expanding TILs from a fine needle aspirate (FNA) or a core needle biopsy, which contain low numbers of TILs, and using these expanded TILs in treatment methods.

SUMMARY

[0004] The present disclosure provides improved and/or shortened methods for expanding TILs isolated from a fine needle aspirate or a core biopsy 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:
(i) performing a first expansion by culturing a first population of TILs obtained from at least one fine needle aspirate (FNA) or at least one small biopsy from a tumor in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the cell culture medium is optionally supplemented with OKT-3 at any one of days 1-3, wherein the first expansion is performed for about 3 days to about 10 days in order to obtain the second population of TILs, wherein the second population of TILs comprises at least 5×10^7 TILs by about 3 days to about 12 days when the first population of TILs is from a small biopsy; and (ii) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the third population of TILs is at least 25-fold greater in number than the second population of TILs, and wherein the second expansion is performed for about 3 days to about 12 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs.

[0005] In one ~~aspect~~example, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

(i) obtaining a first population of TILs from at least one fine needle aspirate (FNA) or at least one small biopsy from a tumor in a patient;

(ii) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the cell culture medium is optionally supplemented with OKT-3 at any one of days 1-3, wherein the first expansion is performed for about 3 days to about 10 days in order to obtain the second population of TILs, wherein the second population of TILs comprises at least 5×10^7 TILs by about 3 days to about 12 days when the first population of TILs is from a small biopsy; and

(iii) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the third population of TILs is at least 25-fold greater in number than the second population of TILs, and wherein the second expansion is performed for about 3 days to about 12 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs.

[0006] In some examples, the cell culture medium comprising IL-2 in step (ii) further comprises OKT-3 and is not optionally supplemented with OKT-3 at any one of days 1-3, and wherein step (i) is a priming first expansion step and step (ii) is a rapid second expansion.

[0007] The method according to claim 1 or 2, wherein after step (iii), the cells are removed from the cell culture and cryopreserved in a storage medium prior to performing step (iv).

[0008] In some examples, the cells are thawed prior to performing step (iv).

population of TILs, wherein the second population of TILs comprises at least 5×10^7 TILs by about 3 days to about 12 days when the first population of TILs is from a small biopsy;

(iii) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the third population of TILs is at least 25-fold greater in number than the second population of TILs, and wherein the second expansion is performed for about 3 days to about 12 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs; and

(iv) administering a therapeutically effective dosage of the third population of TILs to the patient.

[0054a] In another aspect, the present disclosure refers to use of a therapeutic population of tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for treating a subject with cancer, wherein the therapeutic population of TILs is obtained from a method comprising:

(i) ~~obtaining a first population of TILs from a fine needle aspirate (FNA) or a small biopsy obtained from a tumor in a patient;~~

~~(ii)~~ performing a first expansion by culturing ~~the~~ a first population of TILs obtained from a fine needle aspirate (FNA) or a small biopsy from a tumor in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the cell culture medium is supplemented with OKT-3 at any one of days 1-3, wherein the first expansion is performed for about 3 days to about 12 days in order to obtain the second population of TILs, wherein the second population of TILs comprises at least 5×10^7 TILs by about 3 days to about 12 days when the first population of TILs is from a small biopsy; and

(ii~~i~~) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the third population of TILs is at least 25-fold greater in number than the second population of TILs, and wherein the second expansion is performed for about 3 days to about 12 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs.

(a) ~~obtaining a first population of TILs from a fine needle aspirate (FNA) or a small biopsy obtained from a tumor in a patient;~~

~~(b) adding the first population into a closed system;~~

~~(c)~~ performing, within a closed system, a first expansion by culturing ~~the~~ a first population of TILs obtained from a fine needle aspirate (FNA) or a small biopsy from a tumor in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the cell culture medium is supplemented with OKT-3 at any one of days 1-3, wherein the first expansion is performed for about 3 days to about 12 days in order to obtain the second population of TILs, wherein the second population of TILs comprises at least 5×10^7 TILs by about 3 days to about 12 days when the first population of TILs is from a small biopsy, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3 days to about 12 days to obtain the second population of TILs, wherein the second population of TILs is at least 25-fold greater in number than the first population of TILs~~, and wherein the transition from step (b) to step (c) occurs without opening the system~~;

(~~d~~b) performing, without opening the system, a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 3 days to about 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic 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 (c) to step (d) occurs without opening the system~~;

(~~e~~c) harvesting, without opening the system, the therapeutic population of TILs obtained from step (~~d~~b)~~, wherein the transition from step (d) to step (e) occurs without opening the system~~; and

(~~f~~d) transferring, without opening the system, the harvested TIL population from step (e) to an infusion bag~~, wherein the transfer from step (e) to (f) occurs without opening the system~~.

[0096] In some examples, the cell culture medium comprising IL-2 in step (ii) further comprises OKT-3 and is not optionally supplemented with OKT-3 at any one of days 1-3, and wherein step (i) is a priming first expansion step and step (ii) is a rapid second expansion step.

second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 3 days to about 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic 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 (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;

(g) optionally cryopreserving the infusion bag comprising the harvested TIL population from step (f) using a cryopreservation process; and

(h) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (g) to the patient.

[00130a] In another aspect, the present disclosure refers to use of a therapeutic population of tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for treating a subject with cancer, wherein the therapeutic population of TILs is obtained from a method comprising:

(a) ~~obtaining a first population of TILs from a fine needle aspirate (FNA) or a small biopsy from a tumor resected from a subject;~~

~~(b) adding the first population into a closed system;~~

~~(c)~~ performing, within a closed system, a first expansion by culturing the first population of TILs obtained from a fine needle aspirate (FNA) or a small biopsy from a tumor in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the cell culture medium is supplemented with OKT-3 at any one of days 1-3, wherein the first expansion is performed for about 3 days to about 12 days in order to obtain the second population of TILs, wherein the second population of TILs comprises at least 5×10^7 TILs by about 3 days to about 12 days when the first population of TILs is from a small biopsy, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-14 days to obtain the second population of TILs, wherein the second population of TILs is at least 25-fold greater in number than the first population of TILs~~, and wherein the transition from step (b) to step (c) occurs without opening the system~~;

(~~d~~b) performing, without opening the system, a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 3 days to about 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic 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 (c) to step (d) occurs without opening the system~~;

(~~e~~c) harvesting, without opening the system, the therapeutic population of TILs obtained from step (~~d~~b)~~, wherein the transition from step (d) to step (e) occurs without opening the system~~; and

(~~f~~d) transferring, without opening the system, the harvested TIL population from step (e) to an infusion bag~~, wherein the transfer from step (e) to (f) occurs without opening the system~~; and

(~~g~~e) optionally cryopreserving the infusion bag comprising the harvested TIL population from step (~~f~~d) using a cryopreservation process.

[00131] In some examples, the therapeutic population of TILs harvested in step (e) comprises sufficient TILs for administering a therapeutically effective dosage of the TILs in step (h).

[00132] In some examples, the cell culture medium comprising IL-2 in step (ii) further comprises OKT-3 and is not optionally supplemented with OKT-3 at any one of days 1-3, and wherein step (i) is a priming first expansion step and step (ii) is a rapid second expansion step.

[00133] In some examples, the APCs are artificial APCs (aAPCs) or autologous APCs.

[00134] In some examples, the therapeutic population of TILs are infused into a patient.

[00135] In some examples, the first expansion in step (c) is performed by further supplementing the cell culture medium of the second population of TILs with OKT-3, IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

[00136] In some examples, the second expansion in step (d) is performed by further supplementing the cell culture medium of the second population of TILs with OKT-3, IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

[00151] In some examples, the high-dose IL-2 regimen comprises 600,000 or 720,000 IU/kg administered as a 15-minute bolus intravenous infusion every eight hours until tolerance.

[00152] In some examples, the third 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 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.

[00153] In some examples, the effector T cells and/or central memory T cells 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.

[00153a] In another aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

(a) performing a priming first expansion by culturing the first population of TILs obtained from a fine needle aspirate (FNA) a small biopsy, or a core biopsy from a tumor in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed for a first period of about 1 to 14 days in a container comprising a first gas-permeable surface area to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

(b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the rapid second expansion is performed for a second period of about 1 to about 14 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs; and

(c) harvesting the therapeutic population of TILs from step (b).

[00154] In another aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

(a) obtaining a first population of TILs from a fine needle aspirate (FNA), a small biopsy, a core biopsy, or a small biopsy from a tumor in a patient;

(b) performing a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed for a first period of about 1 to 14 days in a container comprising a first gas-permeable surface area to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

(c) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the rapid second expansion is performed for a second period of about 1 to about 14 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs; and

(d) harvesting the therapeutic population of TILs from step (c).

[00155] In some examples, the first period is about 6 to 12 days.

WHAT IS CLAIMED IS:

1. A method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

(i) ~~obtaining a first population of TILs from at least one fine needle aspirate (FNA) or at least one small biopsy from a tumor in a patient;~~

~~(ii)~~ performing a first expansion by culturing ~~the~~ a first population of TILs obtained from at least one fine needle aspirate (FNA) or at least one small biopsy from a tumor in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the cell culture medium is optionally supplemented with OKT-3 at any one of days 1-3, wherein the first expansion is performed for about 3 days to about 10 days in order to obtain the second population of TILs, wherein the second population of TILs comprises at least 5×10^7 TILs by about 3 days to about 12 days when the first population of TILs is from a small biopsy; and

~~(iii)~~ (ii) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the third population of TILs is at least 25-fold greater in number than the second population of TILs, and wherein the second expansion is performed for about 3 days to about 12 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs.

2. The method according to claim 1, wherein the cell culture medium comprising IL-2 in step ~~(ii)~~ (i) further comprises OKT-3 and is not optionally supplemented with OKT-3 at any one of days 1-3, and wherein step (i) is a priming first expansion step and step (ii) is a rapid second expansion.

3. The method according to claim 1 or 2, wherein after step ~~(iii)~~ (ii), the cells are removed from the cell culture and cryopreserved in a storage medium ~~prior to performing step (iv)~~.

4. The method according to claim 3, wherein the cells are thawed ~~prior to performing step (iv)~~ after cryopreservation.

~~5. The method according to any of the preceding claims, wherein step (iv) is repeated one to four times in order to obtain sufficient TILs in the therapeutic population of TILs for a therapeutically effective dosage of the TILs.~~

~~6.~~5. The method of according to any of the preceding claims, wherein steps (i) and (ii) ~~through (iii) or (iv)~~ are performed within a period of about 17 days to about 24 days.

~~7.~~6. The method according to any of the preceding claims, wherein steps (i) and (ii) ~~through (iii) or (iv)~~ are performed within a period of about 18 days to about 22 days.

~~8.~~7. The method according to any of the preceding claims, wherein steps (i) and (ii) ~~through (iii) or (iv)~~ are performed within a period of about 20 days to about 22 days.

~~9.~~8. The method according to any of the preceding claims, wherein steps (i) and (ii) ~~through (iii) or (iv)~~ are performed within about 22 days.

~~10. The method according to any of the preceding claims, wherein the cells from steps (iii) or (iv) express CD4, CD8, and TCR α β at levels similar to freshly harvested cells.~~

9. The method according to claim 1, wherein the cell culture medium of step (i) further comprises APCs.

~~11.~~10. The method according to claim 1, wherein the APCs are peripheral blood mononuclear cells (PBMCs).

~~12.~~11. The method according to ~~claim 11~~ claim 10, wherein the PBMCs are added to the cell culture on any of days 3 through 12 in step ~~(ii)~~ (i) and/or any of days 11 through 14 in step ~~(iii)~~ (ii).

~~13.~~12. The method according to claims ~~2 to 12~~ 2 to 11, wherein the third 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 in the ~~therapeutic~~ third population of TILs ~~in step (iv)~~ exhibit one or more characteristics selected from the group consisting of expression of CD27, expression of

CD28, longer telomeres, increased CD57 expression, and decreased CD56 expression, relative to effector T cells and/or central memory T cells in the ~~third~~ second population of cells.

~~14.~~13. The method according to claim ~~13~~ 12, wherein the effector T cells and/or central memory T cells exhibit increased CD57 expression and decreased CD56 expression.

~~15.~~14. The method according to any of the preceding claims, wherein the APCs are artificial APCs (aAPCs) or autologous APCs.

~~16.~~15. The method according to any of the preceding claims, wherein the therapeutic population of TILs are to be infused into a patient.

~~17.~~16. The method according to claim 1, wherein the first expansion in step (ii) is performed by further supplementing the cell culture medium of the second population of TILs with OKT-3, IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

~~18.~~17. The method according to claim 1, wherein the second expansion in step (iii) is performed by further supplementing the cell culture medium of the second population of TILs with IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

~~19.~~18. The method according to claim 1, wherein the FNA in step (i) comprises at least 400,000 TILs.

~~20.~~19. The method according to claim 1, wherein the small biopsy is obtained from a tumor selected from the group consisting of pancreatic, melanoma, breast, and ovarian.

~~21.~~20. The method according to claim 1, wherein the FNA is obtained from a tumor selected from the group consisting of lung, melanoma, head and neck, cervical, ovarian, pancreatic, glioblastoma, colorectal, and sarcoma.

~~22.~~21. The method according to claim ~~21~~ 20, wherein the lung tumor is a non-small cell lung carcinoma (NSCLC), and optionally wherein the patient has previously undergone surgical treatment.

~~23.~~22. The method according to claim 1, wherein the TILs in step (i) are obtained from a FNA.

~~24.~~23. The method according to claim 1, wherein the FNA is obtained using a 25-18 gauge needle.

~~25.~~24. The method according to claim 1, wherein the TILs in step (i) are obtained from a small biopsy.

~~26.~~25. The method according to claim 1, wherein the small biopsy is obtained using a 16-11 gauge needle.

~~27.~~26. The method according to claim 1, wherein step (iii) is repeated one to four times in order to obtain sufficient TILs in the therapeutic population of TILs for a therapeutically effective dosage of the TILs.

~~28.~~27. The method according to claim ~~27~~ 26, where the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10}.

~~29.~~28. A method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:
(i) performing a first expansion by culturing a first population of TILs from a fine needle aspirate (FNA) or a small biopsy from a tumor in a patient in a cell culture medium comprising IL-2 to obtain a second population of TILs, wherein the cell culture medium is supplemented with OKT-3 at any one of days 1-3, wherein the first expansion is performed for about 3 days to about 12 days in order to obtain the second population of TILs, wherein the second population of TILs comprises at least 5×10^7 TILs by about 3 days to about 12 days when the first population of TILs is from a small biopsy; and
(ii) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs) to obtain a third population of TILs, wherein the third population of TILs is at least 25-fold greater in number than the second population of TILs, and wherein the second expansion is

performed for about 3 days to about 12 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs.

~~30.~~29. The method according to claim ~~29~~ 28, wherein the cell culture medium comprising IL-2 in step (ii) further comprises OKT-3 and is not optionally supplemented with OKT-3 at any one of days 1-3, and wherein step (i) is a priming first expansion step and step (ii) is a rapid second expansion step.

~~31~~30. The method according to claims ~~29 or 30~~ 28 or 29, wherein the cells from the cell culture medium in step (ii) are removed and cryopreserved in a storage medium ~~prior to step (iii)~~.

~~32.~~31. The method according to claim ~~30~~ 29, wherein the cells are thawed ~~prior to step (iii)~~.

~~33.~~32. The method according to any of claims ~~29 to 31~~ 28 to 30, wherein the APCs are artificial APCs (aAPCs) or autologous APCs.

~~34.~~33. The method according to any of claims ~~29 to 32~~ 28 to 31, wherein the therapeutic population of TILs are to be infused into a patient.

~~35.~~34. The method according to claim ~~29~~ 28, wherein the first expansion in step (i) is performed by further supplementing the cell culture medium of the second population of TILs with OKT-3, IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

~~36.~~35. The method according to claim ~~29~~ 28, wherein the second expansion in step (ii) is performed by further supplementing the cell culture medium of the second population of TILs with IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

~~37.~~36. The method according to claim ~~29~~ 28, ~~wherein the~~ further comprising (iii) an additional second expansion ~~in step (iii) is~~ performed by further supplementing the cell culture medium of the third population of TILs with IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

~~38.~~37. The method according to claim ~~29~~ 28, wherein the FNA in step (i) comprises at least 400,000 TILs.

~~39.~~38. The method according to claim ~~29~~ 28, wherein the small biopsy is obtained from a tumor selected from the group consisting of pancreatic, melanoma, breast, and ovarian.

~~40.~~39. The method according to claim ~~29~~ 28, wherein the FNA is obtained from a tumor selected from the group consisting of lung, melanoma, head and neck, cervical, ovarian, pancreatic, glioblastoma, colorectal, and sarcoma.

~~41.~~40. The method according to claim 39, wherein the lung tumor is a non-small cell lung carcinoma (NSCLC), and optionally wherein the patient has previously undergone surgical treatment.

~~42.~~41. The method according to claim ~~29~~ 28, wherein the TILs in step (i) are obtained from a FNA.

~~43.~~42. The method according to claim ~~29~~ 28, wherein the FNA is obtained using a 25-18 gauge needle.

~~44.~~43. The method according to claim ~~29~~ 28, wherein the TILs in step (i) are obtained from a small biopsy.

~~45.~~44. The method according to claim ~~29~~ 28, wherein the small biopsy is obtained using a 16-11 gauge needle.

~~46.~~45. The method according to claim ~~29~~ 28, wherein step (ii) is repeated one to four times in order to obtain sufficient TILs in the therapeutic population of TILs for a therapeutically effective dosage of the TILs.

~~47.~~46. The method according to claim ~~45~~ 44, where the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10}.

~~48.~~47. The method according to any of claims ~~29 to 47~~ 28 to 46, wherein the third 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 exhibit one or more characteristics selected from the group consisting of expression of CD27, expression of CD28, longer telomeres, increased CD57 expression, and decreased CD56 expression, relative to effector T cells and/or central memory T cells in the third population of cells.

~~49.~~48. The method according to claim ~~48~~ 47, wherein the effector T cells and/or central memory T cells exhibit increased CD57 expression and decreased CD56 expression.

~~50.~~49. Use of a therapeutic population of tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for treating a subject with cancer, wherein the therapeutic population of TILs is obtained from a method comprising:

(i) ~~obtaining a first population of TILs from a fine needle aspirate (FNA) or a small biopsy obtained from a tumor in a patient;~~

~~(ii)~~ performing a first expansion by culturing ~~the~~ a first population of TILs obtained from a fine needle aspirate (FNA) or a small biopsy from a tumor in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the cell culture medium is supplemented with OKT-3 at any one of days 1-3, wherein the first expansion is performed for about 3 days to about 12 days in order to obtain the second population of TILs, wherein the second population of TILs comprises at least 5×10^7 TILs by about 3 days to about 12 days when the first population of TILs is from a small biopsy; and

~~(iii)~~ (ii) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the third population of TILs is at least 25-fold greater in number than the second population of TILs, and wherein the second expansion is performed for about 3 days to about 12 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs.

~~51.~~50. The use according to claim ~~50~~ 49, wherein the cell culture medium comprising IL-2 in step ~~(ii)~~ (i) further comprises OKT-3 and is not optionally supplemented with OKT-3 at any one of days 1-3, and wherein step (i) is a priming first expansion step and step (ii) is a rapid second expansion step.

~~52.~~51. The use according to claim ~~50~~ 49, wherein after step (ii) the cells are removed from the cell culture medium and cryopreserved in a storage medium prior to administration of a therapeutically effective dosage of the third population of TILs to the patient.

~~53.~~52. The use according to claim ~~50~~ 49, wherein the cells are thawed prior to administration of a therapeutically effective dosage of the third population of TILs to the patient.

~~54.~~53. The use according to claim ~~50~~ 49, wherein step ~~(iii)~~(ii) is repeated one to four times in order to obtain sufficient TILs in the therapeutic population of TILs for a therapeutically effective dosage of the TILs.

~~55.~~54. The use according to claims ~~50 to 54~~ 49 to 53, wherein the APCs are artificial APCs (aAPCs) or autologous APCs.

~~56.~~55. The use according to claim ~~50~~ 49, wherein the first expansion in step ~~(ii)~~(i) is performed by further supplementing the cell culture medium of the second population of TILs with OKT-3, IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

~~57.~~56. The use according to claim ~~50~~ 49, wherein the second expansion in step ~~(iii)~~(ii) is performed by further supplementing the cell culture medium of the second population of TILs with IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

~~58.~~57. The use according to claim ~~50~~ 49, wherein the FNA in step (i) comprises at least 400,000 TILs.

~~59.~~58. The use according to claim ~~50~~ 49, wherein the FNA is obtained from a tumor selected from the group consisting of lung, melanoma, head and neck, cervical, ovarian, pancreatic, glioblastoma, colorectal, and sarcoma.

~~60.~~59. The use according to claim ~~59~~ 58, wherein the lung tumor is a non-small cell lung carcinoma (NSCLC), and optionally wherein the subject has previously undergone surgical treatment.

~~61.~~60. The use according to claim ~~50~~ 49, wherein the TILs in step (i) are obtained from a FNA.

~~62.~~61. The use according to claim ~~50~~ 49, wherein the FNA is obtained using a 25-18 gauge needle.

~~63.~~62. The use according to claim ~~50~~ 49, wherein the TILs in step (i) are obtained from a small biopsy.

~~64.~~63. The use according to claim ~~50~~ 49, wherein the small biopsy is obtained using a 16-11 gauge needle.

~~65.~~64. The use according to claim ~~50~~ 49, wherein step ~~(iii)~~(ii) is repeated one to four times in order to obtain sufficient TILs in the therapeutic population of TILs for a therapeutically effective dosage of the TILs.

~~66.~~65. The use according to claim ~~65~~ 64, where the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10}.

~~67.~~66. The use according to any of claims ~~50 to 66~~ 49 to 65, wherein the third 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 exhibit one or more characteristics selected from the group consisting of expression of CD27, expression of CD28, longer telomeres, increased CD57 expression, and decreased CD56 expression, relative to effector T cells and/or central memory T cells in the third population of cells.

~~68.~~67. The use according to claim ~~67~~ 66, wherein the effector T cells and/or central memory T cells exhibit increased CD57 expression and decreased CD56 expression.

~~69.~~68. The use according to any of claims ~~50 to 68~~ 49 to 67, wherein the cancer is selected from the group consisting of melanoma, cervical cancer, head and neck cancer, glioblastoma, ovarian cancer, sarcoma, pancreatic cancer, bladder cancer, breast cancer, triple negative breast cancer, and non-small cell lung carcinoma.

~~70.~~69. Use of a therapeutic population of tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for treating a subject with cancer, wherein the therapeutic population of TILs is obtained from a method comprising:
(i) performing a first expansion by culturing a first population of TILs from a fine needle aspirate (FNA) or a small biopsy from a tumor in a patient in a cell culture medium comprising IL-2 to obtain a second population of TILs, wherein the cell culture medium is supplemented with OKT-3 at any one of days 1-3, wherein the first expansion is performed for about 3 days to about 12 days in order to obtain the second population of TILs, wherein the second population of TILs comprises at least 5×10^7 TILs by about 3 days to about 12 days when the first population of TILs is from a small biopsy; and
(ii) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs) to obtain a third population of TILs, wherein the third population of TILs is at least 25-fold greater in number than the second population of TILs, and wherein the second expansion is performed for about 3 days to about 12 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs.

~~71.~~70. The use according to claim ~~70~~ 69, wherein the cell culture medium comprising IL-2 in step (ii) further comprises OKT-3 and is not optionally supplemented with OKT-3 at any one of days 1-3, and wherein step (i) is a priming first expansion step and step (ii) is a rapid second expansion step.

~~72.~~71. The use according to claim ~~70 or 71~~ 69 to 70, wherein the cells from the cell culture medium in step (ii) are removed and cryopreserved in a storage medium prior to administration of a therapeutically effective dosage of the therapeutic population of TILs to the patient.

~~73.~~72. The use according to claim ~~72~~ 71, wherein the cells are thawed prior to administration of a therapeutically effective dosage of the therapeutic population of TILs to the patient.

~~74.~~73. The use according to any of claims ~~70 to 73~~ 69 to 72, wherein the APCs are artificial APCs (aAPCs) or autologous APCs.

~~75.~~74. The use according to any of claims ~~70 to 74~~ 69 to 73, wherein the APCs are peripheral blood mononuclear cells (PBMCs).

~~76.~~75. The use according to any of claims ~~69 to 75~~ 68 to 74, wherein the therapeutic population of TILs are to be infused into a patient.

~~77.~~76. The use according to claim ~~70~~ 69, wherein the first expansion in step (i) is performed by further supplementing the cell culture medium of the second population of TILs with OKT-3, IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

~~78.~~77. The use according to claim ~~70~~ 69, wherein the second expansion in step (ii) is performed by further supplementing the cell culture medium of the second population of TILs with IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

~~79.~~78. The use according to claim ~~70~~ 69, wherein the FNA in step (i) comprises at least 400,000 TILs.

~~80.~~79. The use according to claim ~~70~~ 69, wherein the small biopsy is obtained from a tumor selected from the group consisting of pancreatic, melanoma, breast, and ovarian.

~~81.~~80. The use according to claim ~~70~~ 69, wherein the FNA is obtained from a tumor selected from the group consisting of lung, melanoma, head and neck, cervical, ovarian, pancreatic, glioblastoma, colorectal, and sarcoma.

~~82.~~81. The use according to claim ~~81~~ 80, wherein the lung tumor is a non-small cell lung carcinoma (NSCLC), and optionally wherein the subject has previously undergone surgical treatment.

~~83.~~82. The use according to claim ~~70~~ 69, wherein the TILs in step (i) are obtained from a FNA.

~~84.~~83. The use according to claim ~~70~~ 69, wherein the FNA is obtained using a 25-18 gauge needle.

~~85.~~84. The use according to claim ~~70~~ 69, wherein the TILs in step (i) are obtained from a small biopsy.

~~86.~~85. The use according to claim ~~70~~ 69, wherein the small biopsy is obtained using a 16-11 gauge needle.

~~87.~~86. The use according to claim ~~70~~ 69, wherein step (ii) is repeated one to four times in order to obtain sufficient TILs in the therapeutic population of TILs for a therapeutically effective dosage of the TILs.

~~88.~~87. The use according to claim ~~87~~ 86, where the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10}.

~~89.~~88. The use according to any of claims ~~70 to 88~~ 69 to 87, wherein the third 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 exhibit one or more characteristics selected from the group consisting of expression of CD27, expression of CD28, longer telomeres, increased CD57 expression, and decreased CD56 expression, relative to effector T cells and/or central memory T cells in the third population of cells.

~~90.~~89. The use according to claim ~~89~~ 88, wherein the effector T cells and/or central memory T cells exhibit increased CD57 expression and decreased CD56 expression.

~~91.~~90. A method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

(a) ~~obtaining a first population of TILs from a fine needle aspirate (FNA) or a small biopsy obtained from a tumor in a patient;~~

~~(b) adding the first population into a closed system;~~

~~(c)~~ performing, within a closed system, a first expansion by culturing ~~the~~ a first population of TILs obtained from a fine needle aspirate (FNA) or a small biopsy from a tumor in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the cell culture medium is supplemented with OKT-3 at any one of days 1-3, wherein the first expansion is performed for about 3 days to about 12 days in order to obtain

the second population of TILs, wherein the second population of TILs comprises at least 5 × 10^7 TILs by about 3 days to about 12 days when the first population of TILs is from a small biopsy, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3 days to about 12 days to obtain the second population of TILs, wherein the second population of TILs is at least 25-fold greater in number than the first population of TILs~~, and wherein the transition from step (b) to step (c) occurs without opening the system~~;

~~(d)~~ (b) performing, without opening the system, a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 3 days to about 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic 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 (c) to step (d) occurs without opening the system~~;

~~(e)~~(c) harvesting, without opening the system, the therapeutic population of TILs obtained from step ~~(d)~~(b)~~, wherein the transition from step (d) to step (e) occurs without opening the system~~; and

~~(f)~~(d) transferring, without opening the system, the harvested TIL population from step ~~(e)~~ (c) to an infusion bag~~, wherein the transfer from step (e) to (f) occurs without opening the system~~.

~~92.~~91. The method according to claim ~~91~~ 90, wherein the cell culture medium comprising IL-2 in step ~~(ii)~~ (a) further comprises OKT-3 and is not optionally supplemented with OKT-3 at any one of days 1-3, and wherein step ~~(i)~~(a) is a priming first expansion step and step ~~(ii)~~(b) is a rapid second expansion step.

~~93.~~92. The method according to claims ~~91 or 92~~ 90 to 91, further comprising the step of cryopreserving the infusion bag comprising the harvested TIL population in step ~~(f)~~(d) using a cryopreservation process.

~~94.~~93. The method according to claim ~~93~~ 92, wherein the cryopreservation process is performed using a 1:1 ratio (v/v) of harvested TIL population to CS10 media.

94. The method according to claim 90, wherein the cell culture medium of step (a) further comprises APCs.

95. The method according to any one of claims ~~91 to 94~~ 90 to 94, wherein the APCs are peripheral blood mononuclear cells (PBMCs).

96. The method ~~according to any one~~ of ~~claims 91 to 95~~ claim 95, wherein the PBMCs are irradiated and allogeneic.

97. The method according to claim 96, wherein the PBMCs are added to the cell culture on any of days 3 through 12 in step ~~(c)~~ (a) and/or any of days 3 through 12 in step ~~(d)~~(b).

98. The method according to any one of claims ~~91 to 95~~ 90 to 95, wherein the antigen-presenting cells are artificial antigen-presenting cells (aAPCs) or autologous APCs.

99. The method according to any of claims ~~91 to 98~~ 90 to 98, wherein the therapeutic population of TILs are to be infused into a patient.

100. The method according to claim ~~91~~ 90, wherein the first expansion in step ~~(c)~~(a) is performed by further supplementing the cell culture medium of the second population of TILs with OKT-3, IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

101. The method according to claim ~~91~~ 90, wherein the second expansion in step ~~(d)~~(b) is performed by further supplementing the cell culture medium of the second population of TILs with IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

102. The method according to claim ~~91~~ 90, wherein the FNA ~~in step (a)~~ comprises at least 400,000 TILs.

103. The method according to claim ~~91~~ 90, wherein the small biopsy is obtained from a tumor selected from the group consisting of pancreatic, melanoma, breast and ovarian.

104. The method according to claim ~~91~~ 90, wherein the FNA is obtained from a tumor selected from the group consisting of lung, melanoma, head and neck, cervical, ovarian, pancreatic, glioblastoma, colorectal, and sarcoma.

105. The method according to claim 104, wherein the lung tumor is a non-small cell lung carcinoma (NSCLC), and optionally wherein the subject has previously undergone surgical treatment.

106. The method according to claim ~~91~~ 90, wherein the TILs in step (a) are obtained from a FNA.

107. The method according to claim ~~91~~ 90, wherein the FNA is obtained using a 25-18 gauge needle.

108. The method according to claim ~~91~~ 90, wherein the TILs in step (a) are obtained from a small biopsy.

109. The method according to claim ~~91~~ 90, wherein the small biopsy is obtained using a 16-11 gauge needle.

110. The method according to claim ~~91~~ 90, wherein the harvesting ~~in step (e)~~ is performing using a membrane-based ~~LOVO~~ cell processing system.

111. The method according to claim ~~91~~ 90, wherein the cell culture medium is provided in a container selected from the group consisting of a ~~G-container~~ gas-permeable container and a ~~Xuri~~ cellbag.

112. The method according to claim ~~91~~ 90, wherein the infusion bag ~~in step (f)~~ is a hypothermic biopreservation-containing ~~hypothermosol~~ infusion bag.

113. The method according to claim ~~91~~ 90, wherein steps (a) through ~~(f)~~(d) are performed within a period of about 17 days to about 24 days.

114. The method according to claim ~~91~~ 90, wherein steps (a) through ~~(f)~~(d) are performed within a period of about 18 days to about 22 days.

115. The method according to claim ~~91~~ 90, wherein steps (a) through ~~(f)~~(d) are performed within a period of about 20 days to about 22 days.

116. The method according to claim ~~91~~ 90, wherein steps (a) through ~~(f)~~(d) are performed in 22 days or less.

117. The method according to claim ~~91~~ 90, wherein steps (a) through ~~(f)~~(d) and cryopreservation are performed in 22 days or less.

118. The method according to any one of claims ~~91 to 117~~ 90 to 117, wherein the therapeutic population of TILs harvested ~~in step (e)~~ comprises sufficient TILs for a therapeutically effective dosage of the TILs.

119. The method according to claim 118, wherein the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10}.

120. The method according to any one of claims ~~91 to 119~~ 90 to 119, wherein steps ~~(b) through (e)~~ (a) through (c) are performed in a single container, wherein performing steps ~~(b) through (e)~~ (a) through (c) in a single container results in an increase in TIL yield per resected tumor as compared to performing steps ~~(b) through (e)~~ (a) through (c) in more than one container.

121. The method according to any one of claims ~~91 to 120~~ 90 to 120, wherein the antigen-presenting cells are added to the TILs during the second expansion ~~period in step (d)~~ without opening the system.

122. The method according to any one of claims ~~91 to 121~~ 90 to 121, wherein the third 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 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.

123. The method according to any one of claims ~~91 to 122~~ 90 to 122, wherein the effector T cells and/or central memory T cells obtained from the third 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.

124. The method according to any one of claims ~~91 to 123~~ 90 to 123, wherein the risk of microbial contamination is reduced as compared to an open system.

125. The method according to any one of claims ~~91 to 124~~ 90 to 124, wherein the TILs ~~from step (g)~~ are to be infused into a patient.

126. Use of a therapeutic population of tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for treating a subject with cancer, wherein the therapeutic population of TILs is obtained from a method comprising:

(a) ~~obtaining a first population of TILs from a fine needle aspirate (FNA) or a small biopsy from a tumor resected from a subject;~~

~~(b) adding the first population into a closed system;~~

~~(c)~~ performing, within a closed system, a first expansion by culturing ~~the~~ a first population of TILs obtained from a fine needle aspirate (FNA) or a small biopsy from a tumor in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the cell culture medium is supplemented with OKT-3 at any one of days 1-3, wherein the first expansion is performed for about 3 days to about 12 days in order to obtain the second population of TILs, wherein the second population of TILs comprises at least 5×10^7 TILs by about 3 days to about 12 days when the first population of TILs is from a small biopsy, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-14 days to obtain the second population of TILs, wherein the second population of TILs is at least 25-fold greater in number than the first population of TILs~~, and wherein the transition from step (b) to step (c) occurs without opening the system~~;

~~(d)~~ (b) performing, without opening the system, a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and

antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 3 days to about 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic 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 (c) to step (d) occurs without opening the system~~;

~~(e)~~ (c) harvesting, without opening the system, the therapeutic population of TILs obtained from step ~~(d)~~ (b)~~, wherein the transition from step (d) to step (e) occurs without opening the system~~; and

~~(f)~~ (d) transferring, without opening the system, the harvested TIL population from step ~~(e)~~ (c) to an infusion bag~~, wherein the transfer from step (e) to (f) occurs without opening the system~~; and

~~(g)~~ (e) optionally cryopreserving the infusion bag comprising the harvested TIL population from step ~~(f)~~ (d) using a cryopreservation process.

127. The use according to claim 126, wherein the therapeutic population of TILs harvested ~~in step (e)~~ comprises sufficient TILs for administration of a therapeutically effective dosage of the TILs.

128. The use according to claims 126 or 127, wherein the cell culture medium comprising IL-2 in step ~~(ii)~~(a) further comprises OKT-3 and is not optionally supplemented with OKT-3 at any one of days 1-3, and wherein step ~~(i)~~(a) is a priming first expansion step and step ~~(ii)~~(b) is a rapid second expansion step.

129. The use according to any one of claims 126 to 128, wherein the APCs are artificial APCs (aAPCs) or autologous APCs.

130. The use according to any of claims 126 to 129, wherein the therapeutic population of TILs are to be infused into a patient.

131. The use according to claim 126, wherein the first expansion ~~in step (c)~~ is performed by further supplementing the cell culture medium of the second population of TILs with OKT-3, IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

132. The use according to claim 126, wherein the second expansion in step (d) is performed by further supplementing the cell culture medium of the second population of TILs with OKT-3, IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

133. The use according to claim 126, wherein the FNA in step (a) comprises at least 400,000 TILs.

134. The use according to claim 126, wherein the small biopsy is obtained from a tumor selected from the group consisting of pancreatic, melanoma, breast and ovarian.

135. The use according to claim 126, wherein the FNA is obtained from a tumor selected from the group consisting of lung, melanoma, head and neck, cervical, ovarian, pancreatic, glioblastoma, colorectal, and sarcoma.

136. The use according to claim 135, wherein the lung tumor is a non-small cell lung carcinoma (NSCLC), and optionally wherein the subject has previously undergone surgical treatment.

137. The use according to claim 126, wherein the TILs in step (i) are obtained from a FNA.

138. The use according to claim 137, wherein the FNA is obtained using a 25-18 gauge needle.

139. The use according to claim 126, wherein the TILs in step (i) are obtained from a small biopsy.

140. The use according to claim 139, wherein the small biopsy is obtained using a 16-11 gauge needle.

141. The use according to claim 126, wherein the number of TILs sufficient for administration of a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10}.

142. The method according to claim 126, wherein the cell culture medium of step (a) further comprises APCs.

~~142.~~143. The use according to claim 126, wherein the antigen presenting cells (APCs) are PBMCs.

~~143.~~144. The use according to claim ~~142~~ 142 or 143, wherein the PBMCs are added to the cell culture on any of days 3 through 12 in step ~~(c)~~(a) and/or any of days 3 through 12 in step ~~(d)~~(b).

~~144.~~145. The use according to any of claims ~~126 to 143~~ 126 to 144, wherein prior to administration of a therapeutically effective dosage of TIL cells, a non-myeloablative lymphodepletion regimen has been administered to the patient.

~~145.~~146. The use according to claim ~~144~~ 145, where the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m2/day for two days followed by administration of fludarabine at a dose of 25 mg/m2/day for five days.

~~146.~~147. The use according to any of claims ~~126 to 145~~ 126 to 146, wherein a high-dose IL-2 regimen is to be further administered to the patient, starting on the day after administration of the TIL cells to the patient.

~~147.~~148. The use according to claim ~~146~~ 147, wherein the high-dose IL-2 regimen comprises 600,000 or 720,000 IU/kg to be administered as a 15-minute bolus intravenous infusion every eight hours until tolerance.

~~148.~~149. The use according to any of the claims ~~126 to 147~~ 126 to 148, wherein the third 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 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.

~~149.~~150. The use according to any of claims ~~126 to 148~~ 126 to 149, wherein the effector T cells and/or central memory T cells 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.

~~150.~~151. A method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

(a) ~~obtaining a first population of TILs from a fine needle aspirate (FNA), a small biopsy, a core biopsy, or a small biopsy from a tumor in a patient;~~

~~(b)~~ performing a priming first expansion by culturing the first population of TILs obtained from a fine needle aspirate (FNA) a small biopsy, or a core biopsy from a tumor in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed for a first period of about 1 to 14 days in a container comprising a first gas-permeable surface area to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

~~(c)~~ (b) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the rapid second expansion is performed for a second period of about 1 to about 14 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs; and

~~(d)~~ (c) harvesting the therapeutic population of TILs from step ~~(c)~~ (b).

~~151.~~152. The method according to claim ~~150~~ 151, wherein the first ~~period~~ expansion is about 6 to 12 days.

~~152.~~153. The method according to ~~claim 150 or claim 151~~ claims 151 or 152, wherein the second ~~period~~ expansion is about 6 to 12 days.

~~153.~~154. The method according to claim ~~150~~ 151, wherein the first ~~period~~ expansion is selected from the group consisting of 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, or 12 days.

~~154.~~155. The method according to claim ~~claim 150 or claim 151~~ 151 or 152, wherein the second ~~period~~ expansion is selected from the group consisting of 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, or 12 days.

~~155.~~156. The method of any of claims ~~150 to 154~~ 151 to 155, wherein the APCs are peripheral blood mononuclear cells (PBMCs).

~~156.~~157. The method of claim ~~155~~ 156, wherein the ratio of APCs used in Step ~~(b)~~ (a) to the APCs used in Step ~~(c)~~(b) is about 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1, 1.6:1, 1.7:1, 1.8:1, 1.9:1, 2:1, 2.1:1, 2.2:1, 2.3:1, 2.4:1, 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, 3.5:1, 3.6:1, 3.7:1, 3.8:1, 3.9:1, 4:1, 4.1:1, 4.2:1, 4.3:1, 4.4:1, 4.5:1, 4.6:1, 4.7:1, 4.8:1, 4.9:1, or 5:1, or preferably about 1 to 2.

~~157.~~158. The method of claim ~~156~~ 157, wherein the first population of TILs is obtained from a core biopsy.

~~158.~~159. The method of claim ~~155~~ 158, wherein the core biopsy is obtained from a tumor selected from the group consisting of a melanoma tumor, an ovarian cancer tumor, a cervical cancer tumor, a non-small-cell lung cancer (NSCLC) tumor, a lung cancer tumor, a bladder cancer tumor, a breast cancer tumor, a tumor from a cancer caused by human papilloma virus, a head and neck cancer (including head and neck squamous cell carcinoma (HNSCC)) tumor, a glioblastoma tumor, a gastrointestinal cancer tumor, and a renal cancer tumor.

~~159. A composition comprising TILs expanded using the methods of any of claims 150 to 158.~~

~~160. The composition of claim 159, further comprising a cyropreservant.~~

~~161. The composition of claim 160, wherein the cryopreservant comprises dimethylsulfoxide.~~

~~162. The composition of claim 159, further comprising a cyropreservant and an isotonic agent.~~

~~163. The composition of claim 162, further comprising a cyropreservant comprising dimethylsulfoxide and an isotonic agent comprising sodium chloride, sodium gluconate, and sodium acetate.~~

~~164. The composition of claim 162 further comprising a cyropreservant comprising dimethylsulfoxide and dextran 40 and an isotonic agent comprising sodium chloride, sodium gluconate, and sodium acetate.~~

~~165. The composition of any one of claim 159 to claim 164, wherein the composition is provided in a sterile infusion bag.~~