more efficacious therapies in many hematological malignancies, including chronic lymphocytic leukemia (CLL). There is also an urgent need to provide such therapies using whole blood as a source of lymphocytes with TIL functionality, such as PBLs, to treat patients refractory to other therapies or that have relapsed. Because of the burden of apheresis and the large blood volumes taken from critically ill cancer patients, there is also an urgent need to use as little as patient blood as possible.

[0004] The present invention provides the surprising finding that PBLs expansion processes using low volumes of blood as a source of PBLs can result in efficacious PBL populations obtained from hematological malignancies, such as liquid tumors, including lymphomas or leukemias.

SUMMARY

[0005] In an example of the disclosure, a method for expanding peripheral blood lymphocytes (PBLs) from peripheral blood is disclosed. In one example, the method comprises (a) obtaining a sample of peripheral blood mononuclear cells (PBMCs) from the peripheral blood of a patient, wherein said sample is optionally cryopreserved and the patient is optionally pretreated with an ITK inhibitor; (b) optionally washing the PBMCs by centrifugation; (c) admixing magnetic beads selective for CD3 and CD28 to the PBMCs to form an admixture of the beads and the PBMCs; (d) seeding the admixture of the beads and the PBMCs into a gas-permeable container and co-culturing said PBMCs in media comprising about 3000 IU/mL of IL-2 in for about 4 to about 6 days; (e) feeding said PBMCs using media comprising about 3000 IU/mL of IL-2, and co-culturing said PBMCs for about 5 days, such that the total co-culture period of steps (d) and (e) is about 9 to about 11 days; (f) harvesting PBMCs from media; (g) removing the magnetic beads selective for CD3 and CD28 using a magnet; (h) removing residual B-cells using magnetic-activated cell sorting and beads selective for CD19 to provide a PBL product; (i) washing and concentrating the PBL product using a cell harvester; and (j) formulating and optionally cryopreserving the PBL product. In one example, the ITK inhibitor is optionally an ITK inhibitor that covalently binds to ITK.

[0006] In one example, the method comprises (a) obtaining a sample of peripheral blood mononuclear cells (PBMCs) from the peripheral blood of a patient, wherein said sample is optionally cryopreserved and the patient is optionally pretreated with an ITK inhibitor; (b)

optionally washing the PBMCs by centrifugation; (c) removing B-cells from the PBMCs by selecting against CD19 to provide PBMCs depleted of B-cells; (d) admixing magnetic beads selective for CD3 and CD28 to the PBMCs depleted of B-cells to form an admixture of the beads and the PBMCs; (e) seeding the admixture of the beads and the PBMCs into a gas-permeable container and co-culturing said PBMCs in media comprising about 3000 IU/mL of IL-2 in for about 4 to about 6 days; (f) feeding said PBMCs using media comprising about 3000 IU/mL of IL-2, and co-culturing said PBMCs for about 5 days, such that the total co-culture period of steps (e) and (f) is about 9 to about 11 days; (g) harvesting the PBMCs from media; (h) removing any residual magnetic beads selective for CD3 and CD28 from the PBMCs using a magnet to provide a PBL product; (i) washing and concentrating the PBL product using a cell harvester; and (j) formulating and optionally cryopreserving the PBL product. In one example, the ITK inhibitor is optionally an ITK inhibitor that covalently binds to ITK. In another example, the removal of B-cells in step (c) is performed by using beads selective for CD19 to remove B-cells from the PBMCs. In another example, the removal of B-cells in step (c) is performed by admixing the beads selective for CD19 with the PBMCs to form complexes of beads and B-cells in an admixture with the PBMCs and removing the complexes from the admixture. In another example, the removal of B-cells in step (c) is performed by admixing magnetic beads selective for CD19 with the PBMCs to form complexes of magnetic beads and B-cells in the admixture and using a magnet to remove the complexes from the admixture. In an example of the disclosure, the beads selective for CD19 are beads conjugated to anti-CD19 antibody.

[0007] In an example of the disclosure, the amount of peripheral blood that is obtained from a patient in a method according to the present disclosure is between about 10 mL and 50 mL. In another example, the amount of peripheral blood that is obtained from a patient is less than or equal to about 50 mL.

[0008] In an example of the disclosure, the seeding density of the PBMCs in a method according to the present disclosure is about $2\times10^5/cm^2$ to about $1.6\times10^3/cm^2$ relative to the surface area of the gas-permeable container.

[0009] In an example of the disclosure, a process for the preparation of peripheral blood lymphocytes (PBLs) from a whole blood sample comprises the steps of (a) obtaining peripheral blood mononuclear cells (PBMCs) from less than or equal to about 50 mL of whole blood from a patient having a liquid tumor, wherein the patient is optionally pretreated with an ITK inhibitor;

(b) admixing beads selective for CD3 and CD28 with the PBMCs, wherein the beads are added at a ratio of 3 beads:1 cell, to form an admixture of the PBMCs and the beads; (c) culturing the admixture of the PBMCs and the beads at a density of about 25,000 cells per cm^2 to about 50,000 cells per cm^2 on a gas-permeable surface of one or more containers containing a first cell culture medium and IL-2 for a period of about 4 days; (d) adding to each container IL-2 and a second cell culture medium that is the same as or different from the first cell culture medium and culturing for a period of about 5 days to about 7 days to form an expanded population of PBLs; and (e) harvesting from each container the expanded population of PBLs.

[0009a] In one aspect, the present disclosure refers to a process for the preparation of peripheral blood lymphocytes (PBLs) from a whole blood sample, the process comprising the steps of (a) obtaining peripheral blood mononuclear cells (PBMCs) from less than or equal to about 50 mL of whole blood from a patient having a liquid tumor, wherein the patient is optionally pretreated with an ITK inhibitor; (b) admixing beads selective for CD3 and CD28 with the PBMCs, wherein the beads are added at a ratio of 3 beads:1 cell, to form an admixture of PBMCs and beads; (c) culturing the admixture of PBMCs and beads at a density of about 25,000 cells per cm2 to about 50,000 cells per cm2 on a gas-permeable surface of one or more containers containing a first cell culture medium and IL-2 for a period of about 4 days; (d) adding to each container of step (c) IL-2 and a second cell culture medium that is the same as or different from the first cell culture medium and culturing for a period of about 5 days to about 7 days to form an expanded population of PBLs; and (e) harvesting from each container the expanded population of PBLs.

[0010] In an example of the disclosure, a process for the preparation of peripheral blood lymphocytes (PBLs) from a whole blood sample comprises the steps of (a) obtaining peripheral blood mononuclear cells (PBMCs) from less than or equal to about 50 mL of whole blood from a patient having a liquid tumor, wherein the patient is optionally pretreated with an ITK inhibitor; (b) removing B-cells from the PBMCs by selecting against CD19 to provide PBMCs depleted of B-cells; (c) admixing beads selective for CD3 and CD28 to the PBMCs, wherein the beads are added at a ratio of 3 beads:1 cell, to form an admixture of the PBMCs and the beads; (d) culturing the admixture of the PBMCs and the beads at a density of about 25,000 cells per cm^2 to about 50,000 cells per cm^2 on a gas-permeable surface of one or more containers containing a first cell culture medium and IL-2 for a period of about 4 days; (e) adding to each container IL-2 and a second cell culture medium that is the same as or different from the first cell culture medium and culturing for a period of about 5 days to about 7 days to form an expanded population of PBLs; and (f) harvesting from each container the expanded population of PBLs. In one example, the ITK inhibitor is optionally an ITK inhibitor that covalently binds to ITK. In another example, the patient is pretreated with an ITK inhibitor and the patient is refractory to treatment with the ITK inhibitor. In another example, the removal of B-cells in step (b) is performed by using beads selective for CD19 to remove B-cells from the PBMCs. In another example, the removal of B-cells in step (b) is performed by admixing the beads selective for CD19 with the PBMCs to form complexes of the beads and B-cells in an admixture with the PBMCs and removing the complexes from the admixture. In another example, the removal of B-cells is performed by admixing magnetic beads selective for CD19 to the PBMCs to form complexes of the magnetic beads and B-cells in an admixture with the PBMCs and using

a magnet to remove the complexes from the admixture. In another example, the beads selective for CD19 are beads conjugated to anti-CD19 antibody.

[0011] In an example of the method according to the present disclosure, the total number of cells harvested is from about 8 billion to about 22 billion.

[0012] In an example of the method according to the present disclosure, the total number of cells harvested is from about 1 billion to about 8 billion.

[0013] In an example of the method according to the present disclosure, about 95% to about 99% of the cells harvested are T-cells.

[0014] In an example of the method according to the present disclosure, the step of admixing the beads selective for CD3 and CD28 to the PBMCs to form an admixture of the beads and the PBMCs is replaced with the step of admixing the beads selective for CD3 and CD28 to the PBMCs to form complexes of the beads and the PBMCs in an admixture of the beads and the PBMCs, and the step of culturing the admixture is replaced with the step of separating the complexes of the beads and the PBMCs from the admixture and culturing the complexes of the PBMCs and the beads at a density of about 25,000 cells per cm^2 to about 50,000 cells per cm^2 on a gas-permeable surface in one or more containers containing a first cell culture medium and IL-2 for a period of about 4 days. In another example of the present disclosure, the beads selective for CD3 and CD28 are magnetic beads, and the step of separating the complexes of the beads and the PBMCs from the admixture is performed by using a magnet to remove the complexes from the admixture.

[0015] In an example of the disclosure, the beads selective for CD3 and CD28 are beads conjugated to anti-CD3 antibodies and anti-CD28 antibodies.

[0016] In an example of the method according to the present disclosure, the method further comprises performing a selection to remove any remnant B-cells from the expanded population of PBLs. In another example, the selection is performed by using beads selective for CD19 to remove the remnant B-cells. In another example, the selection is performed by admixing the beads selective for CD19 with the expanded population of PBLs to form complexes of beads and any remnant B-cells and removing the complexes from the admixture. In another example, the selection is performed by admixing magnetic beads selective for CD19 with the expanded population of PBLs to form complexes of magnetic beads and any remnant B-cells and

using a magnet to remove the complexes from the admixture. In an example of the disclosure, the beads selective for CD19 are beads conjugated to anti-CD19 antibody.

[0017] In an example according to the present disclosure, the first cell culture medium contains about 3000 IU/mL of IL-2. In another example, the second cell culture medium contains about 3000 IU/mL of IL-2. In yet another example, the cultures in the culturing steps are incubated at 37°C and under an atmosphere containing 5% CO_2.

[0018] In an example of the disclosure, the method according to the present disclosure is performed over a period of about 9 to about 11 days. In another example, the method is performed over a period of about 9 days. In another example, the method is performed over a period of about 11 days.

[0019] In an example of the disclosure, the patient is pretreated with an ITK inhibitor. In one example, the ITK inhibitor is ibrutinib. In another example, the patient has a liquid tumor. In another example, the patient has a liquid tumor and is pretreated with an ITK inhibitor. In another example, the patient has a liquid tumor, is refractory to treatment with an ITK inhibitor, and is pretreated with the ITK inhibitor.

[0020] In an example of the disclosure, the patient suffers from leukemia. In another example, the leukemia is chronic lymphocytic leukemia.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] 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.

[0022] FIG. 1 illustrates an exemplary embodiment of a PBL manufacturing process with B-cell depletion on Day 9.

[0023] FIG. 2 illustrates the design of experiments to compare the T-cell positive selection method using CTS Dynabeads CD3/28 to the T-cell negative selection method using either research grade Pan-T kit or a sequential anti-CD14, anti CD19 depletion method using CliniMACS microbeads.

[0024] FIG. 3 illustrates total PBL yields (in billions) for two samples extrapolated for an exemplary 9 day manufacturing process.

CLAIMS

We claim:

1. A process for the preparation of peripheral blood lymphocytes (PBLs) from a whole blood sample, the process comprising the steps of:

(a) obtaining peripheral blood mononuclear cells (PBMCs) from less than or equal to about 50 mL of whole blood from a patient having a liquid tumor, wherein the patient is optionally pretreated with an ITK inhibitor;

(b) admixing beads selective for CD3 and CD28 with the PBMCs, wherein the beads are added at a ratio of 3 beads:1 cell, to form an admixture of PBMCs and beads;

(c) culturing the admixture of PBMCs and beads at a density of about 25,000 cells per cm^2 to about 50,000 cells per cm^2 on a gas-permeable surface of one or more containers containing a first cell culture medium and IL-2 for a period of about 4 days;

(d) adding to each container of step (c) IL-2 and a second cell culture medium that is the same as or different from the first cell culture medium and culturing for a period of about 5 days to about 7 days to form an expanded population of PBLs; and

(e) harvesting from each container the expanded population of PBLs.

2. The process of claim 1, wherein in step (e) the total number of cells harvested is from about 8 billion to about 22 billion.

3. The process as in any one of the preceding claims, wherein in step (b) the admixture of beads and PBMCs forms complexes of PBMCs and beads, wherein before step (c) the process comprises the step of separating the complexes from the admixture, and wherein step (c) is replaced by the step of culturing the complexes of PBMCs and beads at a density of about 25,000 cells per cm^2 to about 50,000 cells per cm^2 on a gas-permeable surface in one or more containers containing a first cell culture medium and IL-2 for a period of about 4 days.

4. The process as in any one of the preceding claims, wherein in step (b) magnetic beads selective for CD3 and CD28 are admixed to the PBMCs, and wherein the step of separating the complexes from the admixture is performed by using a magnet to remove the complexes from the admixture.

5. The process as in any one of the preceding claims, wherein the beads selective for CD3 and CD28 are beads conjugated to anti-CD3 antibodies and anti-CD28 antibodies.

6. The process as in any one of the preceding claims, wherein after step (d) the process comprises the step of:

(f) performing a selection to remove any remnant B-cells from the expanded population of PBLs.

7. The process as in any one of the preceding claims, wherein in step (f) the selection is performed by using beads selective for CD19 to remove the remnant B-cells.

8. The process as in any one of the preceding claims, wherein in step (f) the selection is performed by admixing the beads selective for CD19 with the expanded population of PBLs to form complexes of beads and any remnant B-cells and removing the complexes from the admixture.

9. The process as in any one of the preceding claims, wherein in step (f) the selection is performed by admixing magnetic beads selective for CD19 with the expanded population of PBLs to form complexes of magnetic beads and any remnant B-cells and using a magnet to remove the complexes from the admixture.

10. The process as in any one of the preceding claims, wherein the beads selective for CD19 are beads conjugated to anti-CD19 antibody.

11. The process as in any one of the preceding claims, wherein before step (b) the process further comprises the step of removing B-cells from the PBMCs to provide PBMCs depleted of B-cells.

12. The process as in any one of the preceding claims, wherein before step (b) the process further comprises the step of removing B-cells from the PBMCs by selecting against CD19 to provide PBMCs depleted of B-cells.

13. The process as in any one of the preceding claims, wherein before step (b) the process further comprises the step of removing B-cells from the PBMCs by admixing beads selective for CD19 with the PBMCs to form complexes of the beads and CD19+ cells in an admixture and removing the complexes from the admixture to provide PBMCs depleted of B-cells.

14. The process as in any one of the preceding claims, wherein before step (b) the process further comprises the step of removing B-cells from the PBMCs by admixing magnetic beads selective for CD19 with the PBMCs to form complexes of the magnetic beads and CD19+ cells in an admixture and using a magnet to remove the complexes from the admixture to provide PBMCs depleted of B-cells.

15. The process as in any one of the preceding claims, wherein the first cell culture medium contains about 3000 IU/mL of IL-2.

16. The process as in any one of the preceding claims, wherein the second cell culture medium contains about 3000 IU/mL of IL-2.

17. The process as in any one of the preceding claims, wherein in steps (c) and (d) the culture is incubated at 37°C and under an atmosphere containing 5% CO_2.

18. The process as in any one of the preceding claims, wherein the process is performed over about 9 days.

19. The process as in any one of the preceding claims, wherein the patient is pretreated with an ITK inhibitor.

20. The process as in any one of the preceding claims, wherein the patient is pretreated with ibrutinib.

21. The process as in any one of the preceding claims, wherein the patient is suffering from a leukemia or a chronic lymphocytic leukemia.