effector T cells (see Chang, *et al.*, *Nat. Immunol.* **2016**, *17*, 364, hereby expressly incorporated in its entirety, and in particular for the discussion and markers of anaerobic and aerobic metabolism). For example, naïve T cells rely on mitochondrial respiration to produce ATP, while mature, healthy effector T cells such as TIL are highly glycolytic, relying on aerobic glycolysis to provide the bioenergetics substrates they require for proliferation, migration, activation, and anti-tumor efficacy.

[0005] In addition, these expanded cell populations can be cryopreserved, leading to ease of use, long-term storage for multiple reinfusions into patients with recurrent disease, and other considerations. However, current infusion acceptance parameters rely on readouts of the composition of TILs and on fold-expansion and viability of the expanded TIL based product. These measures give little insight into the health of the TIL that will be infused into the patient, and little is known about the effects of cryopreservation on TIL populations.

[0006] Accordingly, the present invention is directed to methods for expanding and re-stimulating TIL populations that lead to improved phenotype and increased metabolic health of the TILs and towards methods of assaying for TIL populations to determine suitability for more efficacious infusion after re-stimulation.

SUMMARY

[0007] The present disclosure provides methods for expanding TILs in larger, sometimes therapeutic, populations in combination with optional cryopreservation.

[0008] According to the present disclosure, a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising the following steps is provided:

(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; 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 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.

[0008a] In one aspect, there is provided 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; 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.

[0009] In some embodiments, 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 embodiments, 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 embodiments, the cells are thawed prior to performing step (iv).

[0012] In some embodiments, 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 embodiments, steps (i) through (iii) or (iv) are performed within a period of about 40 days to about 50 days. In some embodiments, steps (i) through (iii) or (iv) are performed within a period of about 42 days to about 48 days. In some embodiments, steps (i) through (iii) or (iv) are performed within a period of about 42 days to about 45 days. In some embodiments, steps (i) through (iii) or (iv) are performed within about 44 days.

[0014] In some embodiments, the cells from steps (iii) or (iv) express CD4, CD8, and TCR $\alpha\beta$ at levels similar to freshly harvested cells.

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

[0016] In some embodiments, 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 embodiments, the effector T cells and/or central memory T cells exhibit increased CD57 expression and decreased CD56 expression.

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

[0019] In some embodiments, 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 embodiments, 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 embodiments, the therapeutic population of TILs are infused into a patient.

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

[0023] In some embodiments, 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 embodiments, 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, there is provided a population of expanded TILs made according to the method described herein.

[0026] In another aspect, there is provided 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, there is provided 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, there is provided 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, there is provided 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 aspect, there is provided 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, there is provided 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 embodiments, 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 embodiments, 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 embodiments, the cells are thawed prior to step (iii).

[0035] In some embodiments, 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 embodiments, 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 embodiments, the APCs are peripheral blood mononuclear cells (PBMCs).

[0038] In some embodiments, 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 embodiments, 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, there is provided 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 embodiments, 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, there is provided 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 embodiments, 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 embodiments, 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 embodiments, the cells are thawed prior to the additional second expansion as described herein.

[0054] In some embodiments, 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 embodiments, 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 embodiments, the APCs are peripheral blood mononuclear cells (PBMCs).

[0057] In some embodiments, 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.

[0058] In some embodiments, the effector T cells and/or central memory T cells exhibit increased CD57 expression and decreased CD56 expression.

[0059] In some embodiments, 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.

[0060] The present disclosure also provides assay methods for determining TIL viability. The present disclosure provides methods for assaying TILs for viability by expanding tumor infiltrating lymphocytes (TILs) into a larger population of TILs comprising:

(i) obtaining a first population of TILs which has been previously expanded;

(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; 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 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 comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, and wherein the third population is further assayed for viability.

[0061] In some embodiments, 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 population of TILs than obtained in step (iii), wherein the larger population of TILs comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the third population of TILs, and wherein the third population is further assayed for viability.

[0062] In some embodiments, prior to step (i), the cells are cryopreserved.

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, there is provided 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; 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 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 a 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 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 embodiments, 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 embodiments, 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 embodiments, steps (i) through (vii) are performed within a period of about 40 days to about 50 days. In some embodiments, steps (i) through (vii) are performed within a period of about 42 days to about 48 days. In some embodiments, steps (i) through (vii) are performed within a period of about 42 days to about 45 days. In some embodiments, steps (i) through (vii) are performed within about 44 days.

[0086] In some embodiments, the cells from steps (iii) or (vii) express CD4, CD8, and TCR $\alpha\beta$ at levels similar to freshly harvested cells. In some embodiments the cells are TILs.

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

[0088] In some embodiments, 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 embodiments, the effector T cells and/or central memory T cells exhibit increased CD57 expression and decreased CD56 expression.

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

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

[0099] In some embodiments, the method further comprises administering the entire first population of cryopreserved TILs from step (i) to the patient.

[00100] The present disclosure also provides further methods for assaying TILs. In some embodiments, the disclosure provides 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;

(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; and

(v) therapeutically administering the remainder of the first population of TILs 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).

[100a] In another aspect, there is provided 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).

[00101] In some embodiments, 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.

[00102] In some embodiments, the method further comprises performing expansion of the entire first population of cryopreserved TILs from step (i) according to the methods described herein.

[00103] In some embodiments, the method further comprises administering the entire first population of cryopreserved TILs from step (i) to the patient.

[00104] In some embodiments, the method further comprised the step of assessing the metabolic health of the second population of TILs.

[00105] In some embodiments, the method further comprises the step of assessing the phenotype of the second population of TILs.

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; 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.

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 the preceding claims, 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.

6. The method of according to any of the preceding claims, wherein steps (i) through (ii) or (iii) are performed within a period of about 40 days to about 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 about 42 days to about 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 about 42 days to about 45 days.

9. The method according to any of the preceding claims, wherein steps (i) through (ii) or (iii) are performed within about 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. The method according to claim 1, wherein the antigen presenting cells are peripheral blood mononuclear cells (PBMCs).

12. The method according to claim 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 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 exhibit increased CD57 expression and decreased CD56 expression.

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

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

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

20. 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. The method according to claim 20, where the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 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 about 2.3×10^{10} to about 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 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 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 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 37, 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 about 2.3×10^{10} to about 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. A use according to any of claims 37 to 43, 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.

45. The use according to claim 44, wherein the effector T cells and/or central memory T cells 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 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. A use according to claim 47, 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 about 2.3×10^{10} to about 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 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 exhibit increased CD57 expression and decreased CD56 expression.

56. A 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. 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; 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 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 a 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 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. The method according to claim 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. The method according to claim 57, where the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10}.

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

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

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

63. The method according to any of claims 57 to 59, wherein steps (i) through (vii) are performed within about 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. The method according to claim 57, wherein the antigen presenting cells are peripheral blood mononuclear cells (PBMCs).

66. The method according to claim 645, 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 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.

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

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

70. The method according to any of claims 57 to 69, 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. The method according to claim 70, wherein the step of transducing occurs before step (i).

72. The method according to any of claims 57 to 71, 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. The method according to claim 72, wherein the step of transducing occurs before step (i).

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

75. 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. The method according to claim 75, 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. The method according to claims 75 or 76, further comprising performing expansion of the entire first population of cryopreserved TILs from step (i) according to the methods of any of the preceding claims.

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

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

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

81. The method according to claims 75 to 79, wherein the antigen presenting cells are allogeneic peripherial blood mononuclear cells.