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

**[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.

## CLAIMS

1. 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.

2. The process according to claim 1 wherein the first population of TILs is obtained from a tumor which tumor has been resected from a patient.

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

4. The process according to claim 3, 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.

5. The process according to claim 3, 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.

6. The process according to 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.

7. The process according to 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.

8. The process according to claim 1, wherein IL-2 is present at an initial concentration of 10 to 6000 IU/mL in the first cell culture medium.

9. The process according to 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.

10. The process according to claim 8, wherein the initial expansion and the rapid expansion each is performed using a gas permeable container.