

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, wherein the one or more TME stimulators comprises at least:

- ~~— one or more substance(s) of group B selected from the group consisting of ipilimumab and tremelimumab, B: substances that act through the CTLA-4 receptor on T-cells (or ligand),~~

- ~~and 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 and/or are capable of depleting regulatory T-cells thereby favoring ex vivo effector T-cell expansion.~~

- 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, 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, wherein the one or more TME stimulators comprises at least:

- ~~— one or more substance(s) of group B selected from the group consisting of ipilimumab and tremelimumab, B: substances that act through the CTLA-4 receptor on T-cells (or ligand), and 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 and/or are capable of depleting regulatory T-cells thereby favoring ex vivo effector T-cell expansion.~~

- 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, 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, wherein the one or more TME stimulators comprise at least one or more substance(s) of group B selected from the group consisting of ipilimumab and tremelimumab, ÷

~~- B: substances that act through the CTLA-4 receptor on T-cells (or ligand), and 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 and/or are capable of depleting regulatory T-cells thereby favoring ex-vivo effector T-cell expansion;~~

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.

4. The uses of claims 1-2 and method of claim 3, wherein the one or more TME stimulators comprises at least:

- one or more substance(s) of group A selected from the group consisting of pembrolizumab, nivolumab, cemiplimab, sym021, atezolizumab, avelumab and durvalumab~~A: substances that act through the PD-1 receptor on T-cells (or ligand), 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, and~~

- one or more substance(s) of group B selected from the group consisting of ipilimumab and tremelimumab~~B: substances that act through the CTLA-4 receptor on T-cells (or ligand) and 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 and/or are capable of depleting regulatory T-cells thereby favoring ex-vivo effector T-cell expansion.~~

5. The uses and methods of claims 4, 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:~~

~~———of group J selected from the group consisting of urelumab and utomilumab~~; substances that act through the 4-1BB/CD137 receptor on T-cells, and that are capable of agonizing and/or stimulating receptors expressed on T-cells known to cause T-cell upregulation, activation, and/or reinvigoration.

6. The uses and methods of claim 5, wherein group J is used in combination with an anti-CD3 substance.

7. The uses and methods of claims 5-6, wherein the anti-CD3 substance is OKT-3.

~~8. The uses and methods of claims 4-7, wherein the substance of group A is an antibody.~~

~~9. The uses and methods of claims 4-8, wherein the substance of group A is selected from one or more from the group consisting of pembrolizumab, nivolumab, cemiplimab, sym021, atezolizumab, avolumab, and durvalumab.~~

~~108.~~ The uses and methods of claims ~~94-7,~~ wherein the substance of group A is pembrolizumab.

~~11. The uses and methods of claims 1-10, wherein the substance of group B is an antibody.~~

~~12. The uses and methods of claims 1-11, wherein the substance of group B is selected from one or more from the group consisting of ipilimumab and tremelimumab.~~

~~139.~~ The uses and methods of claims ~~121-8,~~ wherein the substance of group B is ipilimumab.

~~14. The uses and methods of claims 5-13, wherein the substance of group J is an antibody.~~

~~15. The uses and methods of claims 5-14, wherein the substance of group J is selected from one or more from the group consisting of urelumab and utomilumab.~~

~~1610.~~ The uses and methods of claims ~~155-9,~~ wherein the substance of wherein the substance of group J is urelumab.

~~1711.~~ The uses and methods of claims 1-~~1610,~~ 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.

~~1812.~~ The uses and methods of claims 1-~~1711,~~ wherein steps (a) through (b) are performed within a period of about 7 days to about 28 days.

~~19~~13. The uses and methods of claims 1-~~18~~12, wherein step (c) is performed within a period of about 7 days to about 21 days.

~~20~~14. The uses and methods of claims 1-~~19~~13, wherein the therapeutic population of T cells is used to 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.

~~21~~15. The uses and methods of claims 1-~~20~~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.

~~22~~16. The uses and methods of claims 1-~~21~~15, wherein the anti-CD3 antibody is OKT3.

~~23~~17. The uses and methods of claims 1-~~22~~16, wherein the TME stimulators are added together or in time lapse, *i.e.* one day apart, or such as 2, 3, 4, 5, 6 or 7 days apart.

~~24~~18. The uses and methods of claims 1-~~23~~17, wherein the antigen-presenting cells (APCs) are selected from the group consisting of allogeneic feeder cells, PBMCs, and artificial antigen-presenting feeder cells.

~~25~~19. The uses and methods of claims 1-~~24~~18, wherein the first step further comprises processing of the resected tumor into multiple tumor fragments, such as 4 to 50 fragments.

~~26~~20. The uses and methods of claim ~~25~~19, wherein the fragments have a size of about 1 to 10 mm³.

~~27~~21. A population of tumor infiltrating lymphocytes (TILs) obtainable by a method of any of the previous claims, wherein the population is at least 1×10^8 to 5×10^{11} cells.

~~28~~22. A therapeutic population of TILs comprising IL-2 and TME stimulators, wherein the TME stimulators comprises at least one or more substance(s) of group B selected from the group consisting of ipilimumab and tremelimumab:

~~— B: substances that act through the CTLA-4 receptor on T-cells (or ligand) and 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 and/or capable of depleting regulatory T-cells thereby favoring ex-vivo effector T-cell expansion, and~~

wherein the population is at least 1×10^8 to 5×10^{11} cells.

~~29~~23. A therapeutic population of TILs comprising IL-2 and TME stimulators, wherein the TME stimulators comprises at least:

- one or more substance(s) of group A selected from the group consisting of pembrolizumab, nivolumab, cemiplimab, sym021, atezolizumab, avelumab and durvalumab~~A: substances that act through the PD-1 receptor on T-cells (or ligand), 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, and~~

~~- B: substances that act through the CTLA-4 receptor on T-cells (or ligand) and 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 and/or are capable of depleting regulatory T-cells thereby favoring ex vivo effector T-cell expansion, and one or more substance(s) of group B selected from the group consisting of ipilimumab and tremelimumab,~~

wherein the population is at least 1×10^8 to 5×10^{11} cells.

~~3024. The therapeutic population of TILs according to claim 2623, further comprising at least one or more substances of group J selected from the group consisting of urelumab and utomilumab~~
~~group consisting of:~~

~~—— J: substances that act through the 4-1BB/CD137 receptor on T-cells, and that are capable of agonizing and/or stimulating receptors expressed on T-cells known to cause T-cell upregulation, activation, and/or reinvigoration, and~~

~~wherein the population is at least 1×10^8 to 5×10^{11} cells.~~