

SUMMARY

[0004] The present disclosure provides improved and/or shortened methods for expanding TILs and producing therapeutic populations of TILs.

[0004a] In one aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (a) adding processed tumor fragments from a tumor resected from a patient into a closed system to obtain a first population of TILs; (b) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, to produce a second population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for 3-14 days to obtain the second population of TILs, and wherein the transition from step (a) to step (b) occurs without opening the system; (c) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for 4 to 6 days to obtain the third population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system; (d) splitting the third population of TILs into a first plurality of five or less sub-populations of TILs, each sub-population comprising at least 1.0×10^9 TILs, and performing a third expansion of the first plurality of sub-populations of TILs by supplementing the cell culture medium of each sub-population of TILs with additional IL-2, optionally OKT-3, to produce a second plurality of sub-populations of TILs, wherein the second plurality of sub-populations of TILs comprise a therapeutic population of TILs, wherein the third expansion is performed for 5 to 7 days, wherein the third expansion for each sub-population is performed in a closed container providing a third gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system; (e) harvesting the therapeutic population of TILs obtained from step (d), wherein the transition from step (d) to step (e) occurs without opening the system; and (f) transferring the harvested TIL population from step (e) to an infusion bag, wherein the transfer from step (e) to (f) occurs without opening the system.

~~**[0004b]** In another aspect, the present disclosure refers to a cryopreservation composition comprising the population of TILs obtained or obtainable by the method of any of the preceding claims, a cryoprotectant medium comprising dimethylsulfoxide (DMSO), and an electrolyte solution.~~

~~**[0004c]** In another aspect, the present disclosure refers to the cryopreservation composition as disclosed herein for use in therapy.~~

~~**[0004d]** In another aspect, the present disclosure refers to the use of the cryopreservation composition as disclosed herein in the manufacture of a medicament for treating cancer in a subject.~~

WHAT IS CLAIMED IS:

1. A method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:
 - (a) adding processed tumor fragments from a tumor resected from a patient into a closed system to obtain a first population of TILs;
 - (b) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, to produce a second population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for 3-14 days to obtain the second population of TILs, and wherein the transition from step (a) to step (b) occurs without opening the system;
 - (c) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for 4 to 6 days to obtain the third population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system;
 - (d) splitting the third population of TILs into a first plurality of five or less sub-populations of TILs, each sub-population comprising at least 1.0×10^9 TILs, and performing a third expansion of the first plurality of sub-populations of TILs by supplementing the cell culture medium of each sub-population of TILs with additional IL-2, optionally OKT-3, to produce a second plurality of sub-populations of TILs, wherein the second plurality of sub-populations of TILs comprise a therapeutic population of TILs, wherein the third expansion is performed for 5 to 7 days, wherein the third expansion for each sub-population is performed in a closed container providing a third gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;
 - (e) harvesting the therapeutic population of TILs obtained from step (d), wherein the transition from step (d) to step (e) occurs without opening the system; and
 - (f) transferring the harvested TIL population from step (e) to an infusion bag, wherein the transfer from step (e) to (f) occurs without opening the system.

2. The method according to claim 1, wherein the therapeutic population of TILs harvested in step (e) comprises sufficient TILs for a therapeutically effective dosage of the TILs.
3. The method according to claim 2, where the number of TILs sufficient for a therapeutically effective dosage is from 2.3×10^{10} to 13.7×10^{10} .
4. The method according to claim 3, further comprising the step of cryopreserving the infusion bag comprising the harvested TIL population using a cryopreservation process.
5. The method according to claim 4, wherein the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.
6. The method according to claim 5, wherein the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).
7. The method according to claim 6, wherein the PBMCs are irradiated and allogeneic.
8. The method according to claim 1, wherein the infusion bag in step (f) is a hypothermic biopreservation infusion bag.
9. The method according to claim 1, wherein step (b) is performed within a period of 10 days, 11 days, or 12 days.
10. The method according to claim 1, wherein step (b) is performed within a period of 11 days.
11. The method according to claim 1, wherein steps (a) through (f) are performed within a period of 10 days to 22 days.
12. The method according to claim 1, wherein steps (a) through (f) are performed within a period of 10 days to 20 days.
13. The method according to claim 1, wherein steps (a) through (f) are performed within a period of 10 days to 15 days.
14. The method according to claim 1, wherein steps (a) through (f) are performed in 22 days or less.
15. The method according to claim 1, wherein steps (a) through (e) are performed in 22 days or less.
16. The method according to claim 1, wherein step (c) is performed for 108 hours to 132 hours.

17. The method according to claim 1, wherein step (c) is performed for 108 hours, 5 days or 132 hours.
18. The method according to claim 1, wherein step (c) and step (d) are performed within a period of 10 days to 12 days.
19. The method according to claim 1, wherein step (c) and step (d) are performed within a period of about 10 days, 11 days or 12 days.
20. The method according to any one of claims 1 to 19, wherein the therapeutic population of TILs comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, wherein the effector T cells and/or central memory T cells obtained in the therapeutic population of TILs exhibit one or more characteristics selected from the group consisting of expressing CD27+, expressing CD28+, longer telomeres, increased CD57 expression, and decreased CD56 expression relative to effector T cells, and/or central memory T cells obtained from the second population of cells.
21. The method according to [claim 20](#)~~any one of claims 1 to 20~~, wherein the effector T cells and/or central memory T cells obtained in the therapeutic population of TILs exhibit increased CD57 expression and decreased CD56 expression relative to effector T cells, and/or central memory T cells obtained from the second population of cells.
22. The method according to any one of claims 1 to 21, wherein the risk of microbial contamination is reduced as compared to an open system.
23. The method according to any of the preceding claims wherein each closed container comprises a single bioreactor.
24. The method according to claim 1, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs.
- ~~25. A cryopreservation composition comprising the population of TILs obtained or obtainable by the method of any of the preceding claims, a cryoprotectant medium comprising dimethylsulfoxide (DMSO), and an electrolyte solution.~~
- ~~26. The cryopreservation composition according to claim 25, further comprising one or more stabilizers and one or more lymphocyte growth factors.~~

27. The cryopreservation composition according to claim 26, wherein the one or more stabilizers comprise human serum albumin (HSA) and the one or more lymphocyte growth factors comprise IL-2.
28. The cryopreservation composition according to claim 27, wherein the composition optionally comprises OKT-3.
29. The cryopreservation composition according to claim 25, wherein the cryoprotectant medium comprising DMSO and the electrolyte solution are present in a ratio of 1:1:1 to 1:1:1.
30. The cryopreservation composition according to claim 29, wherein the cryoprotectant medium comprising DMSO and the electrolyte solution are present in a ratio of about 1:1.
31. The cryopreservation composition according to claim 25, wherein 100 mL of the composition comprises the population of TILs in an amount of 1×10^6 to 9×10^{14} ; the cryoprotectant medium comprising DMSO in an amount of 30 mL to 70 mL, the electrolyte solution in an amount of 30 mL to 70 mL; HSA in an amount of 0.1 g to 1.0 g; and IL-2 in an amount of 0.001 mg to 0.005 mg.
32. The cryopreservation composition according to claim 25, wherein 100 mL of the composition comprises the population of TILs in an amount of 1×10^7 to 1×10^{11} ; the cryoprotectant medium in an amount of 30 mL to 70 mL, wherein the cryoprotectant medium comprises about 10% DMSO; the electrolyte solution in an amount of 30 mL to 70 mL; HSA in an amount of 0.3 g to 0.7 g; and IL-2 in an amount of 0.001 mg to 0.003 mg.
33. The cryopreservation composition according to claim 25, wherein 100 mL of the composition consists essentially of the population of TILs in an amount of 1×10^7 to 1×10^{11} ; the cryoprotectant medium in an amount of 30 mL to 70 mL, wherein the cryoprotectant medium consists essentially of about 10% DMSO; the electrolyte solution in an amount of 30 mL to 70 mL; HSA in an amount of 0.3 g to 0.7 g; and IL-2 in an amount of 0.001 mg to 0.003 mg.
34. The cryopreservation composition according to claim 25 for use in therapy.
35. Use of the cryopreservation composition according to claim 25 in the manufacture of a medicament for treating cancer in a subject.