Dudley, *et al.*, *J. Clin. Oncol.* **2008**, *26,* 5233-39; Rosenberg, *et al.*, *Clin. Cancer Res.* **2011,** *17,* 4550-57. Earlier approaches to expansions of TILs from B cell lymphomas yielded poor results, with only 2 of 12 attempts at TIL growth providing for potential activity against tumors. Schwartzentruber, *et al.*, *Blood* **1993,** *82,* 1204-1211. There is an urgent need to provide for more efficacious therapies in many hematological malignancies, including acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL).

[0004] The present invention provides the surprising finding that TIL expansion processes can result in efficacious TIL populations obtained from hematological malignancies, such as liquid tumors, including lymphomas or leukemias.

SUMMARY

[0004a] In one aspect, the present disclosure refers to an *ex vivo* method for expanding peripheral blood lymphocytes (PBLs) from peripheral blood comprising:

a. Isolating PBLs from a sample of peripheral blood mononuclear cells (PBMCs) obtained from peripheral blood, wherein said sample is optionally cryopreserved, by selecting and removing CD19+ B cells;

b. Optionally co-culturing said PBLs with said CD19+ B cells;

c. Stimulating said PBLs in a first cell culture medium with IL-2 and anti-CD3/anti-CD28 antibodies for a period of from about 2 to about 6 days in a gas permeable container;

d. Culturing the PBLs from step (d) for a period of from about 2 to about 6 days with IL-2 and anti-CD3/anti-CD28 antibodies;

e. Isolating the antibody-bound PBLs from the culture in step (e);

f. Removing the antibodies from the PBLs isolated in step (e); and

g. Harvesting the PBLs.

[0004b] In another aspect, the present disclosure refers to an *ex vivo* method for expanding peripheral blood lymphocytes (PBLs) from peripheral blood comprising:

a. Isolating PBLs from a sample of PBMCs obtained from peripheral blood of a patient, wherein said sample is optionally cryopreserved and the patient is optionally pretreated with an ITK inhibitor, by selecting and removing CD19+ B cells and optionally

pretreating said PBLs with an ITK inhibitor at a concentration of between 0.1 nM and 200 nM;

b. Co-culturing said PBLs with said CD19+ B cells for a period of between 2 to 5 days;

c. Adding from about 2.5x105 to about 5x105 cells to a gas-permeable container in a first cell culture medium and stimulating said PBLs with 3000 IU/ml IL-2 and anti-CD3/anti-CD28 antibodies immobilized on beads, and and optionally an ITK inhibitor at a concentration of between 0.1 nM and 200 nM, for a period of between 3 to 6 days;

d. Exchanging the first cell culture medium with a second cell culture medium and additional IL-2 at a concentration of about 3000 IU/ml and optionally an ITK inhibitor at a concentration of between 0.1 nM and 200 nM;

e. Culturing the PBLs from step (d) for an additional period of between 3 to 6 days with IL-2 and anti-CD3/anti-CD28 antibodies immobilized on beads and optionally an ITK inhibitor at a concentration of between 0.1 nM and 200 nM;

f. Exchanging the second cell culture medium with a third cell culture medium and adding additional IL-2 at a concentration of 3000 IU/mL, and optionally an ITK inhibitor at a concentration of between 0.1 nM and 200 nM, and culturing the cells for an additional period of between 2 to 5 days;

g. Isolating the antibody-bound PBLs from the culture in step (f);

h. Removing the antibodies from the PBLs isolated in step (g); and

i. Harvesting the PBLs,

wherein the ITK inhibitor is an ITK inhibitor that covalently binds to ITK.

[0004c] In another aspect, the present disclosure refers to the use of peripheral blood lymphocytes (PBLs) in the manufacture of a medicament for treating a hematological malignancy, comprising:

a. Isolating PBLs from a sample of PBMCs from peripheral blood of a patient suffering from a hematological malignancy by selecting and removing CD19+ B cells;

b. Optionally co-culturing said PBLs with said CD19+ B cells;

c. Stimulating said PBLs in a first cell culture medium with IL-2 and anti-CD3/anti-CD28 antibodies for a period of from about 2 to about 6 days in a gas permeable container;

d. Culturing the PBLs from step (c) for a period of from about 2 to about 6 days with IL-2 and anti-CD3/anti-CD28 antibodies;

e. Isolating the antibody-bound PBLs from the culture in step (d);

f. Removing the antibodies from the PBLs isolated in step (e); and

g. Harvesting the PBLs; wherein

h. the PBLs are to be administered to the patient in a therapeutically effective amount to treat said hematological malignancy.

[0004d] In another aspect, the present disclosure refers to an *ex vivo* method for expanding peripheral blood lymphocytes (PBLs) from peripheral blood, the method comprising the steps of:

(a) culturing a sample of peripheral blood mononuclear cells (PBMCs) obtained from peripheral blood of a patient who is pre-treated with ibrutinib or another insulin-like tyrosine kinase (ITK) inhibitor and who is refractory to treatment with ibrutinib or such other ITK inhibitor, in a culture comprising a first culture medium with IL-2 and anti-CD3/anti-CD28 antibodies, for a period of time selected from the group consisting of: about 9 days, about 10 days, about 11 days, about 12 days, about 13 days and about 14 days, thereby effecting expansion of peripheral blood lymphocytes (PBLs) from said PBMCs; and

(b) harvesting the PBLs from the culture in step (a).

[0004e] In another aspect, the present disclosure refers to the use of peripheral blood lymphocytes (PBLs) in the manufacture of a medicament for treating a hematological malignancy, comprising:

(a) culturing a sample of peripheral blood mononuclear cells (PBMCs) from peripheral blood of a patient with said hematological malignancy who is pre-treated with ibrutinib or another insulin-like tyrosine kinase (ITK) inhibitor and who is refractory to treatment with ibrutinib or such other ITK inhibitor in a culture comprising a first culture medium with IL-2 and anti-CD3/anti-CD28 antibodies, for a period of time selected from the group consisting of: about 9 days, about 10 days, about 11 days, about 12 days, about 13 days and about 14 days, thereby effecting expansion of peripheral blood lymphocytes (PBLs) from said PBMCs;

(b) harvesting the PBLs from the culture in step (a); wherein

(c) the PBLs are to be administered to the patient in a therapeutically effective amount to treat said malignancy.

[0005] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) optionally pre-treating a patient with a regimen comprising at least one kinase inhibitor;

(b) obtaining a tumor from the patient by resection, biopsy, needle aspiration, or apheresis, the tumor comprising a first population of TILs;

(c) optionally fragmenting or dissociating the tumor to obtain tumor fragments and contacting the tumor fragments with a first cell culture medium;

(d) performing an initial expansion of the first population of TILs in the first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, wherein the first cell culture medium comprises IL-2, and wherein the initial expansion is performed over a period of 21 days or less;

(e) performing a second expansion of the second population of TILs in a second cell culture medium to obtain 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 after 7 days from the start of the second expansion; wherein the second cell culture medium comprises IL-2, OKT-3 (anti-CD3 antibody), and irradiated allogeneic peripheral blood mononuclear cells (PBMCs), and wherein the second expansion is performed over a period of 14 days or less;

(f) harvesting the third population of TILs; and

(g) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer;

wherein the tumor is a liquid tumor, and wherein the cancer is a hematological malignancy.

[0006] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) optionally pre-treating a patient with a regimen comprising at least one kinase inhibitor;

(b) obtaining a tumor from the patient by resection, biopsy, needle aspiration, or apheresis, the tumor comprising a first population of TILs;

(c) optionally fragmenting or dissociating the tumor to obtain tumor fragments and contacting the tumor fragments with a first cell culture medium;

(d) performing an initial expansion of the first population of TILs in the first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, wherein the first cell culture medium comprises IL-2, and wherein the initial expansion is performed over a period of 21 days or less;

(e) performing a second expansion of the second population of TILs in a second cell culture medium to obtain 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 after 7 days from the start of the second expansion; wherein the second cell culture medium comprises IL-2, OKT-3 (anti-CD3 antibody), and irradiated allogeneic peripheral blood mononuclear cells (PBMCs), and wherein the second expansion is performed over a period of 14 days or less;

(f) harvesting the third population of TILs; and

(g) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer;

wherein the tumor is a liquid tumor, and wherein the cancer is a hematological malignancy selected from the group consisting of acute myeloid leukemia (AML), mantle cell lymphoma (MCL), follicular lymphoma (FL), diffuse large B cell lymphoma (DLBCL), activated B cell (ABC) DLBCL, germinal center B cell (GCB) DLBCL, chronic lymphocytic leukemia (CLL), small lymphocytic leukemia (SLL), non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma, relapsed and/or refractory Hodgkin's lymphoma, B cell acute lymphoblastic leukemia (B-ALL), mature B-ALL, Burkitt's lymphoma, Waldenström's macroglobulinemia (WM), multiple myeloma, myelodysplatic syndromes, myelofibrosis, chronic myelocytic leukemia, follicle center lymphoma, indolent NHL, human immunodeficiency virus (HIV) associated B cell lymphoma, and Epstein–Barr virus (EBV) associated B cell lymphoma.

[0007] In an example of the disclosure, the method further comprises addition of an ITK inhibitor. In an example, the ITK inhibitor is added to the cell culture medium during at least one of steps (d) and (e). In an example of the disclosure, the ITK inhibitor is a covalent ITK inhibitor that covalently and irreversibly binds to ITK. In an example of the disclosure, the ITK inhibitor is an allosteric ITK inhibitor that binds to ITK. In another example, the ITK inhibitor is selected from the group consisting of aminothiazole-based ITK inhibitors, benzimidazole-based ITK inhibitors, aminopyrimidine-based ITK inhibitors, 3-aminopyride-2-ones-based ITK inhibitors, indolylndazole-based ITK inhibitors, pyrazolyl-indole-based inhibitors, thienopyrazole inhibitors, and ITK inhibitors targeting cysteine-442 in the ATP pocket. In another example, ITK inhibitor is the ITK inhibitor is ibrutinib, dasatinib, bosutinib, nilotinib, erlotinib BMS509744, CTA056, GSK2250665A, PF06465469 ((*R*)-3-(1-(1-acryloylpiperidin-3-yl)-4-amino-1*H*-pyrazolo[3,4-d]pyrimidin-3-yl)-N-(3-methyl-4-(1-methylethyl))benzamide), and combinations thereof. In another example, the ITK inhibitor is ibrutinib. In another example, the ITK inhibitor is (*R*)-3-(1-(1-acryloylpiperidin-3-yl)-4-amino-1*H*-pyrazolo[3,4-d]pyrimidin-3-yl)-*N*-(3-methyl-4-(1-methylethyl))benzamide. The foregoing ITK inhibitors are available commercially from various sources, including Tocris Bioscience, Inc. (Minneapolis, MN, USA), Selleckchem, Inc. (Houston, TX, USA), and AK Scientific, Inc. (Union City, CA, USA). In another example, the ITK inhibitor is added at a concentration of from about 0.1 nM to about 5 μM. In another example, the ITK inhibitor is added at a concentration of from about 0.1 nM to about 5 μM. In another example, the ITK inhibitor is added at a concentration of from about 0.1 nM to about 100 nM. In another example, the ITK inhibitor is added at a concentration of from about 0.5 nM to about 50 nM. In another example, the ITK inhibitor is added at a concentration of from about 1 nM to about 10 nM. In an example, the ITK inhibitor is added at a concentration of about 0.01 nM, 0.05 nM, 0.1 nM, 0.5 nM, 1 nM, 2 nM, 5 nM, 10 nM, 20 nM, 30 nM, 40 nM, 50 nM, 60 nM, 70 nM, 80 nM, 90 nM, 100 nM, 150 nM, 200 nM, 300 nM, 400 nM, 500 nM, 600 nM, 700 nM, 800 nM, 900 nM, 1 μM, 2 μM, 3 μM, 4 μM, 5 μM, 10 μM, 20 μM, 30 μM, 40 μM, and 50 μM.

[0008] In an example of the disclosure, a method for expanding peripheral blood lymphocytes (PBLs) from peripheral blood comprises:

a. Obtaining a sample of peripheral blood mononuclear cells (PBMCs) from peripheral blood, wherein said sample is optionally cryopreserved;

b. Isolating PBLs from said sample by selecting and removing CD19+ B cells;

c. Optionally co-culturing said PBLs with said CD19+ B cells;

d. Stimulating said PBLs in a first cell culture medium with IL-2 and anti-CD3/anti-CD28 antibodies for a period of from about 2 days ot aobut 6 days in a gas permeable container;

e. Culturing the PBLs from step (d) for a period of from about 2 days to about6 days with IL-2 and anti-CD3/anti-CD28 antibodies;

f. Isolating the antibody-bound PBLs from the culture in step (e);

g. Removing the antibodies from the PBLs isolated in step (e); and

h. Harvesting the PBLs.

[0009] In an example of the disclosure, the method further comprises addition of IL-2 after step (d), and exchanging the first culture medium to a second cell culture medium. In another example, the method further comprises addition of IL-2 after step (e), and exchanging the second culture medium to a third culture medium. In an example, the first cell culture medium, second cell culture medium, or third culture medium is selected from the group consisting of CM-2, CM-4, and AIM-V. In another example, the first and second cell culture media are the same. In another example, the first and second cell culture media are different. In an example of the disclosure, one or more of the first, second and third cell culture media are the same. In another example, the first, second, and third cell culture media are all different.

[0010] In an example, the optional co-culturing of said PBLs with said CD19+ B cells is performed for a period of 1 hour to 3 days.

[0011] In an example of the disclosure, the ratio of T-cells to B-cells in step (c) is from about 0.1:1 to about 10:1 (B-cells:T-cells). In another example, the ratio of B-cells to T-cells in step (c) is selected from the group consisting of 0.1:1, 1:1, and 10:1 (B-cells:T-cells).

[0012] In an example of the disclosure, the starting cell number of PBLs at the beginning of step (d) is at least from about $1x10^5$ to about $10x10^5$ PBLs. In another example, the starting cell number of PBLs at the beginning of step (d) is at least from about $2.5x10^5$ to $10x10^5$ PBLs. In another example, the starting cell number of PBLs at the beginning of step (d) is at least $5x10^5$ PBLs.

[0013] In an example of the disclosure, the IL-2 in each of steps (c) and (d) is used at a concentration of from about 1000 IU/mL to about 6000 IU/mL. In another example, the IL-2 in each of steps (c) and (d) is used at a concentration of about 3000 IU/mL.

[0014] In an example of the disclosure, the anti-CD3/anti-CD28 antibodies are coated onto beads. In an example of the disclosure, the anti-CD3/anti-CD28 antibodies are DynaBeads®. In an example, the method includes co-culturing the anti-CD3/anti-CD28 antibody beads with the PBLs in about a 1:1 bead:PBL ratio in each of steps (c) and (d).

[0015] In an example of the disclosure, the method comprises adding an ITK inhibitor. In an example, the ITK inhibitor is added during at least one of steps (c), (d), and (e). In an example of the disclosure, the ITK inhibitor is selected from the group consisting of aminothiazole-based ITK inhibitors, benzimidazole-based ITK inhibitors, aminopyrimidine-based ITK inhibitors, 3-aminopyride-2-ones-based ITK inhibitors, indolylndazole-based ITK inhibitors, pyrazolyl-indole-based inhibitors, thienopyrazole inhibitors, and ITK inhibitors targeting cysteine-442 in the ATP pocket. In another example, the ITK inhibitor is ibrutinib, dasatinib, bosutinib, nilotinib, erlotinib BMS509744, CTA056, GSK2250665A, PF06465469, and combinations thereof. In another example, the ITK inhibitor is ibrutinib.

[0016] In an example of the disclosure, any of the foregoing methods for preparing PBLs is performed in a closed, sterile system.

[0017] In an example of the present disclosure, a method for expanding peripheral blood lymphocytes (PBLs) from peripheral blood comprises:

a. Obtaining a sample of PBMCs from peripheral blood, wherein said sample is optionally cryopreserved;

b. Isolating PBLs from said sample by selecting and removing CD19+ B cells;

c. Co-culturing said PBLs with said CD19+ B cells for a period of 4 days;

d. Adding from about $2.5x10^5$ to about $5x10^5$ cells to a gas-permeable container in CM-2 cell culture medium and stimulating said PBLs with 3000 IU/ml IL-2 and anti-CD3/anti-CD28 antibodies immobilized on beads for a period of about 4 days;

e. Exchanging the CM-2 with AIM-V cell culture medium and additional IL-2 at about 3000 IU/ml;

f. Culturing the PBLs from step (e) for an additional period of about 3 days with IL-2 and anti-CD3/anti-CD28 antibodies immobilized on beads;

g. Isolating the antibody-bound PBLs from the culture in step (f);

h. Removing the antibodies from the PBLs isolated in step (g); and

i. Harvesting the PBLs.

[0018] In an example of the disclosure, a method for treating a hematological malignancy comprises:

a. Obtaining a sample of PBMCs from peripheral blood of a patient suffering from a hematological malignancy;

b. Isolating PBLs from said sample by selecting and removing CD19+ B cells;

c. Optionally co-culturing said PBLs with said CD19+ B cells;

d. Stimulating said PBLs in a first cell culture medium with IL-2 and anti-CD3/anti-CD28 antibodies for a period of at least about 4 days in a gas permeable container;

e. Culturing the PBLs from step (d) for a period of 3 days with IL-2 and anti-CD3/anti-CD28 antibodies;

f. Isolating the antibody-bound PBLs from the culture in step (e);

g. Removing the antibodies from the PBLs isolated in step (e); and

h. Harvesting the PBLs; and

i. Administering the PBLs to the patient in a therapeutically effective amount to treat said hematological malignancy.

[0019] In an example of the disclosure, the method further comprises obtaining a PBMC sample from a patient that is pre-treated with an ITK inhibitor. In an example of the disclosure, the ITK inhibitor is selected from the group consisting of aminothiazole-based ITK inhibitors, benzimidazole-based ITK inhibitors, aminopyrimidine-based ITK inhibitors, 3-aminopyride-2-ones-based ITK inhibitors, indolylndazole-based ITK inhibitors, pyrazolyl-indole-based inhibitors, thienopyrazole inhibitors, and ITK inhibitors targeting cysteine-442 in the ATP pocket. In an example of the disclosure, the ITK inhibitor is ibrutinib, BMS509744, CTA056, GSK2250665A, PF06465469, and combinations thereof. In another example, the ITK inhibitor

is ibrutinib. In another example, the patient is pre-treated with at least three rounds of an ibrutinib regimen.

[0020] In an example of the disclosure, the hematological malignancy is selected from the group consisting of acute myeloid leukemia (AML), mantle cell lymphoma (MCL), follicular lymphoma (FL), diffuse large B cell lymphoma (DLBCL), activated B cell (ABC) DLBCL, germinal center B cell (GCB) DLBCL, chronic lymphocytic leukemia (CLL), small lymphocytic leukemia (SLL), non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma, relapsed and/or refractory Hodgkin's lymphoma, B cell acute lymphoblastic leukemia (B-ALL), mature B-ALL, Burkitt's lymphoma, Waldenström's macroglobulinemia (WM), multiple myeloma, myelodysplatic syndromes, myelofibrosis, chronic myelocytic leukemia, follicle center lymphoma, indolent NHL, human immunodeficiency virus (HIV) associated B cell lymphoma, and Epstein–Barr virus (EBV) associated B cell lymphoma. In another example, the hematological malignancy is chronic lymphocytic leukemia (CLL). In an example of the disclosure, the PBLs are administered in an amount of from about $0.1x10^9$ to about $15x10^9$ PBLs.

[0021] In an example of the disclosure, a method for expanding marrow-infiltrating lymphocytes (MILs) from bone marrow comprises:

a. Obtaining a sample of peripheral blood mononuclear cells (PBMCs) from bone marrow, wherein said sample is optionally cryopreserved;

b. Sorting a CD3+, CD33+, CD20+ and CD14+ cell fraction (MIL fraction) and a non-CD3+, non-CD33+, non-CD20+, non-CD14+ cell fraction (AML blast cell fraction);

c. Optionally disrupting the AML blast cell fraction;

d. Adding the optionally disrupted AML blast cell fraction to the MIL fraction in a cell number ratio of from about 0.1:1 to about 10:1

e. Culturing one or both cell fractions in a gas permeable container in a first cell culture medium, comprising IL-2;

f. Stimulating the MILs with anti-CD3/anti-CD28 antibodies to obtain expansion of MILs;

g. Restimulating the MILs with IL-2 and anti-CD3/anti-CD28 antibodies for an

additional period of about 2 to about 6 days;

h. Culturing the MILs with additional IL-2 for an additional period of from about 1 to about 3 days; and

i. Harvesting said MILs.

[0022] In an example of the disclosure, the method further comprises addition IL-2 after step (e), and exchanging the culture media to a second cell culture medium. In an example, the first cell culture medium and the second cell culture medium is selected from the group consisting of CM-2, CM-4, and AIM-V. In another example, the first and second cell culture media are the same. In another example, the first and second cell culture media are different.

[0023] In an example, there are at least from about $2x10^4$ to about $5x10^5$ MILs in the gas permeable container at the beginning of step (e). In another example, there are at least from about $2.8x10^4$ to $3.4x10^5$ MILs in the gas permeable container at the beginning of step (e). In another example, there are at least $5x10^5$ MILs in the gas permeable container at the beginning of step (e).

[0024] In an example of the disclosure, the IL-2 is present in a concentration of between 1000 IU/ml and 6000 IL/ml in step (e). In another example, the IL-2 is present in a concentration of about 6000 IU/ml. In another example, the IL-2 is present in a concentration of about 3000 IU/ml in step (g). In another example, the IL-2 is present in a concentration of about 3000 IU/ml in step (h).

[0025] In an example of the disclosure, the culturing in step (e) is performed over a period of about 3 days. In an example, the stimulation in step (f) is performed over a period of about 4 days. In an example, the stimulation in step (g) is performed over a period of about 7 days.

[0026] In an example of the disclosure, the optionally disrupted cell fraction is disrupted using a method selected from the group consisting of sonication, homogenization, vortexing, vibration, and lysis. In an example of the disclosure, the non-CD3+, non-CD33+, non-CD20+, non-CD14+ cell fraction (AML blast fraction) is lysed using a suitable lysis method, including high temperature lysis, chemical lysis (such as organic alcohols), enzyme lysis, and other cell lysis methods known in the art.

[0027] In an example of the disclosure, the anti-CD3/anti-CD28 antibodies are coated onto beads and the MILs:bead ratio is about 1:1 in each of steps (f) and (g).

[0028] In an example of the disclosure, the method is performed in a closed, sterile system.

[0029] In an example of the disclosure, a method for expanding marrow infiltrating lymphocytes (MILs) from bone marrow comprises:

a. Obtaining a sample of peripheral blood mononuclear cells (PBMCs) from bone marrow, wherein said sample is optionally cryopreserved;

b. Sorting a CD3+, CD33+, CD20+ and CD14+ cell fraction (MIL cell fraction) and a non-CD3+, non-CD33+, non-CD20+, non-CD14+ cell fraction (AML blast cell fraction);

c. Disrupting the AML blast cell fractionand adding the disrupted AML blast cell fraction to a MIL cell fraction in a cell number ratio of about 1:1;

d. Culturing the cell fractions in a gas permeable container with a first cell culture medium comprising IL-2 at about 6000 IU/ml for a period of about 3 days;

e. Adding anti-CD3/anti-CD28 antibodies immobilized on beads to the cell culture at a ratio of about 1:1 (MILs:beads) and culturing the MILs and antibodies for a period of about 1 day;

f. Exchanging the first cell culture medium to a second cell culture medium comprising additional IL-2 at about 3000 IU/ml;

g. Culturing the antibodies and MILs for an additional period of about 3 days;

h. Restimulating the MILs with IL-2 and anti-CD3/anti-CD28 antibodies immobilized on beads for an additional period of at least about 4 days;

i. Exchanging the second cell culture medium to a third cell culture medium comprising additional IL-2 at about 3000 IU/ml for an additional period of at least about 3 days;

j. Harvesting said MILs.

[0030] In an example of the disclosure, a method for treating a hematological malignancy comprises:

a. Obtaining a sample of peripheral blood mononuclear cells (PBMCs) from bone marrow, wherein said sample is optionally cryopreserved;

b. Sorting a CD3+, CD33+, CD20+ and CD14+ cell fraction (MIL fraction) and a non-CD3+, non-CD33+, non-CD20+, non-CD14+ cell fraction (AML blast cell fraction);

c. optionally disrupting the AML blast cell fraction;

d. Adding the optionally disrupted AML blast cell fraction to the MIL fraction in a cell number ratio of from about 0.1:1 to about 10:1;

e. Culturing one or both cell fractions in a gas permeable container in a first cell culture medium comprising IL-2;

f. Stimulating said sample with anti-CD3/anti-CD28 antibodies;

g. Restimulating the MILs with IL-2 and anti-CD3/anti-CD28 antibodies for an additional period of at least about 4 days;

h. Culturing the MILs with additional IL-2 for an additional period of at least about 3 days;

i. Harvesting said MILs; and

j. Administering said MILs to a patient in a therapeutically effective amount to treat the hematological malignancy.

[0031] In an example of the disclosure, the hematological malignancy is selected from the group consisting of acute myeloid leukemia (AML), mantle cell lymphoma (MCL), follicular lymphoma (FL), diffuse large B cell lymphoma (DLBCL), activated B cell (ABC) DLBCL, germinal center B cell (GCB) DLBCL, chronic lymphocytic leukemia (CLL), small lymphocytic leukemia (SLL), non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma, relapsed and/or refractory Hodgkin's lymphoma, B cell acute lymphoblastic leukemia (B-ALL), mature B-ALL, Burkitt's lymphoma, Waldenström's macroglobulinemia (WM), multiple myeloma, myelodysplatic syndromes, myelofibrosis, chronic myelocytic leukemia, follicle center lymphoma, indolent NHL, human immunodeficiency virus (HIV) associated B cell lymphoma, and Epstein–Barr virus (EBV) associated B cell lymphoma. In another example, the

hematological malignancy is acute myeloid leukemia (AML). In an example of the disclosure, the MILs are administered in an amount of from about $4x10^8$ to about $2.5x10^9$ MILs.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings.

[0033] FIG. 1 illustrates pathology information for lymphoma tumors.

[0034] FIG. 2 illustrates a comparison of different subsets of lymphoma and melanoma TILs, showing that effector memory (EM) subsets in lymphoma TILs are significantly higher than EM subsets in melanoma TILs.

[0035] FIG. 3 illustrates a comparison of different subsets of lymphoma and melanoma TILs, showing that $CD28^+CD4^+$ subsets in lymphoma TIL are significantly higher than these subsets in melanoma TILs.

[0036] FIG. 4 illustrates a comparison of $CD4^+$ T cell subsets of non-Hodgkin's lymphoma TILs and melanoma TILs, showing differentiation markers. Red lines in the graphs represent

CLAIMS

We claim:

1. An *ex vivo* method for expanding peripheral blood lymphocytes (PBLs) from peripheral blood comprising:

    a. Isolating PBLs from a sample of peripheral blood mononuclear cells (PBMCs) obtained from peripheral blood, wherein said sample is optionally cryopreserved, by selecting and removing CD19+ B cells;

    b. Optionally co-culturing said PBLs with said CD19+ B cells;

    c. Stimulating said PBLs in a first cell culture medium with IL-2 and anti-CD3/anti-CD28 antibodies for a period of from about 2 to about 6 days in a gas permeable container;

    d. Culturing the PBLs from step (d) for a period of from about 2 to about 6 days with IL-2 and anti-CD3/anti-CD28 antibodies;

    e. Isolating the antibody-bound PBLs from the culture in step (e);

    f. Removing the antibodies from the PBLs isolated in step (e); and

    g. Harvesting the PBLs.

2. The method of claim 1, wherein the first cell culture medium is selected from the group consisting of CM-2, CM-4, and AIM-V.

3. The method of claim 1, wherein after step (c), additional IL-2 is added and the first cell culture medium is exchanged with a second cell culture medium.

4. The method of claim 1, wherein after step (d), additional IL-2 is added and the second culture medium is exchanged with a third cell culture medium.

5. The method of claim 3, wherein the second cell culture medium is selected from the group consisting of CM-2, CM-4, and AIM-V.

6. The method of claim 4, wherein the third cell culture medium is selected from the group consisting of CM-2, CM-4, and AIM-V.

7. The method of claim 3, wherein the first cell culture medium and the second cell culture

medium are different.

8. The method of claim 3, wherein the first cell culture medium and the second cell culture medium are the same.

9. The method of claim 1, wherein the ratio of B-cells to PBLs in step (b) is from about 0.1:1 to about 10:1 (B-cells:PBLs).

10. The method of claim 1, wherein the ratio of B-cells to PBLs in step (b) is selected from the group consisting of 0.1:1, 1:1, and 10:1 (B-cells:PBLs).

11. The method of claim 1, wherein there are at least from about $1x10^5$ to about $10x10^5$ PBLs in the gas permeable container at the beginning of step (c).

12. The method of claim 1, wherein there are at least from about $2.5x10^5$ to $10x10^5$ PBLs in the gas permeable container at the beginning of step (c).

13. The method of claim 1, wherein there are at least $5x10^5$ PBLs in the gas permeable container at the beginning of step (c).

14. The method of claim 1, wherein the IL-2 is present in a concentration of between 1000 IU/ml and 6000 IL/ml in steps (b) and (c).

15. The method of claim 14, wherein the IL-2 is present in a concentration of about 3000 IU/ml.

16. The method of claim 1, wherein the anti-CD3/anti-CD28 antibodies are coated onto beads and the PBLs:beads ratio is about 1:1 in each of steps (b) and (c).

17. The method of claim 1, further comprising addition of an ITK inhibitor.

18. The method of claim 17, wherein an ITK inhibitor is added during at least one of step (b), step (c), and step (d).

19. The method of claim 17, wherein the ITK inhibitor is selected from the group consisting of aminothiazole-based ITK inhibitors, benzimidazole-based ITK inhibitors, aminopyrimidine-based ITK inhibitors, 3-aminopyride-2-ones-based ITK inhibitors, indolylndazole-based ITK inhibitors, pyrazolyl-indole-based inhibitors, thienopyrazole inhibitors, and ITK inhibitors targeting cysteine-442 in the ATP pocket.

20. The method of claim 19, wherein the ITK inhibitor is selected from the group consisting of ibrutinib, dasatinib, bosutinib, nilotinib, erlotinib BMS509744, CTA056, GSK2250665A,

PF06465469 ((*R*)-3-(1-(1-acryloylpiperidin-3-yl)-4-amino-1*H*-pyrazolo[3,4-d]pyrimidin-3-yl)-*N*-(3-methyl-4-(1-methylethyl))benzamide), and combinations thereof.

21. The method of claim 20, wherein the ITK inhibitor is ibrutinib.

22. The method of claim 1, wherein the method is performed in a closed, sterile system.

23. An *ex vivo* method for expanding peripheral blood lymphocytes (PBLs) from peripheral blood comprising:

    a. Isolating PBLs from a sample of PBMCs obtained from peripheral blood of a patient, wherein said sample is optionally cryopreserved and the patient is optionally pretreated with an ITK inhibitor, by selecting and removing CD19+ B cells and optionally pretreating said PBLs with an ITK inhibitor at a concentration of between 0.1 nM and 200 nM;

    b. Co-culturing said PBLs with said CD19+ B cells for a period of between 2 to 5 days;

    c. Adding from about $2.5x10^5$ to about $5x10^5$ cells to a gas-permeable container in a first cell culture medium and stimulating said PBLs with 3000 IU/ml IL-2 and anti-CD3/anti-CD28 antibodies immobilized on beads, and and optionally an ITK inhibitor at a concentration of between 0.1 nM and 200 nM, for a period of between 3 to 6 days;

    d. Exchanging the first cell culture medium with a second cell culture medium and additional IL-2 at a concentration of about 3000 IU/ml and optionally an ITK inhibitor at a concentration of between 0.1 nM and 200 nM;

    e. Culturing the PBLs from step (d) for an additional period of between 3 to 6 days with IL-2 and anti-CD3/anti-CD28 antibodies immobilized on beads and optionally an ITK inhibitor at a concentration of between 0.1 nM and 200 nM;

    f. Exchanging the second cell culture medium with a third cell culture medium and adding additional IL-2 at a concentration of 3000 IU/mL, and optionally an ITK inhibitor at a concentration of between 0.1 nM and 200 nM, and culturing the cells for an additional period of between 2 to 5 days;

    g. Isolating the antibody-bound PBLs from the culture in step (f);

h. Removing the antibodies from the PBLs isolated in step (g); and

i. Harvesting the PBLs,

wherein the ITK inhibitor is an ITK inhibitor that covalently binds to ITK.

24. Use of peripheral blood lymphocytes (PBLs) in the manufacture of a medicament for treating a hematological malignancy, comprising:

a. Isolating PBLs from a sample of PBMCs from peripheral blood of a patient suffering from a hematological malignancy by selecting and removing CD19+ B cells;

b. Optionally co-culturing said PBLs with said CD19+ B cells;

c. Stimulating said PBLs in a first cell culture medium with IL-2 and anti-CD3/anti-CD28 antibodies for a period of from about 2 to about 6 days in a gas permeable container;

d. Culturing the PBLs from step (c) for a period of from about 2 to about 6 days with IL-2 and anti-CD3/anti-CD28 antibodies;

e. Isolating the antibody-bound PBLs from the culture in step (d);

f. Removing the antibodies from the PBLs isolated in step (e); and

g. Harvesting the PBLs; wherein

h. the PBLs are to be administered to the patient in a therapeutically effective amount to treat said hematological malignancy.

25. The use of claim 24, wherein the patient is pre-treated with an ITK inhibitor prior to obtaining a PBMC sample.

26. The use of claim 25, wherein the ITK inhibitor is selected from the group consisting of aminothiazole-based ITK inhibitors, benzimidazole-based ITK inhibitors, aminopyrimidine-based ITK inhibitors, 3-aminopyride-2-ones-based ITK inhibitors, indolylndazole-based ITK inhibitors, pyrazolyl-indole-based inhibitors, thienopyrazole inhibitors, and ITK inhibitors targeting cysteine-442 in the ATP pocket.

27. The use of claim 25, wherein the ITK inhibitor is selected from the group consisting of

ibrutinib, dasatinib, bosutinib, nilotinib, erlotinib BMS509744, CTA056, GSK2250665A, PF06465469 ((*R*)-3-(1-(1-acryloylpiperidin-3-yl)-4-amino-1*H*-pyrazolo[3,4-d]pyrimidin-3-yl)-*N*-(3-methyl-4-(1-methylethyl))benzamide), and combinations thereof.

28. The use of claim 27, wherein the ITK inhibitor is ibrutinib.

29. The use of claim 25, wherein the patient is pre-treated with at least three rounds of an ibrutinib regimen.

30. The use of claim 24, wherein the hematological malignancy is selected from the group consisting of acute myeloid leukemia (AML), mantle cell lymphoma (MCL), follicular lymphoma (FL), diffuse large B cell lymphoma (DLBCL), activated B cell (ABC) DLBCL, germinal center B cell (GCB) DLBCL, chronic lymphocytic leukemia (CLL), small lymphocytic leukemia (SLL), non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma, relapsed and/or refractory Hodgkin's lymphoma, B cell acute lymphoblastic leukemia (B-ALL), mature B-ALL, Burkitt's lymphoma, Waldenström's macroglobulinemia (WM), multiple myeloma, myelodysplatic syndromes, myelofibrosis, chronic myelocytic leukemia, follicle center lymphoma, indolent NHL, human immunodeficiency virus (HIV) associated B cell lymphoma, and Epstein–Barr virus (EBV) associated B cell lymphoma.

31. The use of claim 24, wherein the hematological malignancy is chronic lymphocytic leukemia (CLL).

32. The use of claim 24, wherein the first cell culture medium is selected from the group consisting of CM-2, CM-4, and AIM-V.

33. The use of claim 24, wherein after step (c), additional IL-2 is added and the cell culture medium is exchanged with a second cell culture medium.

34. The use of claim 33, wherein after step (d), additional IL-2 is added and the second cell culture medium is exchanged with a third cell culture medium.

35. The use of claim 33, wherein the second cell culture medium is selected from the group consisting of CM-2, CM-4, and AIM-V.

36. The use of claim 34, wherein the third cell culture medium is selected from the group consisting of CM-2, CM-4, and AIM-V.

37. The use of claim 33, wherein the first cell culture medium and the second cell culture

medium are different.

38. The use of claim 33, wherein the first cell culture medium and the second cell culture medium are the same.

39. The use of claim 24, wherein the ratio of B-cells to PBLs in step (b) is from about 0.1:1 to about 10:1 (B-cells:PBLs).

40. The use of claim 24, wherein the ratio of B-cells to PBLs in step (b) is selected from the group consisting of 0.1:1, 1:1, and 10:1 (B-cells:PBLs).

41. The use of claim 24, wherein there are at least from about $1x10^5$ to about $10x10^5$ PBLs in the gas permeable container at the beginning of step (c).

42. The use of claim 24, wherein there are at least from about $2.5x10^5$ to $10x10^5$ PBLs in the gas permeable container at the beginning of step (c).

43. The use of claim 24, wherein there are at least $5x10^5$ PBLs in the gas permeable container at the beginning of step (c).

44. The use of claim 24, wherein the IL-2 is present in a concentration of between 1000 IU/ml and 6000 IL/ml in steps (c) and (d).

45. The use of claim 44, wherein the IL-2 is present in a concentration of about 3000 IU/ml.

46. The use of claim 24, wherein the anti-CD3/anti-CD28 antibodies are coated onto beads and the PBLs:bead ratio is about 1:1 in each of steps (c) and (e).

47. The use of claim 24, wherein the PBLs are to be administered in an amount of from about $0.1x10^9$ to about $15x10^9$ PBLs.

48. An *ex vivo* method for expanding peripheral blood lymphocytes (PBLs) from peripheral blood, the method comprising the steps of:

(a) culturing a sample of peripheral blood mononuclear cells (PBMCs) obtained from peripheral blood of a patient who is pre-treated with ibrutinib or another insulin-like tyrosine kinase (ITK) inhibitor and who is refractory to treatment with ibrutinib or such other ITK inhibitor, in a culture comprising a first culture medium with IL-2 and anti-CD3/anti-CD28 antibodies, for a period of time selected from the group consisting of: about 9 days, about 10 days, about 11 days, about 12 days, about 13 days and about 14

days, thereby effecting expansion of peripheral blood lymphocytes (PBLs) from said PBMCs; and

(b) harvesting the PBLs from the culture in step (a).

49. The method of claim 48, wherein before step (a) said PBMCs are subjected to CD19+ selection to separate said PBMCs into CD19+ PBMCs and non-CD19+ PBMCs, and wherein step (a) is performed by culturing the non-CD19+ PBMCs.

50. The method of claim 48 or 49, wherein in step (a) the anti-CD3/anti-CD28 antibodies are bound to magnetic beads, and wherein the beads to cells ratio is 3:1 in the culture.

51. The method of claim 48 or 49, wherein in step (a) on day 4 of culturing said PBMCs additional IL-2 is added to the culture and the first culture medium is changed in the culture.

52. The method of claim 51, wherein the first culture medium is exchanged with a second culture medium in the culture.

53. The method of claim 52, wherein the first culture medium is different from the second culture medium.

54. The method of claim 53, wherein the first culture medium is CM-2 and the second culture medium is CM-4.

55. The method of claim 50, wherein the magnetic beads in step (a) are DynaBeads®

56. The method of any of claims 48-55, wherein the peripheral blood is from a patient suffering from a hematologic malignancy.

57. The method of claim 56, wherein the hematologic malignancy is a liquid tumor.

58. The method of claim 57, wherein the liquid tumor is leukemia.

59. The method of claim 57, wherein the liquid tumor is chronic lymphocytic leukemia.

60. The method of claim 48 or 49, wherein the first cell culture medium contains about 3000 IU/mL of IL-2.

61. The method of claim 48 or 49 , wherein the culture is incubated at 37°C and at 5% CO_2.

62. The method of claim 48 or 49, wherein the method is performed over about 9 days.

63. The method of claim 48 or 49, wherein the method is performed over about 11 days.

64. The method of claim 48 or 49, wherein the patient is pretreated with ibrutinib and is refractory to treatment with ibrutinib.

65. The method of claim 48 or 49, wherein the patient has not undergone treatment with ibrutinib or another ITK inhibitor for at least 1 month prior to being pre-treated with ibrutinib or such other ITK inhibitor.

66. The method of claim 48 or 49, wherein the patient is pre-treated with ibrutinib or another ITK inhibitor for at least 3 months.

67. The method of claim 48 or 49, wherein the patient is pre-treated with ibrutinib for at least 3 months.

68. Use of peripheral blood lymphocytes (PBLs) in the manufacture of a medicament for treating a hematological malignancy, comprising:

(a) culturing a sample of peripheral blood mononuclear cells (PBMCs) from peripheral blood of a patient with said hematological malignancy who is pre-treated with ibrutinib or another insulin-like tyrosine kinase (ITK) inhibitor and who is refractory to treatment with ibrutinib or such other ITK inhibitor in a culture comprising a first culture medium with IL-2 and anti-CD3/anti-CD28 antibodies, for a period of time selected from the group consisting of: about 9 days, about 10 days, about 11 days, about 12 days, about 13 days and about 14 days, thereby effecting expansion of peripheral blood lymphocytes (PBLs) from said PBMCs;

(b) harvesting the PBLs from the culture in step (a); wherein

(c) the PBLs are to be administered to the patient in a therapeutically effective amount to treat said malignancy.

69. The use of claim 68, wherein before step (a) said PBMCs are subjected to CD19+ selection to separate said PBMCs into CD19+ PBMCs and non-CD19+ PBMCs, and wherein step (b) is performed by culturing the non-CD19+ PBMCs.

70. The use of claim 68 or 69, wherein in step (a) the anti-CD3/anti-CD28 antibodies are bound to magnetic beads, and wherein the beads to cells ratio is 3:1 in the culture.

71. The use of claim 68 or 69, wherein in step (a) on day 4 of culturing said PBMCs additional IL-2 is added to the culture and the first culture medium is changed in the culture.

72. The use of claim 71, wherein the first culture medium is exchanged with a second culture medium in the culture.

73. The use of claim 72, wherein the first culture medium is different from the second culture medium.

74. The use of claim 73, wherein the first culture medium is CM-2 and the second culture medium is CM-4.

75. The use of claim 70, wherein the magnetic beads in step (a) are DynaBeads® .

76. The use of claim 68 or 69, wherein the patient is pre-treated with ibrutinib and is refractory to treatment with ibrutinib.

77. The use of claim 76, wherein the patient is pre-treated with at least three rounds of an ibrutinib regimen.

78. The use of claim 76, wherein the patient is pre-treated with ibrutinib for at least 3 months.

79. The use of claim 68 or 69, wherein the patient has not undergone treatment with ibrutinib or another ITK inhibitor for at least 1 month prior to being pre-treated with ibrutinib or such other ITK inhibitor.

80. The use of claim 68 or 69, wherein the hematological malignancy is selected from the group consisting of acute myeloid leukemia (AML), mantle cell lymphoma (MCL), follicular lymphoma (FL), diffuse large B cell lymphoma (DLBCL), activated B cell (ABC) DLBCL, germinal center B cell (GCB) DLBCL, chronic lymphocytic leukemia (CLL), small lymphocytic leukemia (SLL), non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma, relapsed and/or refractory Hodgkin's lymphoma, B cell acute lymphoblastic leukemia (B-ALL), mature B-ALL, Burkitt's lymphoma, Waldenström's macroglobulinemia (WM), multiple myeloma, myelodysplatic syndromes, myelofibrosis, chronic myelocytic leukemia, follicle center lymphoma, indolent NHL, human immunodeficiency virus (HIV) associated B cell lymphoma, and Epstein–Barr virus (EBV) associated B cell lymphoma.

81. The use of claim 68 or 69, wherein the hematologic malignancy is a liquid tumor.

82. The use of claim 81, wherein the liquid tumor is leukemia.

83. The use of claim 81, wherein the liquid tumor is chronic lymphocytic leukemia.