

CLAIMS

1. 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, 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.

2. The method of claim 1, 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, and
- one or more substance(s) of group B selected from the group consisting of ipilimumab and tremelimumab.

3. The method of claim 2, further comprising one or more substances of group J selected from the group consisting of urelumab and utomilumab.

4. The method of claim 3, wherein group J is used in combination with an anti-CD3 substance.

5. The method according to any of claims 1-4, wherein the anti-CD3 substance is OKT-3.

6. The method according to any of claims 2-5, wherein the substance of group A is pembrolizumab.

7. The method according to any of claims 1-6, wherein the substance of group B is ipilimumab.

8. The method according to any of claims 3-7, wherein the substance of wherein the substance of group J is urelumab.

9. The method according to any of claims 1-8, 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.

10. The method according to any of claims 1-9, wherein steps (a) through (b) are performed within a period of between 7 days to 28 days.

11. The method according to any of claims 1-10, wherein step (c) is performed within a period of between 7 days to 21 days.
12. The method according to any of claims 1-11, wherein the therapeutic population of T cells is to be 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.
13. The method according to any of claims 1-12, 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.
14. The method according to any of claims 1-13, wherein the anti-CD3 antibody is OKT3.
15. The method according to any of claims 1-14, 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.
16. The method according to any of claims 1-15, wherein the antigen-presenting cells (APCs) are selected from the group consisting of allogeneic feeder cells, PBMCs, and artificial antigen-presenting feeder cells.
17. The method according to any of claims 1-16, wherein the first step further comprises processing of the resected tumor into multiple tumor fragments, such as 4 to 50 fragments.
18. The method of claim 17, wherein the fragments have a size of 1 to 10 mm³.