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.*, C*ell 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 therapeutic population of tumor infiltrating lymphocytes (TILs) in the manufacture of a pharmaceutical composition for treating cancer, comprising that a 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 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 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), a first tumor necrosis factor receptor superfamily (TNFRSF) agonist comprising an OX40 agonist antibody or a soluble OX40L fusion protein, and optionally 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, wherein the third population of TILs exhibits an increased ratio of CD8+ TILs to CD4+ TILs in comparison to a reference ratio of CD8+ TILs to CD4+ TILs in the second population of TILs; and wherein a therapeutically effective portion of the third population of TILs is to be administered to the patient as the therapeutic population of TILs.

[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.

(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 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 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), a first tumor necrosis factor receptor superfamily (TNFRSF) agonist comprising an OX40 agonist antibody or the soluble OX40L fusion protein, and optionally a second 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, wherein the third population of TILs exhibits an increased ratio of CD8+ TILs to CD4+ TILs in comparison to a reference ratio of CD8+ TILs to CD4+ TILs in the second population of TILs.

[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

CLAIMS

1. Use of a therapeutic population of tumor infiltrating lymphocytes (TILs) in the manufacture of a pharmaceutical composition for treating cancer, comprising that a 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 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 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), a first tumor necrosis factor receptor superfamily (TNFRSF) agonist comprising an OX40 agonist antibody or a soluble OX40L fusion protein, and optionally 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, wherein the third population of TILs exhibits an increased ratio of CD8+ TILs to CD4+ TILs in comparison to a reference ratio of CD8+ TILs to CD4+ TILs in the second population of TILs; and

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

2. The use of Claim 1, wherein the first cell culture medium comprises an additional TNFRSF agonist.

3. The use of Claim 2, wherein the additional 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.

4. The use of Claim 2, wherein the additional TNFRSF agonist is a 4-1BB agonist, and the 4-1BB agonist is selected from the group consisting of urelumab, utomilumab, EU-101, and combinations thereof.

5. The use of Claim 1, wherein the OX40 agonist antibody or fusion protein is selected from the group consisting of tavolixizumab, GSK3174998, MEDI6469, MEDI6383, MOXR0916, PF 04518600, Creative Biolabs MOM 18455, and combinations thereof.

6. 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.

7. The use of Claim 2, wherein the additional TNFRSF agonist is a 4-1BB agonist, and the TNFRSF agonist is an OX40 agonist.

8. The use of Claim 2, wherein the additional 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.

9. 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.

10. The use of Claim 1, wherein the TNFRSF agonist is added at a concentration sufficient to achieve a concentration in the second cell culture medium of between 0.1 µg/mL and 100 µg/mL.

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

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

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

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

15. 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.

16. 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.

17. The use of Claim 1, 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.

18. The use of Claim 17, wherein the PD-1 inhibitor or PD-L1 inhibitor is selected from the group consisting of nivolumab, pembrolizumab, durvalumab, atezolizumab, avelumab, and combinations thereof.

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

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

21. 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 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 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), a first tumor necrosis factor receptor superfamily (TNFRSF) agonist comprising an OX40 agonist antibody or the soluble OX40L fusion protein, and optionally a second 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, wherein the third population of TILs exhibits an increased ratio of CD8+ TILs to CD4+ TILs in comparison to a reference ratio of CD8+ TILs to CD4+ TILs in the second population of TILs.

22. The process according to claim 21 wherein the first population of TILs is obtained from a tumor which tumor has been resected from a patient.

23. The process of Claim 21, wherein the first cell culture medium comprises an additional TNFRSF agonist.

24. The process according to claim 23, wherein the additional 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.

25. The process according to claim 23, wherein the additional 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.

26. The process according to claim 21, 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.

27. The process according to claim 21, 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.

28. The process according to claim 21, wherein IL-2 is present at an initial concentration of 10 to 6000 IU/mL in the first cell culture medium.

29. The process according to claim 21, wherein OKT-3 antibody is present at an initial concentration of 10 ng/mL to 60 ng/mL in the second cell culture medium.

30. The process according to claim 28, wherein the initial expansion and the rapid expansion each is performed using a gas permeable container.