

Claims

1. Expanded tumor infiltrating lymphocytes (TILs) for use in treating a subject with cancer, the treatment comprising the steps of:
- culturing autologous T cells by obtaining a first population of TILs from a tumor resected from a mammal
 - performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 and one or more TME stimulators to produce a second population of TILs;
 - performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, anti-CD3 antibody, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the third population of TILs is a therapeutic population; and
 - after administering nonmyeloablative lymphodepleting chemotherapy, administering to the mammal the therapeutic population of T cells, wherein the T cells administered to the mammal, whereupon the regression of the cancer in the mammal is promoted.
2. Expanded tumor infiltrating lymphocytes (TILs) for use in promoting regression of a cancer in a subject with cancer, the regression comprising the steps of:
- culturing autologous T cells by obtaining a first population of TILs from a tumor resected from a mammal
 - performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 and one or more TME stimulators to produce a second population of TILs;
 - performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, anti-CD3 antibody, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the third population of TILs is a therapeutic population; and
 - after administering nonmyeloablative lymphodepleting chemotherapy, administering to the mammal the therapeutic population of T cells, wherein the T cells administered to the mammal, whereupon the regression of the cancer in the mammal is promoted.
3. A method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:
- (a) culturing autologous T cells by obtaining a first population of TILs from a tumor resected from a mammal

- (b) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 and one or more TME stimulators to produce a second population of TILs; and
 - (c) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, anti-CD3 antibody, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the third population of TILs is a therapeutic population.
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4. The uses of claims 1-2 and the method of claim 3, wherein one or more TME stimulators are defined as one or more substances that are capable of antagonizing and/or inhibiting receptors expressed on T-cells (or their ligands) known to cause T-cell downregulation, deactivation and/or exhaustion,
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5. The uses and methods of claim 4, wherein the substances that are capable of antagonizing and/or inhibiting receptors expressed on T-cells (or their ligands) known to cause T-cell downregulation, deactivation and/or exhaustion are selected from:
- A: substances that act through the PD-1 receptor on T-cells,
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6. The uses and methods of claim 5, wherein the substances that are capable of antagonizing and/or inhibiting receptors expressed on T-cells (or their ligands) known to cause T-cell downregulation, deactivation and/or exhaustion are selected from at least one from each of the groups consisting of:
- A: substances that act through the PD-1 receptor on T-cells (or ligand), and
 - B: substances that act through the CTLA-4 receptor on T-cells (or ligand).
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7. The uses and methods of claims 5-6, further comprising one or more substances that are capable of agonizing and/or stimulating receptors expressed on T-cells known to cause T-cell upregulation, activation, and/or reinvigoration selected from the group consisting of:
- J: substances that act through the 4-1BB/CD137 receptor on T-cells,
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8. The uses and methods of claims 5-7, wherein the substance of group A is selected from one or more from the group consisting of pembrolizumab, nivolumab, cemiplimab, sym021, atezolizumab, avelumab, and durvalumab.
9. The uses and methods of claims 5-8, wherein the substance of group B is selected from one or more from the group consisting of ipilimumab and tremelimumab.
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10. The uses and methods of claims 5-9, wherein the substance of group J is selected from one or more from the group consisting of urelumab and utomilumab.

11. The uses and methods of claims 5-10, wherein the concentration of the substance is 0.1 µg/mL to 300 µg/mL, such as 1 µg/mL to 100 µg/mL, such as 10 µg/mL to 100 µg/mL, such as 1 µg/mL to 10 µg/mL, such as 2-20 µg/mL.
12. The uses and methods of claims 5-11, wherein steps (a) through (b) are performed within a
5 period of about 7 days to about 28 days.
13. The uses and methods of claims 5-12, wherein step (c) is performed within a period of about 7 days to about 21 days.
14. The uses and methods of claims 5-13, wherein the therapeutic population of T cells is used to
10 treat a cancer type selected from the groups consisting of breast cancer, renal cell cancer, bladder cancer, melanoma, cervical cancer, gastric cancer, colorectal cancer, lung cancer, head and neck cancer, ovarian cancer, Hodgkin lymphoma, pancreatic cancer, liver cancer, and sarcomas.
15. The uses and methods of claims 5-14, wherein step (c) results in 1×10^7 to 1×10^{12} cells, such as 1×10^8 to 5×10^9 cells, such as 1×10^9 to 5×10^9 cells, such as 1×10^8 to 5×10^{10} cells, such as 1×10^9 to 5×10^{11} cells.
- 15 16. The uses and methods of claims 1-15, wherein the anti-CD3 antibody is OKT3.
17. A population of tumor infiltrating lymphocytes (TILs) obtainable by a method of any of the previous claims.
18. A therapeutic population of TILs comprising IL-2 and one or more TME stimulators.
19. The therapeutic population of TILs according to claim 18, wherein the one or more TME
20 stimulators comprises at least one selected from the group consisting of:
- A: substances that act through the PD-1 receptor on T-cells, and
 - B: substances that act through the CTLA-4 receptor on T-cells.
20. The therapeutic population of TILs according to claim 18, further comprising at least one selected from the group consisting of:
- 25 J: substances that act through the 4-1BB/CD137 receptor on T-cells,