USE OF INHIBITORS OF BRUTON'S TYROSINE KINASE (BTK)

CROSS-REFERENCE

[0001] This application claims priority to U.S. Provisional Patent Application No. 61/549,067, titled "THE USE OF INHIBITORS OF BRUTON'S TYROSINE KINASE (BTK)" and filed October 19, 2011, which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Bruton's tyrosine kinase (Btk), a member of the Tec family of non-receptor tyrosine kinases, is a key signaling enzyme expressed in all hematopoietic cells types except T lymphocytes and natural killer cells. Btk plays an essential role in the B-cell signaling pathway linking cell surface B-cell receptor (BCR) stimulation to downstream intracellular responses.

[0003] Btk is a key regulator of B-cell development, activation, signaling, and survival (Kurosaki, *Curr Op Imm,* 2000, 276-281; Schaeffer and Schwartzberg, *Curr Op Imm* 2000, 282-288). In addition, Btk plays a role in a number of other hematopoietic cell signaling pathways, e.g., Toll like receptor (TLR) and cytokine receptor–mediated TNF-α production in macrophages, IgE receptor (FcεRI) signaling in Mast cells, inhibition of Fas/APO-1 apoptotic signaling in B-lineage lymphoid cells, and collagen-stimulated platelet aggregation. *See, e.g.,* C. A. Jeffries, et al., (2003), *Journal of Biological Chemistry* 278:26258-26264; N. J. Horwood, et al., (2003), *The Journal of Experimental Medicine* 197:1603-1611; Iwaki *et al.* (2005), *Journal of Biological Chemistry* 280(48):40261-40270; Vassilev *et al.* (1999), *Journal of Biological Chemistry* 274(3):1646-1656, and Quek *et al.* (1998), *Current Biology* 8(20):1137-1140.

SUMMARY OF THE INVENTION

[0004a] In one aspect, there is provided a combination comprising

a. therapeutically-effective amount of ibrutinib or a pharmaceutically acceptable salt thereof; and

b. a therapeutically-effective amount of 3-((dimethylamino)methyl)-N-(2-(4-(hydroxycarbamoyl)phenoxy)ethyl) benzofuran-2-carboxamide or a pharmaceutically acceptable salt thereof.

~~use of an anti-cancer treatment, in the manufacture of a medicament for treating a hematological malignancy in an individual, wherein the individual is identified as having an increased mobilization of a plurality of cells from the malignancy following administration of an irreversible Btk inhibitor to the individual~~.

[0004b] In one aspect, there is provided use of

(a) ibrutinib or a pharmaceutically acceptable salt thereof, and

(b) 3-((dimethylamino)methyl)-N-(2-(4-(hydroxycarbamoyl)phenoxy)ethyl) benzofuran-2-carboxamide or a pharmaceutically acceptable salt thereof

in the manufacture of a medicament for the treatment of a hematological malignancy in an individual in need thereof.

~~a first treatment comprising an amount of an irreversible Btk inhibitor and a second treatment in the manufacture of a medicament for treating a hematological malignancy in an individual in need thereof, wherein said Btk inhibitor is sufficient to mobilize a plurality of cells from said malignancy and said plurality of cells are analyzed in a sample obtained from the individual~~.

[0004c] In one aspect, there is provided a kit for treating a hematological malignancy in an individual, wherein the kit comprises:

(a) ibrutinib or a pharmaceutically acceptable salt thereof; and

(b) 3-((dimethylamino)methyl)-N-(2-(4-(hydroxycarbamoyl)phenoxy)ethyl) benzofuran-2-carboxamide or a pharmaceutically acceptable salt thereof.

~~use of a first treatment comprising an amount of an irreversible Btk inhibitor in the manufacture of a medicament for treating a hematological malignancy in an individual in need thereof, wherein said Btk inhibitor is sufficient to mobilize a plurality of cells for said malignancy and wherein a biomarker profile is prepared for a population of cells isolated from the plurality of cells~~.

[0004d] Disclosed herein, in certain embodiments, is a method for treating a hematological malignancy in an individual in need thereof, comprising: (a) administering to the individual a first treatment comprising an amount of an irreversible Btk inhibitor sufficient to mobilize a plurality of cells from the malignancy; (b) analyzing the mobilized plurality of cells in a sample obtained from the individual; and (c) administering a second treatment to the individual. In some embodiments, the amount of the irreversible Btk inhibitor is sufficient to induce lymphocytosis of a plurality of cells from the malignancy. In some embodiments, the hematological malignancy is a B-cell malignancy. In some embodiments, the hematological malignancy is a leukemia, lymphoproliferative disorder, or myeloid disorder. In some embodiments, the mobilized cells are myeloid cells or lymphoid cells. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments is a method further

WHAT IS CLAIMED IS:

1. A combination comprising:

   a. a therapeutically-effective amount of ibrutinib or a pharmaceutically acceptable salt thereof; and

   b. a therapeutically-effective amount of 3-((dimethylamino)methyl)-N-(2-(4-(hydroxycarbamoyl)phenoxy)ethyl) benzofuran-2-carboxamide or a pharmaceutically acceptable salt thereof.

2. The combination according to claim 1, wherein ibrutinib is in a dosage amount of about 300 mg/day to about 10
00 mg/day.

3. The combination according to claim 1, wherein ibrutinib is in a dosage amount of about 420 mg/day to about 840 mg/day.

4. The combination according to any one of claims 1-3, wherein ibrutinib and 3-((dimethylamino)methyl)-N-(2-(4-(hydroxycarbamoyl)phenoxy)ethyl)benzofuran-2-carboxamide are in a single or in separate dosage forms.

5. Use of

   (a) ibrutinib or a pharmaceutically acceptable salt thereof, and

   (b) 3-((dimethylamino)methyl)-N-(2-(4-(hydroxycarbamoyl)phenoxy)ethyl) benzofuran-2-carboxamide or a pharmaceutically acceptable salt thereof

   in the manufacture of a medicament for the treatment of a hematological malignancy in an individual in need thereof.

6. The use according to claim 5, wherein the amount of ibrutinib in the medicament or in the package is from about 300 mg/day to about 1000 mg/day.

7. The use according to claim 5 or claim 6, wherein ibrutinib and 3-((dimethylamino)methyl)-N-(2-(4-(hydroxycarbamoyl)phenoxy)ethyl)benzofuran-2-carboxamide are in a single or separate dosage forms.

8. The use according to any one of claims 5-7, wherein the hematological malignancy is selected from leukemia, a lymphoproliferative disorder, or a myeloid disorder.

9. The use according to any one of claims 5-7, wherein the hematological malignancy is chronic lymphocytic leukemia (CLL).

10. The use according to any one of claims 5-7, wherein the hematological malignancy is mantle cell lymphoma.

11. The use according to any one of claims 5-7, wherein the hematological malignancy is Waldenström's macroglobulinemia.

12. The use according to any one of claims 5-7, wherein the hematological malignancy is acute myelogenous leukemia (AML).

13. The use according to any one of claims 5-12, wherein the individual is identified as having an increased presence, expression, or level of expression of a biomarker selected from: CXCR4, Ki67, MDC, MIP-1β, CXCL13, CXCL17, IL-10, MIP-1α, CCL17, and CCL21.

14. A kit for treating a hematological malignancy in an individual, wherein the kit comprises:

(a) ibrutinib or a pharmaceutically acceptable salt thereof; and

(b) 3-((dimethylamino)methyl)-N-(2-(4-(hydroxycarbamoyl)phenoxy)ethyl) benzofuran-2-carboxamide or a pharmaceutically acceptable salt thereof.

15. The kit of claim 14, wherein the kit further comprises

(c) an agent for detecting the presence, expression, or level of expression of a biomarker selected from CXCR4, Ki67, MDC, MIP-1β, CXCL13, CXCL17, IL-10, MIP-1α, CCL17, and CCL21.

16. The kit according to claim 14 or claim 15, wherein the amount of ibrutinib in kit is from about 300 mg/day to about 1000 mg/day.

17. The kit according to any one of claims 14-16, wherein ibrutinib and 3-((dimethylamino)methyl)-N-(2-(4-(hydroxycarbamoyl)phenoxy)ethyl)benzofuran-2-carboxamide are in a single or separate dosage forms.

18. The kit according to any one of claims 14-17, wherein the hematological malignancy is selected from leukemia, a lymphoproliferative disorder, or a myeloid disorder.

19. The kit according to any one of claims 14-17, wherein the hematological malignancy is chronic lymphocytic leukemia (CLL).

20. The kit according to any one of claims 14-17, wherein the hematological malignancy is mantle cell lymphoma.

21. The kit according to any one of claims 14-17, wherein the hematological malignancy is Waldenström's macroglobulinemia.

22. The kit according to any one of claims 14-17, wherein the hematological malignancy is acute myelogenous leukemia (AML).

WHAT IS CLAIMED IS:

1. Use of an anti-cancer treatment, in the manufacture of a medicament for treating a hematological malignancy in an individual, wherein the individual is identified as having an increased mobilization of a plurality of cells from the malignancy following administration of an irreversible Btk inhibitor to the individual.

2. The use of claim 1, wherein the irreversible Btk inhibitor covalently binds to Cys 481 of Btk.

3. The use of claim 1, wherein the irreversible Btk inhibitor is a compound of Formula (D).

4. The use of claim 1, wherein the irreversible Btk inhibitor is (R)-1-(3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one (PCI-32765/ibrutinib).

5. The use of claim 1, wherein the hematological malignancy is a B-cell malignancy.

6. The use of claim 1, wherein the hematological malignancy is a leukemia, lymphoproliferative disorder, or myeloid disorder.

7. The use of claim 1 wherein the hematological malignancy is a non-Hodgkin's lymphoma.

8. The use of claim 1, wherein the hematological malignancy is a chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), high risk CLL, non-CLL/SLL lymphoma, follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), Waldenstrom's macroglobulinemia, multiple myeloma (MM), marginal zone lymphoma, Burkitt's lymphoma, non-Burkitt high grade B cell lymphoma, extranodal marginal zone B cell lymphoma, acute or chronic myelogenous (or myeloid) leukemia, myelodysplastic syndrome, or acute lymphoblastic leukemia.

9. The use of claim 1, wherein the hematological malignancy is relapsed or refractory diffuse large B-cell lymphoma (DLBCL), relapsed or refractory mantle cell lymphoma, relapsed or refractory follicular lymphoma, relapsed or refractory CLL; relapsed or refractory SLL; relapsed or refractory multiple myeloma.

10. The use of claim 1, wherein the mobilized cells are myeloid cells or lymphoid cells.

11. The use of claim 1, wherein the individual has a higher peripheral blood concentration of mobilized cells following administration of the Btk inhibitor as compared to the concentration before administration of the Btk inhibitor.

12. The use of claim 1, where the second treatment is administered after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time.

13. The use of claim 1, wherein diagnosis is based on detection of the presence, expression or level of expression of one or more biomarkers.

14. The use of claim 13, wherein the biomarker is: ZAP70; t(14,18); β-2 microglobulin; p53 mutational status; ATM mutational status; del(17)p; del(11)q; del(6)q; CD5; CD11c; CD19; CD20; CD22; CD25; CD38; CD103; CD138; secreted, surface or cytoplasmic immunoglobulin expression; V_H mutational status; or a combination thereof.

15. The use of claim 1, wherein the second treatment comprises lenalidomide, bortezomib, sorafenib, gemcitabine, dexamethasone, bendamustine, R-406, taxol, vincristine, doxorubicin, temsirolimus, carboplatin, ofatumumab, rituximab, GA101, R-ICE (ifosfamide, carboplatin, etoposide), R-CHOP (rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone), BR (bendamustine and rituximab), FCR (fludarabine, cyclophosphamide, and rituximab) or any combination thereof.

16. Use of a first treatment comprising an amount of an irreversible Btk inhibitor and a second treatment in the manufacture of a medicament for treating a hematological malignancy in an individual in need thereof, wherein said Btk inhibitor is sufficient to mobilize a plurality of cells from said malignancy and said plurality of cells are analyzed in a sample obtained from the individual.

17. The use of claim 16, wherein the amount of the irreversible Btk inhibitor is sufficient to induce lymphocytosis of a plurality of cells from the malignancy.

18. The use of claim 16, wherein the irreversible Btk inhibitor covalently binds to Cys 481 of Btk.

19. The use of claim 16, wherein the irreversible Btk inhibitor is a compound of Formula (D).

20. The use of claim 16, wherein the irreversible Btk inhibitor is (R)-1-(3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one (PCI-32765).

21. The use of claim 16, wherein the hematological malignancy is a B-cell malignancy.

22. The use of claim 16, wherein the hematological malignancy is a leukemia, lymphoproliferative disorder, or myeloid disorder.

23. The use of claim 16, wherein the hematological malignancy is a non-Hodgkin's lymphoma.

24. The use of claim 16, wherein the hematological malignancy is a chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), high risk CLL, non-CLL/SLL

lymphoma, follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), Waldenstrom's macroglobulinemia, multiple myeloma (MM), marginal zone lymphoma, Burkitt's lymphoma, non-Burkitt high grade B cell lymphoma, extranodal marginal zone B cell lymphoma, acute or chronic myelogenous (or myeloid) leukemia, myelodysplastic syndrome, or acute lymphoblastic leukemia.

25. The use of claim 16, wherein the hematological malignancy is relapsed or refractory diffuse large B-cell lymphoma (DLBCL), relapsed or refractory mantle cell lymphoma, relapsed or refractory follicular lymphoma, relapsed or refractory CLL; relapsed or refractory SLL; relapsed or refractory multiple myeloma.

26. The use of claim 16, wherein the mobilized cells are myeloid cells or lymphoid cells.

27. The use of claim 16, wherein analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells.

28. The use of claim 27, further comprising administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells increases as compared to the concentration before administration of the Btk inhibitor.

29. The use of claim 27, wherein administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells.

30. The use of claim 29, wherein analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the peripheral blood concentration of the mobilized plurality of cells as compared to the concentration before administration of the Btk inhibitor.

31. The use of claim 27, further comprising administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time.

32. The use of claim 16, wherein analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, wherein the biomarker profile indicates the expression of a biomarker, the expression level of a biomarker, mutations in a biomarker, or the presence of a biomarker.

33. The use of claim 32, wherein the biomarker is: ZAP70; t(14,18); β-2 microglobulin; p53 mutational status; ATM mutational status; del(17)p; del(11)q; del(6)q; CD5; CD11c; CD19; CD20; CD22; CD25; CD38; CD103; CD138; secreted, surface or cytoplasmic immunoglobulin expression; V_H mutational status; or a combination thereof.

34. The use of claim 33, further comprising predicting the efficacy of the second treatment based on the biomarker profile.

35. The use of claim 16, wherein the second treatment comprises lenalidomide, bortezomib, sorafenib, gemcitabine, dexamethasone, bendamustine, R-406, taxol, vincristine,

doxorubicin, temsirolimus, carboplatin, ofatumumab, rituximab, GA101, R-ICE (ifosfamide, carboplatin, etoposide), R-CHOP (rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone), BR (bendamustine and rituximab), FCR (fludarabine, cyclophosphamide, and rituximab) or any combination thereof.

36. Use of a first treatment comprising an amount of an irreversible Btk inhibitor in the manufacture of a medicament for treating a hematological malignancy in an individual in need thereof, wherein said Btk inhibitor is sufficient to mobilize a plurality of cells for said malignancy and wherein a biomarker profile is prepared for a population of cells isolated from the plurality of cells.

37. The use of claim 36, wherein the amount of the irreversible Btk inhibitor is sufficient to induce lymphocytosis of a plurality of cells from the malignancy.

38. The use of claim 36, wherein the biomarker expression profile is used to diagnose, determine a prognosis, or create a predictive profile of a hematological malignancy.

39. The use of claim 36, wherein the biomarker profile indicates the expression of a biomarker, the expression level of a biomarker, mutations in a biomarker, or the presence of a biomarker.

40. The use of claim 36, wherein the biomarker profile indicates:

(a) that the hematological malignancy or survival of the hematological malignancy involves Btk signaling;

(b) that the hematological malignancy or survival of the hematological malignancy does not involve Btk signaling;

if survival of a hematological malignancy involves Btk signaling;

(c) that the hematological malignancy or survival of the hematological malignancy involves BCR signaling; or

(d) that the hematological malignancy or survival of the hematological malignancy does not involve BCR signaling.

41. The use of claim 36, wherein the biomarker is ZAP70, t(14,18), β-2 microglobulin, p53 mutational status, ATM mutational status, del(17)p, del(11)q, del(6)q, CD5, CD11c, CD19, CD20, CD22, CD25, CD38, CD103, CD138, CXCR4, secreted, surface or cytoplasmic immunoglobulin expression, V_H mutational status, or a combination thereof.

42. The use of claim 36, further comprising providing a second anti-cancer treatment based on the biomarker profile.

43. The use of claim 36, further comprising predicting the efficacy of a second anti-cancer treatment based on the biomarker profile.

44. The use of any of claims 1, 17, or 36 wherein the hematological malignancy is mantle cell lymphoma (MCL), relapsed or refractory MCL, chronic lymphocytic leukemia (CLL), relapsed or refractory CLL, small lymphocytic lymphoma (SLL), relapsed or refractory SLL, diffuse large B-cell lymphoma (DLBCL), or relapsed or refractory DLBCL.

45. The use of any of claims 1, 17 or 36, wherein the concentration of absolute lymphocyte count in the peripheral blood of the individual increases by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 125% 150%, 175%, or 200% following administration of an irreversible Btk inhibitor to the individual.

46. The use of any of claims 1, 17 or 36, wherein the mobilized cells are CD19+CD5+ cells.

47. The use of any of claims 1, 17 or 36, wherein the mobilized cells have decreased expression of CD38 and CXCR4.

48. The use of any of claims 1, 17 or 36, comprising using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual.