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 of a patient pre-treated with ibrutinib or another interleukin-2-inducible T-cell kinase (ITK) inhibitor, 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 2 to 6 days in a gas permeable container;~~

~~d. Culturing the PBLs from step (d) for a period of from 2 to 6 days with IL-2 and anti-CD3/anti-CD28 antibodies, thereby effecting expansion of PBLs from said PBMCs, wherein the expanded PBLs exhibit increased levels of IFNγ production compared to levels of IFNγ production exhibited by PBLs expanded from patients not pre-treated with ibrutinib or other ITK inhibitor;~~

~~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.~~

~~**[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 pre-treated with an~~

~~interleukin-2-inducible T-cell kinase (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 2.5x105 to 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 thereby effecting expansion of PBLs from said PBMCs, wherein the expanded PBLs exhibit increased levels of IFNγ production compared to levels of IFNγ production exhibited by PBLs expanded from patients not pre-treated with ibrutinib or other ITK inhibitor;~~
~~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, wherein the patient is pre-treated with an interleukin-2-inducible T-cell kinase (ITK) inhibitor prior to obtaining a PBMC sample;~~
~~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 2 to 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, thereby effecting expansion of PBLs from said PBMCs, wherein the expanded PBLs exhibit increased levels of IFNγ production compared to levels of IFNγ production exhibited by PBLs expanded from patients not pre-treated with ibrutinib or other ITK inhibitor;~~
~~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~~0004a] 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~~ obtaining a sample of peripheral blood mononuclear cells (PBMCs) obtained from peripheral blood of a patient who is pre-treated with ibrutinib ~~or another interleukin-2-inducible T-cell 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, thereby effecting the expansion of PBLs from said PBMCs; (b) culturing said PBMCs in a culture comprising a first culture medium with IL-2 and anti-CD3/anti-CD28 antibodies, wherein the method is performed over~~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~~, wherein the expanded PBLs exhibit increased levels of IFNγ production compared to levels of IFNγ production exhibited by PBLs expanded from patients not pre-treated with ibrutinib or other ITK inhibitor~~; and (~~b~~c) harvesting the PBLs from the culture in step (~~a~~b).

[~~0004e~~0004b] 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 alignancy, comprising: (a) obtaining a sample of peripheral blood mononuclear cells (PBMCs) obtained from peripheral blood of a patient who is pre-treated with ibrutinib and who is refractory to treatment with ibrutinib, in a culture comprising a first culture medium with IL-2 and anti-CD3/anti-CD28 antibodies, thereby effecting the expansion of PBLs from said PBMCs;~~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 interleukin-2-inducible T-cell 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, wherein the expanded PBLs exhibit increased levels of IFNγ production compared to levels of IFNγ production exhibited by PBLs expanded from patients not pre-treated with ibrutinib or other ITK inhibitor;~~ (b) culturing said PBMCs in a culture comprising a first culture medium with IL-2 and anti-CD3/anti-CD28 antibodies, wherein the method is performed over 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 (c) harvesting the PBLs from the culture in step (b).~~(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;

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 of a patient pre-treated with ibrutinib or another interleukin-2-inducible T-cell kinase (ITK) inhibitor, 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 2 to 6 days in a gas permeable container;

    d. Culturing the PBLs from step (c) for a period of from 2 to 6 days with IL-2 and anti-CD3/anti-CD28 antibodies, thereby effecting expansion of PBLs from said PBMCs, wherein the expanded PBLs exhibit increased levels of IFNγ production compared to levels of IFNγ production exhibited by PBLs expanded from patients not pre-treated with ibrutinib or other ITK inhibitor;

    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.

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 0.1:1 to 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 $1x10^5$ to $10x10^5$ PBLs in the gas permeable container at the beginning of step (c).

12. The method of claim 1, wherein there are at least from $2.5x10^5$ 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 (c) and (d).

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 1:1 in each of steps (c) and (d).

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 pre-treated with an interleukin-2-inducible T-cell kinase (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 $2.5x10^5$ to $5x10^5$ 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 thereby effecting expansion of PBLs from said PBMCs, wherein the expanded PBLs exhibit increased levels of IFNγ production compared to levels of IFNγ production exhibited by PBLs expanded from patients not pre-treated with ibrutinib or other ITK inhibitor;

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, wherein the patient is pre-treated with an interleukin-2-inducible T-cell kinase (ITK) inhibitor prior to obtaining a PBMC sample;

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 2 to 6 days in a gas permeable container;

d. Culturing the PBLs from step (c) for a period of from 2 to 6 days with IL-2 and anti-CD3/anti-CD28 antibodies, thereby effecting expansion of PBLs from said PBMCs, wherein the expanded PBLs exhibit increased levels of IFNγ production compared to levels of IFNγ production exhibited by PBLs expanded from patients not pre-treated with ibrutinib or other ITK inhibitor;

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 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.

26. The use of claim 24, 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.

27. The use of claim 26, wherein the ITK inhibitor is ibrutinib prior to obtaining a PBMC sample.

28. The use of claim 24, wherein the patient is pre-treated with at least three rounds of an ibrutinib regimen.

29. 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.

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

31. 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.

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

33. The use of claim 32, wherein after step (d), additional IL-2 is added and the second cell culture medium is exchanged with a third cell culture medium.

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

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

36. The use of claim 32, wherein the first cell culture medium and the second cell culture medium are different.

37. The use of claim 32, wherein the first cell culture medium and the second cell culture medium are the same.

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

39. 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).

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

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

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

43. 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).

44. The use of claim 43, wherein the IL-2 is present in a concentration of about 3000 IU/ml.

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

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

~~47~~1. An *ex vivo* method for expanding peripheral blood lymphocytes (PBLs) from peripheral blood, the method comprising the steps of:

(a) ~~culturing~~ obtaining a sample of peripheral blood mononuclear cells (PBMCs) obtained from peripheral blood of a patient who is pre-treated with ibrutinib ~~or another interleukin-2-inducible T-cell 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, thereby effecting the expansion of PBLs from said PBMCs;

(b) culturing said PBMCs in a culture comprising a first culture medium with IL-2 and anti-CD3/anti-CD28 antibodies, wherein the method is performed over~~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~~, wherein the expanded PBLs exhibit increased levels of IFNγ production compared to levels of IFNγ production exhibited by PBLs expanded from patients not pre-treated with ibrutinib or other ITK inhibitor~~; and

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

~~48~~2. The method of claim ~~47~~1, 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.

~~49~~3. The method of claim ~~47~~ 1 or ~~48~~2, 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.

~~50~~4. The method of claim ~~47~~ 1 or ~~48~~2, 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.

5. The method of claim 3, wherein the magnetic beads in step (a) are DynaBeads®.

~~51~~6. The method of claim ~~50~~4, wherein the first culture medium is exchanged with a second culture medium in the culture.

~~52~~7. The method of claim ~~51~~6, wherein the first culture medium is different from the second culture medium.

~~53~~8. The method of claim ~~52~~7, wherein the first culture medium is CM-2 and the second culture

medium is CM-4.

~~54. The method of claim 49, wherein the magnetic beads in step (a) are DynaBeads®~~

~~55~~9. The method of any of claims ~~47~~1-~~54~~8, wherein the peripheral blood is from a patient suffering from a hematologic malignancy.

~~56~~10. The method of claim ~~55~~9, wherein the hematologic malignancy is a liquid tumor.

~~57~~11. The method of claim ~~56~~10, wherein the liquid tumor is leukemia.

~~58~~12. The method of claim ~~56~~10, wherein the liquid tumor is chronic lymphocytic leukemia.

~~59~~13. The method of claim ~~47~~ 1 or ~~48~~2, wherein the first cell culture medium contains about 3000 IU/mL of IL-2.

~~60~~14. The method of claim ~~47~~ 1 or ~~48~~2, wherein the culture is incubated at 37°C and at 5% CO_2.

~~61~~15. The method of claim ~~47~~ 1 or ~~48~~2, wherein the method is performed over about 9 days.

~~62~~16. The method of claim ~~47~~ 1 or ~~48~~2, wherein the method is performed over about 11 days.

~~63~~17. The method of claim ~~47~~ 1 or ~~48~~2, wherein the patient is pre-treated with ibrutinib and is refractory to treatment with ibrutinib.

~~64~~18. The method of claim ~~47~~ 1 or ~~48~~2, 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~~.

~~65~~19. The method of claim ~~47~~ 1 or ~~48~~2, wherein the patient is pre-treated with ibrutinib or another ITK inhibitor for at least 3 months.

~~66~~20. The method of claim ~~47~~ 1 or ~~48~~2, wherein the patient is pre-treated with ~~ibrutinib for~~ at least 3 rounds of an ibrutinib regimen~~months~~.

21. The method according to any preceding claim, wherein the patient suffers from 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.

22. The method of claim 21, wherein the hematologic malignancy is a liquid tumor.

23. The method of claim 22, wherein the liquid tumor is leukemia.

24. The method of claim 22, wherein the liquid tumor is chronic lymphocytic leukemia.

~~67~~25. Use of peripheral blood lymphocytes (PBLs) in the manufacture of a medicament for treating a hematological malignancy, comprising:

(a) obtaining a sample of peripheral blood mononuclear cells (PBMCs) obtained from peripheral blood of a patient who is pre-treated with ibrutinib and who is refractory to treatment with ibrutinib, in a culture comprising a first culture medium with IL-2 and anti-CD3/anti-CD28 antibodies, thereby effecting the expansion of PBLs from said PBMCs;

(b) culturing said PBMCs in a culture comprising a first culture medium with IL-2 and anti-CD3/anti-CD28 antibodies, wherein the method is performed over 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

(c) harvesting the PBLs from the culture in step (b).~~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 interleukin-2-inducible T-cell 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, wherein the expanded PBLs exhibit increased levels of IFNγ production compared to levels of IFNγ production exhibited by PBLs expanded from patients not pre-treated with ibrutinib~~

~~or other ITK inhibitor;~~

~~(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~~.

~~68. The use of claim 67, 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.~~

~~69. The use of claim 67 or 68, 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.~~

~~70. The use of claim 67 or 68, 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.~~

~~71. The use of claim 70, wherein the first culture medium is exchanged with a second culture medium in the culture.~~

~~72. The use of claim 71, wherein the first culture medium is different from the second culture medium.~~

~~73. The use of claim 72, wherein the first culture medium is CM-2 and the second culture medium is CM-4.~~

~~74. The use of claim 69, wherein the magnetic beads in step (a) are DynaBeads®.~~

~~75. The use of claim 67 or 68, wherein the patient is pre-treated with ibrutinib and is refractory to treatment with ibrutinib.~~

~~76. The use of claim 75, wherein the patient is pre-treated with at least three rounds of an ibrutinib regimen.~~

~~77. The use of claim 75, wherein the patient is pre-treated with ibrutinib for at least 3 months.~~

~~78~~26. The use of claim ~~67 or 68~~25, 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~~.

~~79~~27. The use of claim ~~67 or 68~~25, 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.

~~80~~28. The use of claim ~~67~~ 25 or ~~68~~26, wherein the hematologic malignancy is a liquid tumor.

~~81~~29. The use of claim ~~80~~28, wherein the liquid tumor is leukemia.

~~82~~30. The use of claim ~~80~~28, wherein the liquid tumor is chronic lymphocytic leukemia.