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

SUMMARY

[0004] The present disclosure provides improved and/or shortened methods for expanding TILs isolated from a fine needle aspirate or a core biopsy and producing therapeutic populations of TILs.

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

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

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

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

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

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

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

[0009] 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.

[0010] In some examples, steps (i) through (iii) or (iv) are performed within a period of about 17 days to about 24 days.

[0011] In some examples, steps (i) through (iii) or (iv) are performed within a period of about 18 days to about 22 days.

[0012] In some examples, steps (i) through (iii) or (iv) are performed within a period of about 20 days to about 22 days.

[0013] In some examples, steps (i) through (iii) or (iv) are performed within about 22 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 APCs are peripheral blood mononuclear cells (PBMCs).

[0016] In some examples, the PBMCs are added to the cell culture on any of days 3 through 12in step (ii) and/or any of days 11 through 14 in step (iii).

[0017] In some examples, the third population of TILs comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, wherein the effector T cells and/or central memory T cells in the therapeutic population of TILs 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.

[0018] In some examples, the effector T cells and/or central memory T cells exhibit increased CD57 expression and decreased CD56 expression.

[0019] In some examples, the APCs are artificial APCs (aAPCs) or autologous APCs.

[0020] In some examples, the therapeutic population of TILs are infused into a patient.

[0021] In some examples, the first expansion in step (ii) is performed by further supplementing the cell culture medium of the second population of TILs with OKT-3, IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

[0022] In some examples, the second expansion in step (iii) is performed by further supplementing the cell culture medium of the second population of TILs with IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

[0023] In some examples, the FNA in step (i) comprises at least 400,000 TILs.

[0024] In some examples, the small biopsy is obtained from a tumor selected from the group consisting of pancreatic, melanoma, breast, and ovarian.

[0025] In some examples, the FNA is obtained from a tumor selected from the group consisting of lung, melanoma, head and neck, cervical, ovarian, pancreatic, glioblastoma, colorectal, and sarcoma.

[0026] In some examples, the lung tumor is a non-small cell lung carcinoma (NSCLC), and optionally wherein the patient has previously undergone surgical treatment.

[0027] In some examples, the TILs in step (i) are obtained from a FNA.

[0028] In some examples, the FNA is obtained using a 25-18 gauge needle.

[0029] In some examples, the TILs in step (i) are obtained from a small biopsy.

[0030] In some examples, the small biopsy is obtained using a 16-11 gauge needle.

[0031] 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.

[0032] 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}.

[0033] In another 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 fine needle aspirate (FNA) or a small biopsy from a tumor in a patient in a cell culture medium comprising IL-2 to obtain a second population of TILs, wherein the cell culture medium is supplemented with OKT-3 at any one of days 1-3, wherein the first expansion is performed for about 3 days to about 12 days in order to obtain the second population of TILs, wherein the second population of TILs comprises at least 5×10^7 TILs by about 3 days to about 12 days when the first population of TILs is from a small biopsy; and

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

[0034] In some examples, the cell culture medium comprising IL-2 in step (ii) further comprises OKT-3 and is not optionally supplemented with OKT-3 at any one of days 1-3, and wherein step (i) is a priming first expansion step and step (ii) is a rapid second expansion step.

[0035] 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).

[0036] In some examples, the cells are thawed prior to step (iii).

[0037] In some examples, the APCs are artificial APCs (aAPCs) or autologous APCs.

[0038] In some examples, the therapeutic population of TILs are infused into a patient.

[0039] In some examples, the first expansion in step (i) is performed by further supplementing the cell culture medium of the second population of TILs with OKT-3, IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

[0040] In some examples, the second expansion in step (ii) is performed by further supplementing the cell culture medium of the second population of TILs with IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

[0041] In some examples, the additional second expansion in step (iii) is performed by further supplementing the cell culture medium of the third population of TILs with IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

[0042] In some examples, the FNA in step (i) comprises at least 400,000 TILs.

[0043] In some examples, the small biopsy is obtained from a tumor selected from the group consisting of pancreatic, melanoma, breast, and ovarian.

[0044] In some examples, the FNA is obtained from a tumor selected from the group consisting of lung, melanoma, head and neck, cervical, ovarian, pancreatic, glioblastoma, colorectal, and sarcoma.

[0045] In some examples, the lung tumor is a non-small cell lung carcinoma (NSCLC), and optionally wherein the patient has previously undergone surgical treatment.

[0046] In some examples, the TILs in step (i) are obtained from a FNA.

[0047] In some examples, the FNA is obtained using a 25-18 gauge needle.

[0048] In some examples, the TILs in step (i) are obtained from a small biopsy.

[0049] In some examples, the small biopsy is obtained using a 16-11 gauge needle.

[0050] 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.

[0051] 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}.

[0052] In some examples, the third population of TILs comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, wherein the effector T cells and/or central memory T cells 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.

[0053] In some examples, the effector T cells and/or central memory T cells exhibit increased CD57 expression and decreased CD56 expression.

[0054] 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 fine needle aspirate (FNA) or a small biopsy obtained from a tumor in a patient;

(ii) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the cell culture medium is supplemented with OKT-3 at any one of days 1-3, wherein the first expansion is performed for about 3 days to about 12 days in order to obtain the second

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

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

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

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

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

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

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

[0055] In some examples, rein the cell culture medium comprising IL-2 in step (ii) further comprises OKT-3 and is not optionally supplemented with OKT-3 at any one of days 1-3, and wherein step (i) is a priming first expansion step and step (ii) is a rapid second expansion step.

[0056] In some examples, after step (ii) the cells are removed from the cell culture medium and cryopreserved in a storage medium prior to step (iv).

[0057] In some examples, the cells are thawed prior to step (iv).

[0058] 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.

[0059] In some examples, the APCs are artificial APCs (aAPCs) or autologous APCs.

[0060] In some examples, the first expansion in step (ii) is performed by further supplementing the cell culture medium of the second population of TILs with OKT-3, IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

[0061] In some examples, the second expansion in step (iii) is performed by further supplementing the cell culture medium of the second population of TILs with IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

[0062] In some examples, the FNA in step (i) comprises at least 400,000 TILs.

[0063] In some examples, the FNA is obtained from a tumor selected from the group consisting of lung, melanoma, head and neck, cervical, ovarian, pancreatic, glioblastoma, colorectal, and sarcoma.

[0064] In some examples, the lung tumor is a non-small cell lung carcinoma (NSCLC), and optionally wherein the subject has previously undergone surgical treatment.

[0065] In some examples, the TILs in step (i) are obtained from a FNA.

[0066] In some examples, the FNA is obtained using a 25-18 gauge needle.

[0067] In some examples, the TILs in step (i) are obtained from a small biopsy.

[0068] In some examples, the small biopsy is obtained using a 16-11 gauge needle.

[0069] 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.

[0070] 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}.

[0071] The present disclosure also provides a method according to any of claims 50 to 66, wherein the third population of TILs comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, wherein the effector T cells and/or central memory T cells exhibit one or more characteristics selected from the group consisting of expression of CD27, expression of CD28, longer telomeres, increased CD57 expression, and decreased CD56 expression, relative to effector T cells and/or central memory T cells in the third population of cells.

[0072] In some examples, the effector T cells and/or central memory T cells exhibit increased CD57 expression and decreased CD56 expression.

[0073] In some examples, 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.

[0074] The present disclosure also provides a method for treating a subject with cancer comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

(i) performing a first expansion by culturing a first population of TILs from a fine needle

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

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

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

[0074a] In another aspect, the present disclosure refers to use of a therapeutic population of tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for treating a subject with cancer, wherein the therapeutic population of TILs is obtained from a method comprising:

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

(ii) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs) to obtain a third population of TILs, wherein the third population of TILs is at least 25-fold greater in number than the second population of TILs, and wherein the second expansion is

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

[0075] In some examples, the cell culture medium comprising IL-2 in step (ii) further comprises OKT-3 and is not optionally supplemented with OKT-3 at any one of days 1-3, and wherein step (i) is a priming first expansion step and step (ii) is a rapid second expansion step.

[0076] In some examples, the cells from the cell culture medium in step (ii) are removed and cryopreserved in a storage medium prior step (iii).

[0077] In some examples, the cells are thawed prior to step (iii).

[0078] In some examples, the APCs are artificial APCs (aAPCs) or autologous APCs.

[0079] In some examples, the APCs are peripheral blood mononuclear cells (PBMCs).

[0080] In some examples, the therapeutic population of TILs are infused into a patient.

[0081] In some examples, the first expansion in step (i) is performed by further supplementing the cell culture medium of the second population of TILs with OKT-3, IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

[0082] In some examples, the second expansion in step (iii) is performed by further supplementing the cell culture medium of the second population of TILs with IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

[0083] In some examples, the FNA in step (i) comprises at least 400,000 TILs.

[0084] In some examples, the small biopsy is obtained from a tumor selected from the group consisting of pancreatic, melanoma, breast, and ovarian.

[0085] In some examples, the FNA is obtained from a tumor selected from the group consisting of lung, melanoma, head and neck, cervical, ovarian, pancreatic, glioblastoma, colorectal, and sarcoma.

[0086] In some examples, the lung tumor is a non-small cell lung carcinoma (NSCLC), and optionally wherein the subject has previously undergone surgical treatment.

[0087] In some examples, the TILs in step (i) are obtained from a FNA.

[0088] In some examples, the FNA is obtained using a 25-18 gauge needle.

[0089] In some examples, the TILs in step (i) are obtained from a small biopsy.

[0090] In some examples, the small biopsy is obtained using a 16-11 gauge needle.

[0091] 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.

[0092] 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}.

[0093] In some examples, the third population of TILs comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, wherein the effector T cells and/or central memory T cells 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.

[0094] In some examples, the effector T cells and/or central memory T cells exhibit increased CD57 expression and decreased CD56 expression.

[0095] In another aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

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

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

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

(d) 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 about 3 days to about 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

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

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

[0097] In some examples, the method further comprises the step of cryopreserving the infusion bag comprising the harvested TIL population in step (f) using a cryopreservation process.

[0098] In some examples, the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to CS10 media.

[0099] In some examples, the APCs are peripheral blood mononuclear cells (PBMCs).

[00100] In some examples, the PBMCs are irradiated and allogeneic.

[00101] In some examples, the PBMCs are added to the cell culture on any of days 3 through 12 in step (c) and/or any of days 3 through 12 in step (d).

[00102] In some examples, the antigen-presenting cells are artificial antigen-presenting cells (aAPCs) or autologous APCs.

[00103] In some examples, the therapeutic population of TILs are infused into a patient.

[00104] In some examples, the first expansion in step (c) is performed by further supplementing the cell culture medium of the second population of TILs with OKT-3, IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

[00105] In some examples, the second expansion in step (d) is performed by further supplementing the cell culture medium of the second population of TILs with IL-15, OX40 agonistic antibody and/or 4-1BB agonistic antibody.

[00106] In some examples, the FNA in step (a) comprises at least 400,000 TILs.

[00107] In some examples, the small biopsy is obtained from a tumor selected from the group consisting of pancreatic, melanoma, breast and ovarian.

[00108] In some examples, the FNA is obtained from a tumor selected from the group consisting of lung, melanoma, head and neck, cervical, ovarian, pancreatic, glioblastoma, colorectal, and sarcoma.

[00109] In some examples, the lung tumor is a non-small cell lung carcinoma (NSCLC), and optionally wherein the subject has previously undergone surgical treatment.

[00110] In some examples, the TILs in step (a) are obtained from a FNA.

[00111] In some examples, the FNA is obtained using a 25-18 gauge needle.

[00112] In some examples, the TILs in step (a) are obtained from a small biopsy.

[00113] In some examples, the small biopsy is obtained using a 16-11 gauge needle.

[00114] In some examples, the harvesting in step (e) is performing using a LOVO cell processing system.

[00115] In some examples, the cell culture medium is provided in a container selected from the group consisting of a G-container and a Xuri cellbag.

[00116] In some examples, the infusion bag in step (f) is a hypothermosol infusion bag.

[00117] In some examples, steps (a) through (f) are performed within a period of about 17 days to about 24 days.

[00118] In some examples, steps (a) through (f) are performed within a period of about 18 days to about 22 days.

[00119] In some examples, steps (a) through (f) are performed within a period of about 20 days to about 22 days.

[00120] In some examples, steps (a) through (f) are performed in 22 days or less.

[00121] In some examples, steps (a) through (f) and cryopreservation are performed in 22 days or less.

[00122] In some examples, the therapeutic population of TILs harvested in step (e) comprises sufficient TILs for a therapeutically effective dosage of the TILs.

[00123] 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}.

[00124] In some examples, steps (b) through (e) are performed in a single container, wherein performing steps (b) through (e) in a single container results in an increase in TIL yield per resected tumor as compared to performing steps (b) through (e) in more than one container.

[00125] In some examples, the antigen-presenting cells are added to the TILs during the second period in step (d) without opening the system.

[00126] In some examples, the third population of TILs comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, wherein the effector T cells and/or central memory T cells in the therapeutic population of TILs exhibit one or more characteristics selected from the group consisting of expressing CD27+, expressing CD28+, longer telomeres, increased CD57 expression, and decreased CD56 expression relative to effector T cells, and/or central memory T cells obtained from the second population of cells.

[00127] In some examples, the effector T cells and/or central memory T cells obtained from the third population of TILs exhibit increased CD57 expression and decreased CD56 expression relative to effector T cells and/or central memory T cells obtained from the second population of cells.

[00128] In some examples, the risk of microbial contamination is reduced as compared to an open system.

[00129] In some examples, the TILs from step (g) are infused into a patient.

[00130] The present disclosure also provides a method for treating a subject with cancer, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

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

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

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

(d) 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 about 3 days to about 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

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

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

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

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

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

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

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

(d) 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 about 3 days to about 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

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

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

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

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

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

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

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

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

[00137] In some examples, the FNA in step (a) comprises at least 400,000 TILs.

[00138] In some examples, the small biopsy is obtained from a tumor selected from the group consisting of pancreatic, melanoma, breast and ovarian.

[00139] In some examples, the FNA is obtained from a tumor selected from the group consisting of lung, melanoma, head and neck, cervical, ovarian, pancreatic, glioblastoma, colorectal, and sarcoma.

[00140] In some examples, the lung tumor is a non-small cell lung carcinoma (NSCLC), and optionally wherein the subject has previously undergone surgical treatment.

[00141] In some examples, the TILs in step (i) are obtained from a FNA.

[00142] In some examples, the FNA is obtained using a 25-18 gauge needle.

[00143] In some examples, the TILs in step (i) are obtained from a small biopsy.

[00144] In some examples, the small biopsy is obtained using a 16-11 gauge needle.

[00145] In some examples, the number of TILs sufficient for administering a therapeutically effective dosage in step (h) is from about 2.3×10^{10} to about 13.7×10^{10}.

[00146] In some examples, the antigen presenting cells (APCs) are PBMCs.

[00147] In some examples, the PBMCs are added to the cell culture on any of days 3 through 12 in step (c) and/or any of days 3 through 12 in step (d).

[00148] In some examples, prior to administering a therapeutically effective dosage of TIL cells in step (h), a non-myeloablative lymphodepletion regimen has been administered to the patient.

[00149] In some examples, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for five days.

[00150] In some examples, the method further comprises the step of treating the patient with a high-dose IL-2 regimen starting on the day after administration of the TIL cells to the patient in step (h).

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

[00152] In some examples, the third population of TILs comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, wherein the effector T cells and/or central memory T cells in the therapeutic population of TILs exhibit one or more characteristics selected from the group consisting of expressing CD27+, expressing CD28+, longer telomeres, increased CD57 expression, and decreased CD56 expression relative to effector T cells, and/or central memory T cells obtained from the second population of cells.

[00153] In some examples, the effector T cells and/or central memory T cells in the therapeutic population of TILs exhibit increased CD57 expression and decreased CD56 expression relative to effector T cells and/or central memory T cells obtained from the second population of cells.

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

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

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

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

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

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

[00156] In some examples, the second period is about 6 to 12 days.

[00157] In some examples, the first period is selected from the group consisting of 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, or 12 days.

[00158] In some examples, the second period is selected from the group consisting of 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, or 12 days.

[00159] In some examples, the APCs are peripheral blood mononuclear cells (PBMCs).

[00160] In some examples, the ratio of APCs used in Step (b) to the APCs used in Step (c) is about 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1, 1.6:1, 1.7:1, 1.8:1, 1.9:1, 2:1, 2.1:1, 2.2:1, 2.3:1, 2.4:1, 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, 3.5:1, 3.6:1, 3.7:1, 3.8:1, 3.9:1, 4:1, 4.1:1, 4.2:1, 4.3:1, 4.4:1, 4.5:1, 4.6:1, 4.7:1, 4.8:1, 4.9:1, or 5:1, or preferably about 1 to 2.

[00161] In some examples, the first population of TILs is obtained from a core biopsy.

[00162] In some examples, the core biopsy is obtained from a tumor selected from the group consisting of a melanoma tumor, an ovarian cancer tumor, a cervical cancer tumor, a non-small-cell lung cancer (NSCLC) tumor, a lung cancer tumor, a bladder cancer tumor, a breast cancer tumor, a tumor from a cancer caused by human papilloma virus, a head and neck cancer (including head and neck squamous cell carcinoma (HNSCC)) tumor, a glioblastoma tumor, a gastrointestinal cancer tumor, and a renal cancer tumor.

[00163] The present disclosure also provides compositions comprising TILs expanded using the methods as described herein.

[00163a] In another aspect, the present disclosure refers to a composition comprising TILs expanded using the methods as disclosed herein.

[00164] In some examples, the composition further comprises a cyropreservant.

[00165] In some examples, the cryopreservant comprises dimethylsulfoxide.

[00166] In some examples, the composition further comprises a cyropreservant and an isotonic agent.

[00167] In some examples, the composition further comprises a cyropreservant comprising dimethylsulfoxide and an isotonic agent comprising sodium chloride, sodium gluconate, and sodium acetate.

[00168] In some examples, the composition further comprises a cyropreservant comprising dimethylsulfoxide and dextran 40 and an isotonic agent comprising sodium chloride, sodium gluconate, and sodium acetate.

[00169] In some examples, the composition comprising the TILs is provided in a sterile infusion bag.

BRIEF DESCRIPTION OF THE DRAWINGS

[00170] Figure 1: Exemplary Process 2A chart providing an overview of Steps A through F.

[00171] Figure 2: Process Flow Chart of Process 2A.

[00172] Figure 3: Shows a diagram of an embodiment of a cryopreserved TIL exemplary manufacturing process (~22 days).

[00173] Figure 4: Shows a diagram of an embodiment of process 2A, a 22-day process for TIL manufacturing.

[00174] Figure 5: Comparison table of Steps A through F from exemplary embodiments of process 1C and process 2A.

[00175] Figure 6: Detailed comparison of an embodiment of process 1C and an embodiment of process 2A.

[00176] Figure 7: Exemplary Small Biopsy chart providing an overview of Steps A through F of the Small Biopsy Expansion process.

[00177] Figure 8: Image of viable cells expanded from core biopsies taken from a patient with pancreatic cancer (P7015). A) Core biopsies treated with IL-2, and B) core biopsies treated with a combination of IL-2, IL-15, and IL-21.

[00178] Figure 9: FACS analysis of TILs expanded from pancreatic core biopsies. A) T cells expanded from core biopsies treated with IL-2 are CD4+ and CD8+. B) CD8+ T cells expanded from core biopsies treated with IL-2 are CD107a+. C) T cells expanded from core biopsies treated with a combination of IL-2, IL-15, and IL-21 are CD4+ and CD8+. D) CD8+ T cells expanded from core biopsies treated with a combination of IL-2, IL-15, and IL-21 are CD107a+.

WHAT IS CLAIMED IS:

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

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

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

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

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

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

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

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

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

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

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

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

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

11. The method according to claim 1, wherein the APCs 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 3 through 12in step (ii) and/or any of days 11 through 14 in step (iii).

13. The method according to claims 2 to 12, wherein the third population of TILs comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, wherein the effector T cells and/or central memory T cells in the therapeutic population of TILs 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.

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) or autologous APCs.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

TILs is a therapeutic population of TILs.

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

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

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

54. The use according to claim 50, 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.

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

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

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

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

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

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

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

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

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

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

65. The use according to claim 50, 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.

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

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

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

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

(i) performing a first expansion by culturing a first population of TILs from a fine needle aspirate (FNA) or a small biopsy from a tumor in a patient in a cell culture medium comprising IL-2 to obtain a second population of TILs, wherein the cell culture medium is supplemented with OKT-3 at any one of days 1-3, wherein the

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

89. The use according to any of claims 70 to 88, wherein the third population of TILs comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, wherein the effector T cells and/or central memory T cells exhibit one or more characteristics selected from the group consisting of expression of CD27, expression of CD28, longer telomeres, increased

CD57 expression, and decreased CD56 expression, relative to effector T cells and/or central memory T cells in the third population of cells.

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

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

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

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

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

(d) 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 about 3 days to about 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

112. The method according to claim 91, wherein the infusion bag in step (f) is a hypothermosol infusion bag.

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

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

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

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

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

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

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

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

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

122. The method according to any one of claims 91 to 121, wherein the third population of TILs comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, wherein the effector T cells and/or central memory T cells in the therapeutic population of TILs exhibit one or more characteristics selected from the group consisting of expressing CD27+, expressing CD28+, longer telomeres, increased CD57 expression, and decreased CD56 expression relative to effector T cells, and/or central memory T cells obtained from the second population of cells.

123. The method according to any one of claims 91 to 122, wherein the effector T cells and/or central memory T cells obtained from the third population of TILs exhibit increased CD57 expression and decreased CD56 expression relative to effector T cells and/or central memory T cells obtained from the second population of cells.

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

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

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

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

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

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

(d) 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 about 3 days to about 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

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

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

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

128. The use according to claims 126 or 127, wherein the cell culture medium comprising IL-2 in step (ii) further comprises OKT-3 and is not optionally supplemented with

OKT-3 at any one of days 1-3, and wherein step (i) is a priming first expansion step and step (ii) is a rapid second expansion step.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

145. The use according to claim 144, where the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for five days.

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

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

148. The use according to any of the claims 126 to 147, wherein the third population of TILs comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, wherein the effector T cells and/or central memory T cells in the therapeutic population of TILs exhibit one or more characteristics selected from the group consisting of expressing CD27+, expressing CD28+, longer telomeres, increased CD57 expression, and decreased CD56 expression relative to effector T cells, and/or central memory T cells obtained from the second population of cells.

149. The use according to any of claims 126 to 148, wherein the effector T cells and/or central memory T cells in the therapeutic population of TILs exhibit increased CD57

expression and decreased CD56 expression relative to effector T cells and/or central memory T cells obtained from the second population of cells.

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

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

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

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

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

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

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

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

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

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

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

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

158. The method of claim 157, wherein the core biopsy is obtained from a tumor selected from the group consisting of a melanoma tumor, an ovarian cancer tumor, a cervical cancer tumor, a non-small-cell lung cancer (NSCLC) tumor, a lung cancer tumor, a bladder cancer tumor, a breast cancer tumor, a tumor from a cancer caused by human papilloma virus, a head and neck cancer (including head and neck squamous cell carcinoma (HNSCC)) tumor, a glioblastoma tumor, a gastrointestinal cancer tumor, and a renal cancer tumor.

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

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

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

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

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

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

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