[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) 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).

CLAIMS

We claim:

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

(a) obtaining a sample of peripheral blood mononuclear cells (PBMCs) obtainedfrom 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).

2. The method of claim 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.

3. The method of claim 1 or 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.

4. The method of claim 1 or 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®.

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

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

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

medium is CM-4.

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

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

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

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

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

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

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

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

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

18. The method of claim 1 or 2, wherein the patient has not undergone treatment with ibrutinib for at least 1 month prior to being pre-treated with ibrutinib.

19. The method of claim 1 or 2, wherein the patient is pre-treated with ibrutinib for at least 3 months.

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

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.