 comprises a Fc fragment domain and hinge domain, and wherein the fusion protein is a dimeric structure according to structure I-A or structure I-B.

[0022] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is a CD27 agonist, and the CD27 agonist is varlilumab, or a fragment, derivative, variant, or biosimilar thereof.

[0023] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is a CD27 agonist, and wherein the CD27 agonist is an CD27 agonist fusion protein.

[0024] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF

agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is a CD27 agonist, and the CD27 agonist fusion protein comprises (i) a first soluble CD27 binding domain, (ii) a first peptide linker, (iii) a second soluble CD27 binding domain, (iv) a second peptide linker, and (v) a third soluble CD27 binding domain, further comprising an additional domain at the N-terminal and/or C-terminal end, and wherein the additional domain comprises a Fc fragment domain and hinge domain, and wherein the fusion protein is a dimeric structure according to structure I-A or structure I-B.

[0025] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is a GITR agonist, and the GITR agonist is selected from the group consisting of TRX518, 6C8, 36E5, 3D6, 61G6, 6H6, 61F6,

1D8, 17F10, 35D8, 49A1, 9E5, 31H6, 2155, 698, 706, 827, 1649, 1718, 1D7, 33C9, 33F6, 34G4, 35B10, 41E11, 41G5, 42A11, 44C1, 45A8, 46E11, 48H12, 48H7, 49D9, 49E2, 48A9, 5H7, 7A10, 9H6, and fragments, derivatives, variants, biosimilars, and combinations thereof.

[0026] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is an GITR agonist, and the GITR agonist is a GITR agonist fusion protein.

[0027] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is a GITR agonist fusion protein, and the GITR agonist fusion protein comprises (i) a first soluble GITR binding domain, (ii) a first peptide linker, (iii) a second soluble GITR binding domain, (iv) a second peptide linker, and (v) a third soluble GITR binding domain, further comprising an additional domain at the N-terminal and/or C-terminal end, and wherein the additional domain comprises a Fc fragment domain and hinge domain, and wherein the fusion protein is a dimeric structure according to structure I-A or structure I-B.

[0028] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is a HVEM agonist.

[0029] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is an HVEM agonist, and the HVEM agonist is a HVEM agonist fusion protein.

[0030] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is a HVEM agonist fusion protein, and wherein the HVEM agonist fusion protein comprises (i) a first soluble HVEM binding domain, (ii) a first peptide linker, (iii) a second soluble HVEM binding domain, (iv) a second peptide linker, and (v) a third soluble HVEM binding domain, further comprising an additional domain at the N-terminal and/or C-terminal end, and wherein the additional domain comprises a Fc fragment domain and hinge domain, and wherein the fusion protein is a dimeric structure according to structure I-A or structure I-B.

[0031] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, further comprising the step of treating the patient with the TNFRSF agonist starting on the day after administration of the third population of TILs to the patient, wherein the TNFRSF agonist is administered intravenously at a dose of between 0.1 mg/kg and 50 mg/kg every four weeks for up to eight cycles.

[0032] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, further comprising the step of treating the patient with the TNFRSF agonist prior to the step of resecting of a tumor from the patient, wherein the TNFRSF agonist is administered intravenously at a dose of between 0.1 mg/kg and 50 mg/kg every four weeks for up to eight cycles.

[0033] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is selected from the group consisting of urelumab, utomilumab, EU-101, tavolixizumab, Creative Biolabs MOM-18455, and fragments, derivatives, variants, biosimilars, and combinations thereof.

[0034] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the first cell culture medium comprises a second TNFRSF agonist.

[0035] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is added to the first cell culture medium during the initial expansion at an interval selected from the group consisting of every day, every two days, every three days, every four days, every five days, every six days, every seven days, and every two weeks.

[0036] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the TNFRSF agonist is added to the second cell culture medium during the rapid expansion at an interval selected from the group consisting of every

day, every two days, every three days, every four days, every five days, every six days, every seven days, and every two weeks.

[0037] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein 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.

[0038] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein 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.

[0039] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein IL-2 is present at an initial concentration of about 10 to about 6000 IU/mL in the first cell culture medium.

[0040] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein IL-2 is present at an initial concentration of about 3000 IU/mL in the first cell culture medium.

[0041] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, The method of Claim 31, wherein IL-2 is present at an initial concentration of about 800 to about 1100 IU/mL in the first cell culture medium.

[0042] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein IL-2 is present at an initial concentration of about 1000 IU/mL in the first cell culture medium.

[0043] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein IL-2 is present at an initial concentration of about 10 to about 6000 IU/mL in the second cell culture medium.

[0044] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein IL-2 is present at an initial concentration of about 3000 IU/mL in the second cell culture medium.

[0045] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein IL-2 is present at an initial concentration of about 800 to about 1100 IU/mL in the second cell culture medium.

[0046] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein IL-2 is present at an initial concentration of about 1000 IU/mL in the second cell culture medium.

[0047] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein IL-15 is present in the first cell culture medium.

[0048] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein IL-15 is present at an initial concentration of about 5 ng/mL to about 20 ng/mL in the first cell culture medium.

[0049] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein IL-15 is present in the second cell culture medium.

[0050] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein IL-15 is present at an initial concentration of about 5 ng/mL to about 20 ng/mL in the second cell culture medium.

[0051] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein IL-21 is present in the first cell culture medium.

[0052] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein IL-21 is present at an initial concentration of about 5 ng/mL to about 20 ng/mL in the first cell culture medium.

[0053] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein IL-21 is present in the second cell culture medium.

[0054] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a

patient with the cancer, wherein IL-21 is present at an initial concentration of about 5 ng/mL to about 20 ng/mL in the second cell culture medium.

[0055] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein OKT-3 antibody is present at an initial concentration of about 10 ng/mL to about 60 ng/mL in the second cell culture medium.

[0056] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein OKT-3 antibody is present at an initial concentration of about 30 ng/mL in the second cell culture medium.

[0057] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the initial expansion is performed using a gas permeable container.

[0058] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the rapid expansion is performed using a gas permeable container.

[0059] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

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

[0060] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, 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, wherein the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for five days.

[0061] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, further comprising the step of treating the patient with a decrescendo IL-2 regimen starting on the day after administration of the third population of TILs to the patient, wherein the decrescendo IL-2 regimen comprises aldesleukin administered intravenously at a dose of 18,000,000 IU/m^2 on day 1, 9,000,000 IU/m^2 on day 2, and 4,500,000 IU/m^2 on days 3 and 4.

[0062] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, further comprising the step of treating the patient with pegylated IL-2 after administration of the third population of TILs to the patient at a dose of 0.10 mg/day to 50 mg/day.

[0063] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist,

and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, further comprising the step of treating the patient with a high-dose IL-2 regimen starting on the day after administration of the third population of TILs to the patient.

[0064] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, further comprising the step of treating the patient with a high-dose IL-2 regimen starting on the day after administration of the third population of TILs to the patient, wherein the high-dose IL-2 regimen comprises 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.

[0065] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, lung cancer, bladder cancer, breast cancer, head and neck cancer, renal cell carcinoma, acute myeloid leukemia, colorectal cancer, cholangiocarcinoma, and sarcoma.

[0066] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, wherein the cancer is selected from the group consisting of non-small cell lung cancer (NSCLC), triple negative breast cancer, double-refractory melanoma, and uveal (ocular) melanoma.

[0067] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, further comprising the step of treating the patient with a PD-1 inhibitor or PD-L1 inhibitor prior to resecting the tumor from the patient.

[0068] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, further comprising the step of treating the patient with a PD-1 inhibitor or PD-L1 inhibitor prior to resecting the tumor from the patient, wherein the PD-1 inhibitor or PD-L1 inhibitor is selected from the group consisting of nivolumab,

pembrolizumab, durvalumab, atezolizumab, avelumab, and fragments, derivatives, variants, biosimilars, and combinations thereof.

[0069] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, further comprising the step of treating the patient with a PD-1 inhibitor or PD-L1 inhibitor after resecting the tumor from the patient.

[0070] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, further comprising the step of treating the patient with a PD-1 inhibitor or PD-L1 inhibitor after resecting the tumor from the patient, wherein the PD-1 inhibitor or PD-L1 inhibitor is selected from the group consisting of nivolumab, pembrolizumab, durvalumab, atezolizumab, avelumab, and fragments, derivatives, variants, biosimilars, and combinations thereof.

[0071] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF

agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, further comprising the step of treating the patient with a PD-1 inhibitor or PD-L1 inhibitor after administering the third population of TILs to the patient.

[0072] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer, further comprising the step of treating the patient with a PD-1 inhibitor or PD-L1 inhibitor after administering the third population of TILs to the patient, wherein the PD-1 inhibitor or PD-L1 inhibitor is selected from the group consisting of nivolumab, pembrolizumab, durvalumab, atezolizumab, avelumab, and fragments, derivatives, variants, biosimilars, and combinations thereof.

[0073] In an example, the disclosure provides a process for the preparation of a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(b) obtaining a first population of TILs;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less; and

(e) harvesting the third population of TILs.

[0074] In an example, the disclosure provides a population of tumor infiltrating lymphocytes (TILs) obtainable from a process comprising the steps of:

(b) obtaining a first population of TILs;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less; and

(e) harvesting the third population of TILs.

[0074a] In another aspect, the present disclosure refers to a process for the preparation of a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) performing an initial expansion of a first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(b) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less; and

(c) harvesting the third population of TILs.

[0074b] In yet another aspect, the present disclosure refers to a population of tumor infiltrating lymphocytes (TILs) obtainable from a process as described herein.

[0074c] In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising a population of tumor infiltrating lymphocytes (TILs) for use in therapy.

[0075] In an example, the disclosure provides a population of TILs is for use in the treatement of cancer. In an example, the disclosure provides a pharmaceutical composition comprising a population of tumor infiltrating lymphocytes (TILs) for use in treating a cancer wherein the population of tumor infiltrating lymphocytes (TILs) is obtainable by a process comprising the steps of:

(b) obtaining a first population of TILs;

(c) performing an initial expansion of the first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(d) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less; and

(e) harvesting the third population of TILs.

[0076] In an example, the first population of TILs is obtained from a tumor. In an example, the tumor is firstly resected from a patient. In an example, the first population of TILs is obtained from the tumor which has been resected from a patient. In an example, the population of TILs is for adminsitration in a therapeutically effective amount to a patient with cancer.

[0077] In an example, the disclosure provides a method of expanding a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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, and wherein the initial expansion is performed over a period of 11 days or less;

(d) 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, peripheral blood mononuclear cells (PBMCs), and a TNFRSF agonist, and wherein the rapid expansion is performed over a period of 11

days or less;

(e) harvesting the third population of TILs; and

(f) optionally cryopreserving the third population of TILs in a dimethylsulfoxide-based media.

[0078] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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, and wherein the initial expansion is performed over a period of 11 days or less;

(d) 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, peripheral blood mononuclear cells (PBMCs), and a TNFRSF agonist, and wherein the rapid expansion is performed over a period of 11 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to the patient.

[0079] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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, and wherein the initial expansion is performed over a period of 11 days or less;

(d) 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, peripheral blood mononuclear cells (PBMCs), and a TNFRSF agonist, and wherein the rapid expansion is performed over a period of 11 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to the patient,

wherein the TNFRSF agonist is selected from the group consisting of a 4-1BB agonist, an OX40 agonist, and a combination thereof.

[0080] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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, and wherein the initial expansion is performed over a period of 11 days or less;

(d) 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, peripheral blood mononuclear cells (PBMCs), and a TNFRSF agonist, and wherein the rapid expansion is performed over a period of 11 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to the patient,

wherein the TNFRSF agonist is selected from the group consisting of a 4-1BB agonist, an OX40 agonist, and a combination thereof, and

wherein 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.

[0081] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) resecting a tumor from a patient;

(b) obtaining a first population of TILs from the tumor;

(c) performing an initial expansion of the first population of TILs in a 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, and wherein the initial expansion is performed over a period of 11 days or less;

(d) 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, peripheral blood mononuclear cells (PBMCs), and a TNFRSF agonist, and wherein the rapid expansion is performed over a period of 11 days or less;

(e) harvesting the third population of TILs; and

(f) administering a therapeutically effective portion of the third population of TILs to the patient,

wherein the TNFRSF agonist is selected from the group consisting of a 4-1BB agonist, an OX40 agonist, and a combination thereof, and

wherein the TNFRSF agonist is a OX40 agonist, and the OX40 agonist is selected from the group consisting of tavolixizumab, GSK3174998, MEDI6469, MEDI6383, MOXR0916, PF-04518600, Creative Biolabs MOM-18455, and fragments, derivatives, variants, biosimilars, and combinations thereof.

wherein the OX4 agonist is present at the start of step (d) at a concentration between 1 μg/mL and 30 μg/mL.

[0082] In an example, the disclosure provides a method of any of the foregoing examples, wherein the TNFRSF agonist is present at the start of step (d) at a concentration between 5 μg/mL and 20 μg/mL.

[0083] In an example, the disclosure provides a method of any of the foregoing examples, wherein the TNFRSF agonist is present at the start of step (d) at a concentration of about 10 μg/mL.

[0084] In an example, the disclosure provides a method of any of the foregoing examples, wherein the TNFRSF agonist is maintained throughout step (d) at a concentration between 1 μg/mL and 30 μg/mL.

[0085] In an example, the disclosure provides a method of any of the foregoing examples, wherein the TNFRSF agonist is maintained throughout step (d) at a concentration between 5 μg/mL and 20 μg/mL.

[0086] In an example, the disclosure provides a method of any of the foregoing examples, wherein the TNFRSF agonist is maintained throughout step (d) at a concentration of about 10 μg/mL.

[0087] In an example, the disclosure provides a method of any of the foregoing examples, wherein the third population of TILs exhibits an increased ratio of $CD8^+$ TILs to $CD4^+$ TILs

in comparison to the reference ratio of $CD8^+$ TILs to $CD4^+$ TILs in the second population of TILs. In an example, the increased ratio is selected from the group consisting of at least 1% greater than the reference ratio, at least 2% greater than the reference ratio, at least 5% greater than the reference ratio, at least 10% greater than the reference ratio, at least 15% greater than the reference ratio, at least 20% greater than the reference ratio, at least 25% greater than the reference ratio, at least 30% greater than the reference ratio, at least 35% greater than the reference ratio, at least 40% greater than the reference ratio, at least 45% greater than the reference ratio, and at least 50% greater than the reference ratio. In an example, the increased ratio is between 5% and 80% greater than the reference ratio. In an example, the increased ratio is between 10% and 70% greater than the reference ratio. In an example, the increased ratio is between 15% and 60% greater than the reference ratio. In an of the foregoing examples, the reference ratio is obtained from a third TIL population that is a responder to the TNFRSF agonist.

[0088] In an example, the disclosure provides a method of any of the foregoing examples, wherein the cancer is selected from the group consisting of melanoma, uveal (ocular) melanoma, ovarian cancer, cervical cancer, lung cancer, bladder cancer, breast cancer, head and neck cancer (head and neck squamous cell cancer), renal cell carcinoma, colorectal cancer, pancreatic cancer, glioblastoma, cholangiocarcinoma, and sarcoma. In an example, the disclosure provides a method of any of the foregoing examples, wherein the cancer is selected from the group consisting of cutaneous melanoma, uveal (ocular) melanoma, platinum-resistant ovarian cancer, pancreatic ductal adenocarcinoma, osteosarcoma, triple-negative breast cancer, and non-small-cell lung cancer.

[0089] In an example, any of the foregoing examples may be combined with any of the following examples.

[0090] In an example, the process is an *in vitro* or an *ex vivo* process.

[0091] In an example, the TNFRSF agonist is selected from the group consisting of a 4-1BB agonist, an OX40 agonist, a CD27 agonist, a GITR agonist, a HVEM agonist, a CD95 agonist, and combinations thereof.

[0092] In an example, the TNFRSF agonist is a 4-1BB agonist.

[0093] In an example, the TNFRSF agonist is a 4-1BB agonist, and the 4-1BB agonist is selected from the group consisting of urelumab, utomilumab, EU-101 and fragments, derivatives, variants, biosimilars, and combinations thereof.

[0094] In an example, the TNFRSF agonist is a 4-1BB agonist, and the 4-1BB agonist is a 4-1BB agonist fusion protein.

[0095] In an example, the TNFRSF agonist is a 4-1BB agonist fusion protein, and the 4-1BB agonist fusion protein comprises (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 comprises a Fc fragment domain and hinge domain, and wherein the fusion protein is a dimeric structure according to structure I-A or structure I-B.

[0096] In an example, the TNFRSF agonist is a OX40 agonist.

[0097] In an example, the TNFRSF agonist is a OX40 agonist, and the OX40 agonist is selected from the group consisting of tavolixizumab, GSK3174998, MEDI6469, MEDI6383, MOXR0916, PF-04518600, Creative Biolabs MOM-18455, and fragments, derivatives, variants, biosimilars, and combinations thereof.

[0098] In an example, the TNFRSF agonist is an OX40 agonist, and the OX40 agonist is an OX40 agonist fusion protein.

[0099] In an example, the TNFRSF agonist is an OX40 agonist fusion protein, and the OX40 agonist fusion protein comprises (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 comprises a Fc fragment domain and hinge domain, and wherein the fusion protein is a dimeric structure according to structure I-A or structure I-B.

[00100] In an example, the TNFRSF agonist is a CD27 agonist.

[00101] In an example, the TNFRSF agonist is a CD27 agonist, and the CD27 agonist is varlilumab, or a fragment, derivative, variant, or biosimilar thereof.

[00102] In an example, the TNFRSF agonist is a CD27 agonist, and wherein the CD27 agonist is an CD27 agonist fusion protein.

[00103] In an example, the TNFRSF agonist is a CD27 agonist, and the CD27 agonist fusion protein comprises (i) a first soluble CD27 binding domain, (ii) a first peptide linker, (iii) a second soluble CD27 binding domain, (iv) a second peptide linker, and (v) a third soluble CD27 binding domain, further comprising an additional domain at the N-terminal and/or C-terminal end, and wherein the additional domain comprises a Fc fragment domain and hinge domain, and wherein the fusion protein is a dimeric structure according to structure I-A or structure I-B.

[00104] In an example, the TNFRSF agonist is a GITR agonist.

[00105] In an example, the TNFRSF agonist is a GITR agonist, and the GITR agonist is selected from the group consisting of TRX518, 6C8, 36E5, 3D6, 61G6, 6H6, 61F6, 1D8, 17F10, 35D8, 49A1, 9E5, 31H6, 2155, 698, 706, 827, 1649, 1718, 1D7, 33C9, 33F6, 34G4, 35B10, 41E11, 41G5, 42A11, 44C1, 45A8, 46E11, 48H12, 48H7, 49D9, 49E2, 48A9, 5H7, 7A10, 9H6, and fragments, derivatives, variants, biosimilars, and combinations thereof.

[00106] In an example, the TNFRSF agonist is an GITR agonist, and the GITR agonist is a GITR agonist fusion protein.

[00107] In an example, the TNFRSF agonist is a GITR agonist fusion protein, and the GITR agonist fusion protein comprises (i) a first soluble GITR binding domain, (ii) a first peptide linker, (iii) a second soluble GITR binding domain, (iv) a second peptide linker, and (v) a third soluble GITR binding domain, further comprising an additional domain at the N-terminal and/or C-terminal end, and wherein the additional domain comprises a Fc fragment domain and hinge domain, and wherein the fusion protein is a dimeric structure according to structure I-A or structure I-B.

[00108] In an example, the TNFRSF agonist is a HVEM agonist.

[00109] In an example, the TNFRSF agonist is an HVEM agonist, and the HVEM agonist is a HVEM agonist fusion protein.

[00110] In an example, the TNFRSF agonist is a HVEM agonist fusion protein, and wherein the HVEM agonist fusion protein comprises (i) a first soluble HVEM binding domain, (ii) a

first peptide linker, (iii) a second soluble HVEM binding domain, (iv) a second peptide linker, and (v) a third soluble HVEM binding domain, further comprising an additional domain at the N-terminal and/or C-terminal end, and wherein the additional domain comprises a Fc fragment domain and hinge domain, and wherein the fusion protein is a dimeric structure according to structure I-A or structure I-B.

[00111] In an example, the TNFRSF agonist is selected from the group consisting of urelumab, utomilumab, EU-101, tavolixizumab, Creative Biolabs MOM-18455, and fragments, derivatives, variants, biosimilars, and combinations thereof.

[00112] In an example, the first cell culture medium comprises a second TNFRSF agonist.

[00113] In an example, the TNFRSF agonist is added to the first cell culture medium during the initial expansion at an interval selected from the group consisting of every day, every two days, every three days, every four days, every five days, every six days, every seven days, and every two weeks.

[00114] In an example, the TNFRSF agonist is added to the second cell culture medium during the rapid expansion at an interval selected from the group consisting of every day, every two days, every three days, every four days, every five days, every six days, every seven days, and every two weeks.

[00115] In an example, 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.

[00116] In an example, 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.

[00117] Further details of the TNFRSF agonists are provided herein.

[00118] In an example, IL-2 is present at an initial concentration of about 10 to about 6000 IU/mL in the first cell culture medium.

[00119] In an example, IL-2 is present at an initial concentration of about 3000 IU/mL in the first cell culture medium.

[00120] In an example, IL-2 is present at an initial concentration of about 800 to about 1100 IU/mL in the first cell culture medium.

[00121] In an example, IL-2 is present at an initial concentration of about 1000 IU/mL in the first cell culture medium.

[00122] In an example, IL-2 is present at an initial concentration of about 10 to about 6000 IU/mL in the second cell culture medium.

[00123] In an example, IL-2 is present at an initial concentration of about 3000 IU/mL in the second cell culture medium.

[00124] In an example, IL-2 is present at an initial concentration of about 800 to about 1100 IU/mL in the second cell culture medium.

[00125] In an example, IL-2 is present at an initial concentration of about 1000 IU/mL in the second cell culture medium.

[00126] In an example, IL-15 is present in the first cell culture medium.

[00127] In an example, IL-15 is present at an initial concentration of about 5 ng/mL to about 20 ng/mL in the first cell culture medium.

[00128] In an example, IL-15 is present in the second cell culture medium.

[00129] In an example, IL-15 is present at an initial concentration of about 5 ng/mL to about 20 ng/mL in the second cell culture medium.

[00130] In an example, IL-21 is present in the first cell culture medium.

[00131] In an example, IL-21 is present at an initial concentration of about 5 ng/mL to about 20 ng/mL in the first cell culture medium.

[00132] In an example, IL-21 is present in the second cell culture medium.

[00133] In an example, IL-21 is present at an initial concentration of about 5 ng/mL to about 20 ng/mL in the second cell culture medium.

[00134] In an example, OKT-3 antibody is present at an initial concentration of about 10 ng/mL to about 60 ng/mL in the second cell culture medium.

[00135] In an example, OKT-3 antibody is present at an initial concentration of about 30 ng/mL in the second cell culture medium.

[00136] In an example, the initial expansion is performed using a gas permeable container.

[00137] In an example, the rapid expansion is performed using a gas permeable container.

[00138] In an example, the disclosure provides a population of tumor infiltrating lymphocytes (TILs) for use in treating a cancer wherein the population of tumor infiltrating lymphocytes (TILs) is obtainable by a process of the disclosure as described herein.

[00139] In an example, the disclosure provides a pharmaceutical composition comprising a population of tumor infiltrating lymphocytes (TILs) for use in a method of treating a cancer wherein the population of tumor infiltrating lymphocytes (TILs) is obtainable by a process of the disclosure as described herein.

[00140] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with a TNFRSF.

[00141] In an example, the disclosure provides a combination of a population of TILs obtainable by a process of the disclosure as described herein and a TNFRSF for use in the treatment of cancer.

[00142] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with a TNFRSF agonist wherein the TNFRSF agonist is for administration on the day after administration of the third population of TILs to the patient, and wherein the TNFRSF agonist is administered intravenously at a dose of between 0.1 mg/kg and 50 mg/kg every four weeks for up to eight cycles.

[00143] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with a TNFRSF agonist wherein the TNFRSF agonist is for administration prior to the step of resecting of a tumor from the patient, and wherein the TNFRSF agonist for administration intravenously at a dose of between 0.1 mg/kg and 50 mg/kg every four weeks for up to eight cycles.

[00144] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with a non-myeloablative lymphodepletion regimen.

[00145] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with a non-myeloablative lymphodepletion regimen prior to administering the third population of TILs and/or a pharmaceutical composition comprising the third population of TILs to the patient.

[00146] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with a non-myeloablative lymphodepletion regimen prior to administering the third population of TILs and/or a pharmaceutical composition comprising the third population of TILs to the patient, wherein the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for five days. Further details of the non-myeloablative lymphodepletion regimen are provided herein, e.g., under the Heading "Non-Myeloablative Lymphodepletion with Chemotherapy".

[00147] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with a IL-2 regimen.

[00148] In an example, the IL-2 regimen is a decrescendo IL-2 regimen.

[00149] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with a decrescendo IL-2 regimen starting on the day after administration of the third population of TILs and/or a pharmaceutical composition comprising the third population of TILs to the patient, wherein the decrescendo IL-2 regimen comprises aldesleukin administered intravenously at a dose of 18,000,000 IU/m^2 on day 1, 9,000,000 IU/m^2 on day 2, and 4,500,000 IU/m^2 on days 3 and 4.

[00150] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with pegylated IL-2.

[00151] In an example, the population of TILs and/or the pharmaceutical composition is for use in a method of treating cancer in combination with pegylated IL-2 administered after administration of the third population of TILs and/or a pharmaceutical composition comprising the third population of TILs to the patient at a dose of 0.10 mg/day to 50 mg/day.

[00152] In an example, the population of TILs and/or the pharmaceutical composition is for use in a method of treating cancer in combination with a high-dose IL-2 regimen.

[00153] In an example, the population of TILs and/or the pharmaceutical composition is for use in a method of treating cancer in combination with a high-dose IL-2 regimen starting on

the day after administration of the third population of TILs and/or a pharmaceutical composition comprising the third population of TILs to the patient.

[00154] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with a high-dose IL-2 regimen starting on the day after administration of the third population of TILs and/or a pharmaceutical composition comprising the third population of TILs to the patient, wherein the high-dose IL-2 regimen comprises 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.

[00155] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer, wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, lung cancer, bladder cancer, breast cancer, head and neck cancer, renal cell carcinoma, acute myeloid leukemia, colorectal cancer, cholangiocarcinoma, and sarcoma.

[00156] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer, wherein the cancer is selected from the group consisting of non-small cell lung cancer (NSCLC), triple negative breast cancer, double-refractory melanoma, and uveal (ocular) melanoma.

[00157] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with a PD-1 inhibitor or PD-L1 inhibitor.

[00158] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with a PD-1 inhibitor or PD-L1 inhibitor, wherein the PD-1 inhibitor or PD-L1 inhibitor is selected from the group consisting of nivolumab, pembrolizumab, durvalumab, atezolizumab, avelumab, and fragments, derivatives, variants, biosimilars, and combinations thereof.

[00159] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with a PD-1 inhibitor or PD-L1 inhibitor, wherein the PD-1 inhibitor or PD-L1 inhibitor is for administration prior to resecting the tumor from the patient.

[00160] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with a PD-1 inhibitor or PD-L1 inhibitor prior to resecting the tumor from the patient, wherein the PD-1 inhibitor or PD-L1 inhibitor is selected from the group consisting of nivolumab, pembrolizumab, durvalumab, atezolizumab, avelumab, and fragments, derivatives, variants, biosimilars, and combinations thereof.

[00161] In an example, the population of TILs and/or the pharmaceutical composition is for use in method of treating cancer in combination with a PD-1 inhibitor or PD-L1 inhibitor.

[00162] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with a PD-1 inhibitor or PD-L1 inhibitor, wherein the PD-1 inhibitor or PD-L1 inhibitor is selected from the group consisting of nivolumab, pembrolizumab, durvalumab, atezolizumab, avelumab, and fragments, derivatives, variants, biosimilars, and combinations thereof.

[00163] In an example, the population of TILs and/or the pharmaceutical composition is for use in a method of treating cancer in combination with a PD-1 inhibitor or PD-L1 inhibitor after resecting the tumor from the patient.

[00164] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with a PD-1 inhibitor or PD-L1 inhibitor after resecting the tumor from the patient, wherein the PD-1 inhibitor or PD-L1 inhibitor is selected from the group consisting of nivolumab, pembrolizumab, durvalumab, atezolizumab, avelumab, and fragments, derivatives, variants, biosimilars, and combinations thereof.

[00165] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with a PD-1 inhibitor or PD-L1 inhibitor, wherein the PD-1 or PD-L1 inhibitor is for administration after administering the third population of TILs and/or a pharmaceutical composition comprising the third population of TILs to the patient.

[00166] In an example, the population of TILs and/or the pharmaceutical composition is for use in treating cancer in combination with a PD-1 inhibitor or PD-L1 inhibitor which is for administrationafter administering the third population of TILs to the patient, wherein the PD-1 inhibitor or PD-L1 inhibitor is selected from the group consisting of nivolumab,

pembrolizumab, durvalumab, atezolizumab, avelumab, and fragments, derivatives, variants, biosimilars, and combinations thereof. Further details of the PD-1 inhibitor and the PD-L1 inhibitor are described herein e.g. under the heading "Combinations with PD-1 and PD-L1 Inhibitors".In some examples, the population of TILs and/ or the pharmaceutical composition comprising a population of TILs further comprise one or more features as described herein, for example, under the headings "Pharmaceutical Compositions, Dosages, and Dosing Regimens for TILs" and "Pharmaceutical Compositions, Dosages, and Dosing Regimens for TNFRSF Agonists".

BRIEF DESCRIPTION OF THE DRAWINGS

[00167] The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings.

[00168] FIG. 1 illustrates a TIL expansion and treatment process. TNFRSF agonists of the present disclosure may be used in both the pre-REP stage (top half of figure) or REP stage (bottom half of figure) and may be added when IL-2 is added to each cell culture. Step 1 refers to the addition of 4 tumor fragments into 10 G-Rex 10 flasks. At step 2, approximately 40×10^6 TILs or greater are obtained. At step 3, a split occurs into 36 G-Rex 100 flasks for REP. TILs are harvested by centrifugation at step 4. Fresh TIL product is obtained at step 5 after a total process time of approximate 43 days, at which point TILs may be infused into a patient.

[00169] FIG. 2 illustrates a treatment protocol for use with TILs expanded with TNFRSF agonists of the present disclosure. Surgery (and tumor resection) occurs at the start, and

CLAIMS

1. Use of a first population of tumor infiltrating lymphocytes (TILs) in the manufacture of a pharmaceutical composition for treating cancer, comprising that the first population of TILs is to be obtained from a tumor resected from a patient, an initial expansion of the first population of TILs is performed in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

   a rapid expansion of the second population of TILs is performed 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, peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist and a second TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less;

   wherein the third population of TILs is harvested; and

   wherein a therapeutically effective portion of the third population of TILs is to be administered to the patient.

2. The use of Claim 1, wherein the TNFRSF agonist is selected from the group consisting of a 4-1BB agonist, an OX40 agonist, a CD27 agonist, a GITR agonist, a HVEM agonist, a CD95 agonist, and combinations thereof.

3. The use of Claim 1, wherein 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.

4. The use of Claim 1, wherein the TNFRSF agonist is an OX40 agonist, and the OX40 agonist is selected from the group consisting of tavolixizumab, GSK3174998, MEDI6469,

MEDI6383, MOXR0916, PF 04518600, Creative Biolabs MOM 18455, an OX40 agonsist fusion protein, and fragments, derivatives, variants, biosimilars, and combinations thereof.

5. The use of Claim 1, wherein the TNFRSF agonist is a CD27 agonist, and the CD27 agonist is selected from the group consisting of varlilumab, a CD27 agonist fusion protein, and fragments, derivatives, variants, or biosimilars thereof.

6. The use of Claim 1, wherein the TNFRSF agonist is a GITR agonist, and the GITR agonist is selected from the group consisting of TRX518, 6C8, 36E5, 3D6, 61G6, 6H6, 61F6, 1D8, 17F10, 35D8, 49A1, 9E5, 31H6, 2155, 698, 706, 827, 1649, 1718, 1D7, 33C9, 33F6, 34G4, 35B10, 41E11, 41G5, 42A11, 44C1, 45A8, 46E11, 48H12, 48H7, 49D9, 49E2, 48A9, 5H7, 7A10, 9H6, and fragments, derivatives, variants, biosimilars, and combinations thereof.

7. The use of Claim 1, further comprising that the patient is to be treated with the TNFRSF agonist starting on the day after administration of the third population of TILs to the patient, wherein the TNFRSF agonist is to be administered intravenously at a dose of between 0.1 mg/kg and 50 mg/kg every four weeks for up to eight cycles.

8. The use of Claim 1, wherein the TNFRSF agonist is selected from the group consisting of urelumab, utomilumab, EU-101, tavolixizumab, Creative Biolabs MOM-18455, and fragments, derivatives, variants, biosimilars, and combinations thereof.

9. The use of Claim 1, wherein the first cell culture medium comprises a second TNFRSF agonist.

10. The use of Claim 1, wherein the TNFRSF agonist is a 4-1BB agonist, and the second TNFRSF agonist is an OX40 agonist.

11. The use of Claim 1, wherein the TNFRSF agonist is added to the first cell culture medium during the initial expansion at an interval selected from the group consisting of every day, every two days, every three days, every four days, every five days, every six days, every seven days, and every two weeks.

12. The use of Claim 1, wherein the TNFRSF agonist is added to the second cell culture medium during the rapid expansion at an interval selected from the group consisting of

every day, every two days, every three days, every four days, every five days, every six days, every seven days, and every two weeks.

13. The use of Claim 1, wherein 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.

14. The use of Claim 1, wherein IL-2 is present at an initial concentration of about 10 to about 6000 IU/mL in the first cell culture medium.

15. The use of Claim 1, wherein IL-2 is present at an initial concentration of about 10 to about 6000 IU/mL in the second cell culture medium.

16. The use of Claim 1, wherein OKT-3 antibody is present at an initial concentration of about 10 ng/mL to about 60 ng/mL in the second cell culture medium.

17. The use of Claim 1, wherein the initial expansion and the rapid expansion each is performed using a gas permeable container.

18. The use of Claim 1, further comprising that the patient is to be treated with a non-myeloablative lymphodepletion regimen prior to administration of the third population of TILs to the patient.

19. The use of Claim 1, wherein the cancer is selected from the group consisting of melanoma, cutaneous melanoma, double-refractory melanoma, uveal (ocular) melanoma, ovarian cancer, platinum-resistant ovarian cancer, cervical cancer, lung cancer, non-small cell lung cancer (NSCLC), bladder cancer, breast cancer, triple negative breast cancer, head and neck cancer, head and neck squamous cell cancer, renal cell carcinoma, acute myeloid leukemia, colorectal cancer, pancreatic ductal adenocarcinoma, glioblastoma, cholangiocarcinoma, osteosarcoma, and sarcoma.

20. The use of Claim1, wherein the patient is to be treated with a PD-1 inhibitor or PD-L1 inhibitor after administration of the third population of TILs to the patient.

21. The use of Claim 20, wherein the PD-1 inhibitor or PD-L1 inhibitor is selected from the group consisting of nivolumab, pembrolizumab, durvalumab, atezolizumab, avelumab, and fragments, derivatives, variants, biosimilars, and combinations thereof.

22. The use of Claim 1, wherein the initial expansion is performed over a period of 11 days or less.

23. The use of Claim 1, wherein the rapid expansion is performed over a period of 11 days or less.

24. A process for the preparation of a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) performing an initial expansion of a first population of TILs in a 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 and a tumor necrosis factor receptor superfamily (TNFRSF) agonist, and wherein the initial expansion is performed over a period of 21 days or less;

(b) 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), peripheral blood mononuclear cells (PBMCs), and optionally the TNFRSF agonist, and wherein the rapid expansion is performed over a period of 14 days or less; and

(c) harvesting the third population of TILs.

25. The process according to claim 24 wherein the first population of TILs is obtained from a tumor which tumor has been resected from a patient.

26. The process according to claim 24 , wherein the TNFRSF agonist is selected from the group consisting of a 4-1BB agonist, an OX40 agonist, a CD27 agonist, a GITR agonist, a HVEM agonist, a CD95 agonist, and combinations thereof.

27. The process according to claim 24, wherein the TNFRSF agonist is added to the first cell culture medium during the initial expansion at an interval selected from the group consisting of every day, every two days, every three days, every four days, every five days, every six days, every seven days, and every two weeks.

28. The process according to claim 24, wherein the TNFRSF agonist is added to the second cell culture medium during the rapid expansion at an interval selected from the group consisting of every day, every two days, every three days, every four days, every five days, every six days, every seven days, and every two weeks.

29. The process according to claim 24, wherein 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.

30. The process according to claim 24, wherein IL-2 is present at an initial concentration of about 10 to about 6000 IU/mL in the first cell culture medium.

31. The process according to claim 24, wherein OKT-3 antibody is present at an initial concentration of about 10 ng/mL to about 60 ng/mL in the second cell culture medium.

32. The process according to claim 31, wherein the initial expansion and the rapid expansion each is performed using a gas permeable container.

33. A population of tumor infiltrating lymphocytes (TILs) obtainable from a process according to claim 24.

34. A pharmaceutical composition comprising a population of tumor infiltrating lymphocytes (TILs) for use in therapy.