population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs.

[0008a] 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 from a tumor resected from a patient in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is performed for 11 days to 12 days in order to produce the second population of TILs; 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 100-fold greater in number than the second population of TILs,~~ and wherein the second expansion is performed for ~~at least~~ 14 days to 22 days in order to obtain the third population of TILs~~, wherein the third population of TILs is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs~~.

[0009] In some examples, the method further comprises:

(iv) performing an additional second expansion by supplementing the cell culture medium of the third population of TILs with additional IL-2, additional OKT-3, and additional APCs, wherein the additional second expansion is performed for at least 14 days to obtain a larger therapeutic population of TILs than obtained in step (iii), wherein the larger therapeutic population of TILs comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the third population of TILs.

[0010] In some examples, after step (iii), the cells are removed from the cell culture and cryopreserved in a storage medium prior to performing step (iv).

[0011] In some examples, the cells are thawed prior to performing step (iv).

[0012] In some examples, 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.

[0013] In some examples, steps (i) through (iii) or (iv) are performed within a period of about 40 days to about 50 days. In some examples, steps (i) through (iii) or (iv) are performed within a period of about 42 days to about 48 days. In some examples, steps (i) through (iii) or (iv) are performed within a period of about 42 days to about 45 days. In some examples, steps (i) through (iii) or (iv) are performed within about 44 days.

[0014] In some examples, the cells from steps (iii) or (iv) express CD4, CD8, and TCR α β at levels similar to freshly harvested cells.

[0015] In some examples, the antigen presenting cells are peripheral blood mononuclear cells (PBMCs). In some examples, the PBMCs are added to the cell culture on any of days 9 through 17 in step (iii).

[0016] In some examples, the effector T cells and/or central memory T cells in the therapeutic 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 population of cells.

[0017] In some examples, the effector T cells and/or central memory T cells exhibit increased CD57 expression and decreased CD56 expression.

[0018] In some examples, the APCs are artificial APCs (aAPCs).

[0019] In some examples, the method further comprises the step of transducing the first population of TILs with an expression vector comprising a nucleic acid encoding a high-affinity T cell receptor.

[0020] In some examples, the method further comprises the step of transducing the first population of TILs with an expression vector comprising a nucleic acid encoding a chimeric antigen receptor (CAR) comprising a single chain variable fragment antibody fused with at least one endodomain of a T-cell signaling molecule.

[0021] In some examples, the therapeutic population of TILs are infused into a patient.

[0022] In some examples, step (iii) further comprises a step of removing the cells from the cell culture medium.

[0023] In some examples, 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.

[0024] In some examples, the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10}.

[0025] In another aspect, the present disclosure refers to a population of expanded TILs made according to the method described herein.

[0026] In another aspect, the present disclosure refers to a population of expanded TILs made according to the method described herein, wherein the expanded TILs have at least a two-fold increase in basal glycolysis as compared to thawed cryopreserved TILs.

[0027] In another ~~aspect~~example, the present disclosure refers to a method for assessing the metabolic activity of a TIL cell population made according to the methods described herein, comprising measuring the basal glycolysis of the cells.

[0028] In another ~~aspect~~example, the present disclosure refers to a method for assessing the metabolic activity of a TIL cell population made according to the methods described herein, comprising measuring the basal respiration of the cells.

[0029] In another ~~aspect~~example, the present disclosure refers to a method for assessing the metabolic activity of a TIL cell population made according to the methods described herein, comprising measuring the spare respiratory capacity (SRC) of the cells.

[0030] In another example~~aspect~~, the present disclosure refers to a method for assessing the metabolic activity of a TIL cell population made according to the methods described herein, comprising measuring the glycolytic reserve of the cells.

[0031] In another ~~aspect~~example, 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 from a tumor resected from a patient in a cell culture medium comprising IL-2 to obtain a second population of TILs; 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 50-fold or 100-fold greater in number than the second population of TILs, and wherein the second expansion is performed for at least 14 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs.

[0032] In some examples, the method further comprises:

(iii) performing an additional second expansion of the third population of TILs by supplementing the cell culture medium of the third population of TILs with additional IL-2, additional OKT-3, and additional APCs, wherein the additional second expansion is performed for at least 14 days to obtain a larger therapeutic population of TILs than obtained in step (ii), wherein the larger therapeutic population of TILs exhibits an increased subpopulation of effector T cells and/or central memory T cells relative to the third population of TILs.

[0033] In some examples, the cells from the cell culture medium in step (ii) are removed and cryopreserved in a storage medium prior to step (iii).

[0034] In some examples, the cells are thawed prior to step (iii).

[0035] In some examples, 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.

[0036] In some examples, the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10}.

[0037] In some examples, the APCs are peripheral blood mononuclear cells (PBMCs).

[0038] In some examples, 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.

[0039] In some examples, the effector T cells and/or central memory T cells exhibit increased CD57 expression and decreased CD56 expression.

[0040] The present disclosure also provides a method for treating a subject with cancer comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

(i) obtaining a first population of TILs from a tumor resected from 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;

(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 50-fold or 100-fold greater in number than the second population of TILs, and wherein the second expansion is performed for at least 14 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs; and

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

[0040a] In another ~~aspect~~example, the present disclosure refers to use of a therapeutically effective dosage of a third population of expanded tumor infiltrating lymphocytes (TILs) obtained by a method comprising:
(i) performing a first expansion by culturing a first population of TILs from a tumor resected from a patient in a cell culture medium comprising IL-2 to produce a second population of TILs; 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 100-fold greater in number than the second population of TILs, and wherein the second expansion is performed for at least 14 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs; in the manufacture of a medicament for treating a subject with cancer.

[0041] In some examples, the method further comprises prior to step (iv) a step of performing an additional second expansion by supplementing the cell culture medium of the third population of TILs with additional IL-2, additional OKT-3, and additional APCs,

(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 50-fold or 100-fold greater in number than the second population of TILs, and wherein the second expansion is performed for at least 14 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs; and

(iii) administering a therapeutically effective dosage of the therapeutic population of TILs to the patient.

[0050a] In another ~~aspect~~example, the present disclosure refers to use of a therapeutically effective dosage of a therapeutic population of expanded tumor infiltrating lymphocytes (TILs) obtained by a method comprising:

(i) performing a first expansion by culturing a first population of TILs from a tumor resected from a patient in a cell culture medium comprising IL-2 to obtain a second population of TILs; 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 100-fold greater in number than the second population of TILs, and wherein the second expansion is performed for at least 14 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs;

in the manufacture of a medicament for treating cancer in a subject

[0051] In some examples, the method further comprises prior to step (iii) a step of performing an additional second expansion by supplementing the cell culture medium of the third population of TILs with additional IL-2, additional OKT-3, and additional APCs, wherein the additional second expansion is performed for at least 14 days to obtain a larger therapeutic population of TILs than obtained in step (ii), wherein the larger therapeutic population of TILs comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the third population of TILs.

[0052] In some examples, the cells from the cell culture medium in step (ii) are removed and cryopreserved in a storage medium prior to the additional second expansion as described herein.

[0053] In some examples, the cells are thawed prior to the additional second expansion as described herein.

[0054] In some examples, 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.

[0055] In some examples, the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10}.

[0056] In some examples, the APCs are peripheral blood mononuclear cells (PBMCs).

[0057] In some examples, 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

TILs is suitable for administration to a patient;

(ix) administering a therapeutically effective dosage of the thawed third population of TILs to the patient when the ratio of the number of TILs in the fourth population of TILs to the number of TILs in the third population of TILs is determined to be greater than 5:1 in step (viii).

[0082a] In another aspect, the present disclosure refers to a method for assaying TILs comprising: (i) obtaining a first population of TILs; (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 first expansion is performed for 11 to 12 days in order to produce the second population of TILs; 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 second expansion is performed for 14 days to 22 days in order to obtain the third population of TILs~~wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs~~; (iv) harvesting, washing, and cryopreserving the third population of TILs; (v) storing the cryopreserved TILs at a cryogenic temperature; (vi) thawing the third population of TILs to provide a thawed third population of TILs; and (vii) performing an additional second expansion of a portion of the thawed third population of TILs by supplementing the cell culture medium of the third population with IL-2, OKT-3, and APCs for an antigen restimulation rapid expansion protocol (reREP) period of at least 3 days, wherein the third expansion is performed to obtain a fourth population of TILs, wherein the number of TILs in the fourth population of TILs is compared to the number of TILs in the third population of TILs to obtain a ratio; (viii) determining based on the ratio in step (vii) whether the thawed third population of TILs is suitable for administration to a patient,

wherein a therapeutically effective dosage of the thawed third population of TILs is to be administered to the patient when the ratio of the number of TILs in the fourth population of TILs to the number of TILs in the third population of TILs is determined to be greater than 5:1 in step (viii).

[0083] In some examples, the reREP period is performed until the ratio of the number of TILs in the fourth population of TILs to the number of TILs in the third population of TILs is greater than 50:1.

[0084] In some examples, the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10}.

[0085] In some examples, steps (i) through (vii) are performed within a period of about 40 days to about 50 days. In some examples, steps (i) through (vii) are performed within a period of about 42 days to about 48 days. In some examples, steps (i) through (vii) are performed within a period of about 42 days to about 45 days. In some examples, steps (i) through (vii) are performed within about 44 days.

[0086] In some examples, the cells from steps (iii) or (vii) express CD4, CD8, and TCR α β at levels similar to freshly harvested cells. In some examples the cells are TILs.

[0087] In some examples, the antigen presenting cells are peripheral blood mononuclear cells (PBMCs). In some examples, the PBMCs are added to the cell culture on any of days 9 through 17 in step (iii).

[0088] In some examples, the effector T cells and/or central memory T cells in the larger population of TILs in steps (iii) or (vii) 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.

[0089] In some examples, the effector T cells and/or central memory T cells exhibit increased CD57 expression and decreased CD56 expression.

[0090] In some examples, the APCs are artificial APCs (aAPCs).

[0091] In some examples, the step of transducing the first population of TILs with an expression vector comprising a nucleic acid encoding a high-affinity T cell receptor.

WHAT IS CLAIMED IS:

1. 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 tumor resected from a patient in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is performed for 11 days to 12 days in order to produce the second population of TILs; 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 100-fold greater in number than the second population of TILs,~~ and wherein the second expansion is performed for ~~at least~~ 14 days to 22 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs~~which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs~~.

2. The method according to claim 1, wherein the method further comprises:

(iii) performing an additional second expansion by supplementing the cell culture medium of the third population of TILs with additional IL-2, additional OKT-3, and additional APCs, wherein the additional second expansion is performed for at least 14 days ~~to obtain a larger therapeutic population of TILs than obtained in step (ii), wherein the larger therapeutic population of TILs comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the third population of TILs~~.

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

4. The method according to claim 3, wherein the cells are thawed prior to performing step (iii).

5. The method according to any of claims 2 to 4, wherein step (iii) is repeated one to four times in order to obtain sufficient TILs in the larger therapeutic population of TILs for a therapeutically effective dosage of the TILs.

6. The method according to any of the preceding claims, wherein steps (i) through (ii) or (iii) are performed within a period of 40 days to 50 days.

7. The method according to any of the preceding claims, wherein steps (i) through (ii) or (iii) are performed within a period of 42 days to 48 days.

8. The method according to any of the preceding claims, wherein steps (i) through (ii) or (iii) are performed within a period of 42 days to 45 days.

9. The method according to any of the preceding claims, wherein steps (i) through (ii) or (iii) are performed within 44 days.

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

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

~~12.~~11. The method according to claim 10~~11~~, wherein the PBMCs are added to the cell culture on any of days 9 through 17 in step (ii).

~~13. The method according to claims 2 to 12, wherein the effector T cells and/or central memory T cells in the larger therapeutic population of TILs in step (iii) 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.~~

~~14. The method according to claim 13, wherein the effector T cells and/or central memory T cells in the larger therapeutic population of TILs in step (iii) exhibit increased CD57 expression and decreased CD56 expression.~~

~~15.~~12. The method according to any of ~~the preceding~~ claims 1-9, wherein the APCs are artificial APCs (aAPCs).

~~16.~~13. The method according to any of the preceding claims, further comprising the step of transducing the first population of TILs with an expression vector comprising a nucleic acid encoding a high-affinity T cell receptor.

~~17.~~14. The method according to any of the preceding claims, further comprising the step of transducing the first population of TILs with an expression vector comprising a nucleic

acid encoding a chimeric antigen receptor (CAR) comprising a single chain variable fragment antibody fused with at least one endodomain of a T-cell signaling molecule.

~~18.~~15. The method according to any of the preceding claims, wherein the therapeutic population of TILs are to be infused into a patient.

~~19.~~16. The method according to claim 1, wherein step (ii) further comprises a step of removing the cells from the cell culture medium.

~~20.~~17. The method according to claim 1, 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.

~~21.~~18. The method according to claim 17~~20~~, where the number of TILs sufficient for a therapeutically effective dosage is from 2.3×10^{10} to 13.7×10^{10}.

~~22. A population of expanded TILs made according to the method of claim 1.~~

~~23. A population of expanded TILs made according to the method of claim 1, wherein the expanded TILs have at least a two-fold increase in basal glycolysis as compared to thawed cryopreserved TILs.~~

~~24. A method for assessing the metabolic activity of a TIL cell population made according to the method of claim 1, comprising measuring the basal glycolysis of the cells.~~

~~25. A method for assessing the metabolic activity of a TIL cell population made according to the method of claim 1, comprising measuring the basal respiration of the cells.~~

~~26. A method for assessing the metabolic activity of a TIL cell population made according to the method of claim 1, comprising measuring the spare respiratory capacity (SRC) of the cells.~~

~~27. A method for assessing the metabolic activity of a TIL cell population made according to the method of claim 1, comprising measuring the glycolytic reserve of the cells.~~

~~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 tumor resected from a patient in a cell culture medium comprising IL-2 to obtain a second population of TILs; 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 100-fold greater in number than the second population of TILs, and wherein the second expansion is performed for at least 14 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs.

29. The method of claim 28, wherein the method further comprises:

(iii) performing an additional second expansion of the third population of TILs by supplementing the cell culture medium of the third population of TILs with additional IL-2, additional OKT-3, and additional APCs, wherein the additional second expansion is performed for at least 14 days to obtain a larger therapeutic population of TILs than obtained in step (ii), wherein the larger therapeutic population of TILs exhibits an increased subpopulation of effector T cells and/or central memory T cells relative to the third population of TILs.

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

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

32. The method according to claim 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.

33. The method according to claim 32, where the number of TILs sufficient for a therapeutically effective dosage is from 2.3×10^{10} to 13.7×10^{10}.

34. The method according to claims 28 to 33, wherein the APCs are peripheral blood mononuclear cells (PBMCs).

35. The method according to any of claims 29 to 34, wherein the effector T cells and/or central memory T cells in the larger therapeutic population of TILs in step (iii) 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.

36. The method according to claim 35, wherein the effector T cells and/or central memory T cells in the larger therapeutic population of TILs in step (iii) exhibit increased CD57 expression and decreased CD56 expression.

37. Use of a therapeutically effective dosage of a third population of expanded tumor infiltrating lymphocytes (TILs) obtained by a method comprising:

(i) performing a first expansion by culturing a first population of TILs from a tumor resected from a patient in a cell culture medium comprising IL-2 to produce a second population of TILs; 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 100-fold greater in number than the second population of TILs, and wherein the second expansion is performed for at least 14 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs; in the manufacture of a medicament for treating a subject with cancer.

38. The use of claim 37, wherein the method further comprises prior to administration of the medicament, a step (iii) of performing an additional second expansion by supplementing the cell culture medium of the third population of TILs with additional IL-2, additional OKT-3, and additional APCs, wherein the additional second expansion is performed for at least 14 days to obtain a larger therapeutic population of TILs than obtained in step (ii), wherein the larger therapeutic population of TILs comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the third population of TILs.

39. The use according to claim 38, wherein after step (i) the cells are removed from the cell culture medium and cryopreserved in a storage medium prior to the additional second expansion of claim 38.

40. The use according to claim 39, wherein the cells are thawed prior to the additional second expansion of claim 38.

41. The use according to claim 37, 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.

42. The use according to claim 41, where the number of TILs sufficient for a therapeutically effective dosage is from 2.3×10^{10} to 13.7×10^{10}.

43. The use according to any of claims 37 to 42, wherein the APCs are peripheral blood mononuclear cells (PBMCs).

44. The use according to any of claims 37 to 43, wherein the effector T cells and/or central memory T cells in the larger therapeutic population of TILs in step (iii) 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.

45. The use according to claim 44, wherein the effector T cells and/or central memory T cells in the larger therapeutic population of TILs in step (iii) exhibit increased CD57 expression and decreased CD56 expression.

46. The use according to any of claims 37 to 45, 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.

47. Use of a therapeutically effective dosage of a therapeutic population of expanded tumor infiltrating lymphocytes (TILs) obtained by a method comprising:

(i) performing a first expansion by culturing a first population of TILs from a tumor resected from a patient in a cell culture medium comprising IL-2 to obtain a second population of TILs; 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 100-fold greater in number than the second population of TILs, and wherein the second expansion is performed for at least 14 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs;

in the manufacture of a medicament for treating cancer in a subject.

48. The use of claim 47, wherein the method further comprises prior to administration of the medicament, a step (iii) of performing an additional second expansion by supplementing the cell culture medium of the third population of TILs with additional IL-2, additional OKT-3, and additional APCs, wherein the additional second expansion is performed for at least 14 days to obtain a larger therapeutic population of TILs than obtained in step (ii), wherein the larger therapeutic population of TILs comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the third population of TILs.

49. The use according to claim 48, wherein the cells from the cell culture medium in step (ii) are removed and cryopreserved in a storage medium prior to the additional second expansion in claim 48.

50. The use according to claims 49, wherein the cells are thawed prior to the additional second expansion in claim 48.

51. The use according to claim 47, 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.

52. The use according to claim 51, where the number of TILs sufficient for a therapeutically effective dosage is from 2.3×10^{10} to 13.7×10^{10}.

53. The use according to any of claims 47 to 52, wherein the APCs are peripheral blood mononuclear cells (PBMCs).

54. The use according to any of claims 47 to 53, wherein the effector T cells and/or central memory T cells in the larger therapeutic population of TILs in step (iii) 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.

55. The use according to claim 54, wherein the effector T cells and/or central memory T cells in the larger therapeutic population of TILs in step (iii) exhibit increased CD57 expression and decreased CD56 expression.

56. The use according to any of claims 47 to 55, 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.~~

~~57.~~19. ~~-~~A method for assaying TILs comprising:

(i) obtaining a first population of TILs;

(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 first expansion is performed for 11 to 12 days in order to produce the second population of TILs; 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 second expansion is performed for 14 days to 22 days in order to obtain the third population of TILs ~~wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs~~;

(iv) harvesting, washing, and cryopreserving the third population of TILs;

(v) storing the cryopreserved TILs at a cryogenic temperature;

(vi) thawing the third population of TILs to provide a thawed third population of TILs; and

(vii) performing an additional second expansion of a portion of the thawed third population of TILs by supplementing the cell culture medium of the third population with IL-2, OKT-3, and APCs for an antigen restimulation rapid expansion protocol (reREP) period of at least 3 days, wherein the third expansion is performed to obtain a fourth population of TILs, wherein the number of TILs in the fourth population of TILs is compared to the number of TILs in the third population of TILs to obtain a ratio;

(viii) determining based on the ratio in step (vii) whether the thawed third population of TILs is suitable for administration to a patient,

wherein a therapeutically effective dosage of the thawed third population of TILs is to be administered to the patient when the ratio of the number of TILs in the fourth population of TILs to the number of TILs in the third population of TILs is determined to be greater than 5:1 in step (viii).

~~58.~~20. The method according to claim 19~~57~~, wherein the reREP period is performed until the ratio of the number of TILs in the fourth population of TILs to the number of TILs in the third population of TILs is greater than 50:1.

~~59.~~21. The method according to claim ~~57~~19, where the number of TILs sufficient for a therapeutically effective dosage is from 2.3×10^{10} to 13.7×10^{10}.

~~60.~~22. The method of according to any of claims ~~57~~ 19 to ~~59~~21, wherein steps (i) through (vii) are performed within a period of 40 days to 50 days.

~~61.~~23. The method according to any of claims ~~57~~ 19 to ~~59~~21, wherein steps (i) through (vii) are performed within a period of 42 days to 48 days.

~~62.~~24. The method according to any of claims ~~57~~ 19 to ~~59~~21, wherein steps (i) through (vii) are performed within a period of 42 days to 45 days.

~~63.~~25. The method according to any of claims ~~57~~ 19 to ~~59~~21, wherein steps (i) through (vii) are performed within 44 days.

~~64. The method according to any of claims 57 to 63, wherein the cells from steps (iii) or (vii) express CD4, CD8, and TCR α β at levels similar to freshly harvested cells.~~

~~65.~~26. The method according to claim ~~57~~19, wherein the antigen presenting cells are peripheral blood mononuclear cells (PBMCs).

~~66.~~27. The method according to claim ~~65~~26, wherein the PBMCs are added to the cell culture on any of days 9 through 17 in step (iii).

~~67. The method according any of claims 57 to 66, wherein the effector T cells and/or central memory T cells in the third population of TILs in steps (iii) or fourth population of TILs in step (vii) 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. The method according to claim 67, wherein the effector T cells and/or central memory T cells in the third population of TILs in steps (iii) or fourth population of TILs in step (vii) exhibit increased CD57 expression and decreased CD56 expression.~~

~~69.~~28. The method according to any of claims ~~57~~ 19 to ~~68~~25, wherein the APCs are artificial APCs (aAPCs).

~~70.~~29. The method according to any of claims ~~57~~ 19 to ~~69~~28, further comprising the step of transducing the first population of TILs with an expression vector comprising a nucleic acid encoding a high-affinity T cell receptor.

~~71.~~30. The method according to claim ~~70~~29, wherein the step of transducing occurs before step (i).

~~72.~~31. The method according to any of claims ~~57~~ 19 to ~~71~~30, further comprising the step of transducing the first population of TILs with an expression vector comprising a nucleic acid encoding a chimeric antigen receptor (CAR) comprising a single chain variable fragment antibody fused with at least one endodomain of a T-cell signaling molecule.

~~73.~~32. The method according to claim ~~72~~31, wherein the step of transducing occurs before step (i).

~~74.~~33. The method according to any of claims ~~57~~ 19 to ~~75~~32, wherein the TILs are assayed for viability after step (vii).

~~75.~~34. A method for assaying TILs comprising:

(i) obtaining a portion of a first population of cryopreserved TILs;

(ii) thawing the portion of the first population of cryopreserved TILs;

(iii) performing a first expansion by culturing the portion of the first population of TILs in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) for a reREP period of at least 3 days, to produce a second population of TILs, wherein the portion from the first population of TILs is compared to the second population of TILs to obtain a ratio of the number of TILs, wherein the ratio of the number of TILs in the second population of TILs to the number of TILs in the portion of the first population of TILs is greater than 5:1; and

(iv) determining based on the ratio in step (iii) whether the first population of TILs is suitable for use in therapeutic administration to a patient,

wherein the remainder of the first population of TILs is to be therapeutically administered to the patient when the ratio of the number of TILs in the second population of TILs to the number of TILs in the first population of TILs is determined to be greater than 5:1 in step (iv).

~~76.~~35. The method according to claim ~~75~~34, wherein the ratio of the number of TILs in the second population of TILs to the number of TILs in the portion of the first population of TILs is greater than 50:1.

~~77.~~36. The method according to claims ~~75~~ 34 or ~~76~~35, ~~further comprising~~wherein the performing a first expansion of the entire first population of cryopreserved TILs is ~~from step (i)~~ according to the methods of performing a fist expansion of the first population of cryopreserved TILs as defined in ~~any of~~ claim~~s~~ 1 or 19~~to 21, 24 to 36, and 57 to 76~~.

~~78.~~37. The method according to claims ~~75~~ 34 or ~~76~~36, wherein the entire first population of cryopreserved TILs from step (i) is to be administered to the patient.

~~79.~~38. The method according to claims ~~75~~ 34 to ~~77~~37, further comprising the step of assessing the metabolic health of the second population of TILs.

~~80.~~39. The method according to claims ~~75~~ 34 to ~~78~~38, further comprising the step of assessing the phenotype of the second population of TILs.

~~81.~~40. The method according to claims ~~75~~ 34 to ~~79~~39, wherein the antigen presenting cells are allogeneic peripheral blood mononuclear cells.