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

[0004] 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

comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, the hematological malignancy is a chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), high risk CLL, or a non-CLL/SLL lymphoma. In some embodiments, the hematological malignancy is follicular lymphoma, diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma, Waldenström's macroglobulinemia, multiple myeloma, marginal zone lymphoma, Burkitt's lymphoma, non-Burkitt high grade B cell lymphoma, or extranodal

marginal zone B cell lymphoma. In some embodiments, the hematological malignancy is acute or chronic myelogenous (or myeloid) leukemia, myelodysplastic syndrome, or acute lymphoblastic leukemia. In some embodiments, 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. In some embodiments, the irreversible Btk inhibitor covalently binds to Cys 481 of Btk. In some embodiments, the irreversible Btk inhibitor is a compound of (A), (A1), (B), (B1), (C), (C1), (D), (D1), (E) or (F). In some embodiments, the irreversible Btk inhibitor is a compound of Formula (D). In some embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second treatment comprises lenalidomide. In some embodiments, the second treatment comprises bortezomib. In some embodiments, the second treatment comprises sorafenib. In some embodiments, the second treatment comprises gemcitabine. In some embodiments, the second treatment comprises dexamethasone. In some embodiments, the second treatment comprises bendamustine. In some embodiments, the second treatment comprises R-406. In some embodiments, the second treatment comprises taxol. In some embodiments, the second treatment comprises vincristine. In some embodiments, the second treatment comprises doxorubicin. In some embodiments, the second treatment comprises temsirolimus. In some embodiments, the second treatment comprises carboplatin. In some embodiments, the second treatment comprises ofatumumab. In some embodiments, the second treatment comprises rituximab. In some embodiments, the second treatment comprises GA101. In some embodiments, the second treatment comprises R-ICE (ifosfamide, carboplatin, etoposide). In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual. In some embodiments, the 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. In some embodiments, the absolute lymphocyte count in the peripheral blood of the individual increases by at least about 10%-50% following administration of an irreversible Btk inhibitor to the individual. In some embodiments, the mobilized cells have decreased expression of CD38 and CXCR4. In some embodiments, the mobilized cells are CD19+CD5+ cells.

[0005] Disclosed herein, in certain embodiments, is a method for treating a hematological malignancy in an individual in need thereof, comprising administering to the individual an anti-cancer treatment, 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. In some embodiments, the irreversible Btk inhibitor covalently binds to Cys 481 of Btk. In some embodiments, the irreversible Btk inhibitor is a compound of (A), (A1), (B), (B1), (C), (C1), (D), (D1), (E) or (F). In some embodiments, the irreversible Btk inhibitor is a compound of Formula (D). In some embodiments, 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). 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 hematological malignancy is a non-Hodgkin's lymphoma. In some embodiments, 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. In some embodiments, 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. In some embodiments, the mobilized cells are myeloid cells or lymphoid cells. In some embodiments, 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. In some embodiments, the second treatment is administered after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, identification of cell mobilization is based on detection of the presence, expression or level of expression of one or more biomarkers. In some embodiments, 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. In some embodiments, 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. In some embodiments, the 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. In some embodiments, the absolute lymphocyte count in the peripheral blood of the individual increases by at least about 10%-50% following administration of an irreversible Btk inhibitor to the individual. In some embodiments, the mobilized cells have decreased expression of CD38 and CXCR4. In some embodiments, the mobilized cells are CD19+CD5+ cells.

[0006] Disclosed herein, in certain embodiments, is a method for treating an indolent 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 indolent hematological 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 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, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as

compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, the irreversible Btk inhibitor covalently binds to Cys 481 of Btk. In some embodiments, the irreversible Btk inhibitor is a compound of (A), (A1), (B), (B1), (C), (C1), (D), (D1), (E) or (F). In some embodiments, the irreversible Btk inhibitor is a compound of Formula (D). In some embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second treatment comprises lenalidomide. In some embodiments, the second treatment comprises rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone (R-CHOP). In some embodiments, the second treatment comprises temsirolimus. In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual. In some embodiments, the 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. In some embodiments, the absolute lymphocyte count in the peripheral blood of the individual increases by at least about 10%-50% following administration of an irreversible Btk inhibitor to the individual. In some embodiments, the mobilized cells have decreased expression of CD38 and CXCR4. In some embodiments, the mobilized cells are CD19+CD5+ cells.

[0007] Disclosed herein, in certain embodiments, is a method for treating a non-Hodgkin's lymphoma 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 non-Hodgkin's lymphoma; (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 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, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, the irreversible

Btk inhibitor covalently binds to Cys 481 of Btk. In some embodiments, the irreversible Btk inhibitor is a compound of (A), (A1), (B), (B1), (C), (C1), (D), (D1), (E) or (F). In some embodiments, the irreversible Btk inhibitor is a compound of Formula (D). In some embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second treatment comprises bortezomib. In some embodiments, the second treatment comprises bendamustine and rituximab (BR). In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual. In some embodiments, the 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. In some embodiments, the absolute lymphocyte count in the peripheral blood of the individual increases by at least about 10%-50% following administration of an irreversible Btk inhibitor to the individual. In some embodiments, the mobilized cells have decreased expression of CD38 and CXCR4. In some embodiments, the mobilized cells are CD19+CD5+ cells.

[0008] Disclosed herein, in certain embodiments, is a method for treating a diffuse large b-cell lymphoma (DLBCL) 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 DLBCL; (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 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, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments,

analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, the irreversible Btk inhibitor covalently binds to Cys 481 of Btk. In some embodiments, the irreversible Btk inhibitor is a compound of (A), (A1), (B), (B1), (C), (C1), (D), (D1), (E) or (F). In some embodiments, the irreversible Btk inhibitor is a compound of Formula (D). In some embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second treatment comprises bortezomib. In some embodiments, the second treatment comprises lenalidomide. In some embodiments, the second treatment comprises rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone (R-CHOP). In some embodiments, the second treatment comprises temsirolimus. In some embodiments, the DLBCL is DLBCL, ABC subtype (ABC-DLBCL). In some embodiments, the DLBCL is DLBCL, GCB subtype (GCB-DLBCL). In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual. In some embodiments, the 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. In some embodiments, the absolute lymphocyte count in the peripheral blood of the individual increases by at least about 10%-50% following administration of an irreversible Btk inhibitor to the individual. In some embodiments, the mobilized cells have decreased expression of CD38 and CXCR4. In some embodiments, the mobilized cells are CD19+CD5+ cells.

[0009] Disclosed herein, in certain embodiments, is a method for treating a follicular lymphoma (FL) 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 follicular lymphoma; (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 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, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the

peripheral blood has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, the irreversible Btk inhibitor covalently binds to Cys 481 of Btk. In some embodiments, the irreversible Btk inhibitor is a compound of (A), (A1), (B), (B1), (C), (C1), (D), (D1), (E) or (F). In some embodiments, the irreversible Btk inhibitor is a compound of Formula (D). In some embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second treatment comprises lenalidomide. In some embodiments, the second treatment comprises rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone (R-CHOP). In some embodiments, the second treatment comprises temsirolimus. In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual. In some embodiments, the 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. In some embodiments, the absolute lymphocyte count in the peripheral blood of the individual increases by at least about 10%-50% following administration of an irreversible Btk inhibitor to the individual. In some embodiments, the mobilized cells have decreased expression of CD38 and CXCR4. In some embodiments, the mobilized cells are CD19+CD5+ cells.

[0010] Disclosed herein, in certain embodiments, is a method for treating a CLL or SLL 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 CLL or SLL; (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 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, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, the irreversible Btk inhibitor covalently binds to Cys 481 of Btk. In some embodiments, the irreversible Btk inhibitor is a compound of (A), (A1), (B), (B1), (C), (C1), (D), (D1), (E) or (F). In some embodiments, the irreversible Btk inhibitor is a compound of Formula (D). In some

embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second treatment comprises lenalidomide. In some embodiments, the second treatment comprises bendamustine and rituximab (BR). In some embodiments, the second treatment comprises fludarabine, cyclophosphamide, and rituximab (FCR). In some embodiments, the second treatment comprises ofatumumab. In some embodiments, the second treatment comprises rituximab. In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual. In some embodiments, the 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. In some embodiments, the absolute lymphocyte count in the peripheral blood of the individual increases by at least about 10%-50% following administration of an irreversible Btk inhibitor to the individual. In some embodiments, the mobilized cells have decreased expression of CD38 and CXCR4. In some embodiments, the mobilized cells are CD19+CD5+ cells.

[0011] Disclosed herein, in certain embodiments, is a method for treating a mantel cell lymphoma 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 mantel cell lymphoma; (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 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, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality

of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, the irreversible Btk inhibitor covalently binds to Cys 481 of Btk. In some embodiments, the irreversible Btk inhibitor is a compound of (A), (A1), (B), (B1), (C), (C1), (D), (D1), (E) or (F). In some embodiments, the irreversible Btk inhibitor is a compound of Formula (D). In some embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second treatment comprises temsirolimus. In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual. In some embodiments, the 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. In some embodiments, the absolute lymphocyte count in the peripheral blood of the individual increases by at least about 10%-50% following administration of an irreversible Btk inhibitor to the individual. In some embodiments, the mobilized cells have decreased expression of CD38 and CXCR4. In some embodiments, the mobilized cells are CD19+CD5+ cells.

[0012] Disclosed herein, in certain embodiments, is a method for treating a Waldenstrom's macroglobulinemia 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 mantel cell lymphoma; (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 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, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, the irreversible Btk inhibitor covalently binds to Cys 481 of Btk. In some embodiments, the irreversible Btk inhibitor is a compound of (A), (A1), (B), (B1), (C), (C1), (D), (D1), (E) or (F). In some embodiments, the irreversible Btk inhibitor is a compound of Formula (D). In some embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second treatment comprises rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone (R-CHOP). In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual. In some embodiments, the 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. In some embodiments, the absolute lymphocyte count in the peripheral blood of the individual increases by at least about 10%-50% following administration of an irreversible Btk inhibitor to the individual. In some embodiments, the mobilized cells have decreased expression of CD38 and CXCR4. In some embodiments, the mobilized cells are CD19+CD5+ cells.

[0013] Disclosed herein, in certain embodiments, is a method for treating a multiple myeloma (MM) 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 MM; (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 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, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, the irreversible Btk inhibitor covalently binds to Cys 481 of Btk. In some embodiments, the irreversible Btk inhibitor is a compound of (A), (A1), (B), (B1), (C), (C1), (D), (D1), (E) or (F). In some embodiments, the irreversible Btk inhibitor is a compound of Formula (D). In some embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second treatment comprises lenalidomide. In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual. In some embodiments, the 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. In some embodiments, the absolute lymphocyte count in the peripheral blood of the individual increases by at least about 10%-50% following administration of an irreversible Btk inhibitor to the individual. In some embodiments, the mobilized cells have decreased expression of CD38 and CXCR4. In some embodiments, the mobilized cells are CD19+CD5+ cells.

[0014] 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; and (b) preparing a biomarker profile for a population of cells isolated from the plurality of cells. 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 biomarker expression profile is used to diagnose, determine a prognosis, or create a predictive profile of a hematological malignancy. In some embodiments, 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. In some embodiments, the biomarker profile indicates if a hematological malignancy involves Btk signaling. In some embodiments, the biomarker profile indicates if survival of a hematological malignancy involves Btk signaling. In some embodiments, the biomarker profile indicates that a hematological malignancy does not involve Btk signaling. In some embodiments, the biomarker profile indicates that survival of a hematological malignancy does not involve Btk signaling. In some embodiments, the biomarker profile indicates if a hematological malignancy involves BCR signaling. In some embodiments, the biomarker profile indicates if survival of a hematological malignancy involves BCR signaling. In some embodiments, the biomarker profile indicates that a hematological malignancy does not involve BCR signaling. In some embodiments, the biomarker profile indicates that survival of a hematological malignancy does not involve BCR signaling. In some embodiments, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second

treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile.

BRIEF DESCRIPTION OF THE FIGURES

[0015] Figure 1 depicts the role of Btk activity in a number of processes in a chronic lymphocytic leukemia (CLL) cell that contribute to the pathogenesis of the disease.

[0016] Figure 2 depicts the lymph node (LN) response in a patient suffering from CLL. Left panel depicts LN prior to treatment with an irreversible Btk inhibitor (PCI-32765) and Right panel depicts LN post-treatment with an irreversible Btk inhibitor (PCI-32765).

[0017] Figure 3 depicts the percentage change in tumor burden over the course treatment in a clinical trial involving administration of an irreversible Btk inhibitor (PCI-32765) in relapsed refractory (R/R) CLL/SLL patients at 420 mg/day or 840 mg/day.

[0018] Figure 4 presents the absolute lymphocyte count (ALC) and the sum of the product of the diameters (SPD) of the lymph nodes (LN) during the course of treatment with an irreversible Btk inhibitor (PCI-32765) in treatment naïve (dotted line) or R/R CLL/SLL (solid line) patients administered 420 mg/day PCI-32765.

[0019] Figure 5 presents the cumulative best response in treatment naïve patients administered 420 mg/day PCI-32765 over successive cycles of treatment. CR= complete response. PR=partial response.

[0020] Figure 6 presents the cumulative best response in R/R CLL/SLL patients administered 420 mg/day PCI-32765 over successive cycles of treatment. CR= complete response. PR=partial response.

[0021] Figure 7 presents a comparison between the cumulative best response in R/R CLL/SLL patients (RR) versus treatment naïve (TN) patients administered 420 mg/day PCI-32765 over successive cycles of treatment. CR= complete response. PR=partial response.

[0022] Figure 8 depicts the absolute lymphocyte count (ALC)/109 L vs. Cycle Day after administering a Btk inhibitor to individuals with follicular lymphoma who achieved complete or partial response (CR/PR). The Y Axis shows the Absolute Lymphocyte Counts (ALC) at each time point by cycle number and day in the X axis. All Patients (except Pt 32009) were treated on schedule of 4 weeks on treatment followed by one week off. Thus, day1 of each cycle follows one week off drug for these patients. Note the increases of ALC during most cycles of most patients, and the fall of ALC at the beginning of subsequent cycles. This pattern is often blunted in later cycles as patients responded to treatment. Patient 32009 received treatment without interruption and did not show this cyclic pattern, but did show an increase at Cycle 1, day15, and gradual increases during Cycles 2 to 5.

[0023] Figure 9 depicts the absolute lymphocyte count (ALC)/109 L vs. Cycle Day after administering a Btk inhibitor to individuals with follicular lymphoma who had Stable Disease (SD) during treatment. The Y Axis shows the Absolute Lymphocyte Counts (ALC) at each time point by cycle number and day in the X axis. All Patients were treated on schedule of 4 weeks on treatment followed by one week off. Thus, day1 of each cycle follows one week off drug for these patients. Note the gradual increase of blood ALC mobilization of Patient 32004, who initially was stable but later had Progressive Disease (PD).

[0024] Figure 10 depicts the absolute lymphocyte count (ALC)/109 L vs. Cycle Day after administering a Btk inhibitor to PD individuals with follicular lymphoma. The Y Axis shows the Absolute Lymphocyte Counts (ALC) at each time point by cycle number and day in the X axis. All Patients except 38010 were treated on schedule of 4 weeks on treatment followed by one week off. Thus, day1 of each cycle follows one week off drug for these patients. Note lack of mobilization, especially patients 38010 and 32001. Patient 323001 had limited treatment before being taken off study. The lymphocyte response suggests that this patient might had responded if it had been possible to stay on treatment longer.

[0025] Figure 11 depicts the absolute lymphocyte count (ALC)/109 L vs. Cycle Day after administering a Btk inhibitor to PR and SD individuals with DLBCL. The Y Axis shows the Absolute Lymphocyte Counts (ALC) at each time point by cycle number and day in the X axis. Patient 38011 was treated on schedule of 4 weeks on treatment followed by one week off. Thus, day1 of each cycle follows one week off drug for this patient. Patients 38008 and 324001 were treated with continuous daily doses.

[0026] Figure 12 depicts the absolute lymphocyte count (ALC)/109 L vs. Cycle Day after administering a Btk inhibitor to PD individuals with DLBCL. The Y Axis shows the Absolute Lymphocyte Counts (ALC) at each time point by cycle number and day in the X axis. All Patients were treated on schedule of 4 weeks on treatment followed by one week off. Thus, day1 of each cycle follows one week off drug for these patients. Note lack of mobilization for 3 of the 4 patients. Patient 32002 received only one cycle of treatment.

[0027] Figure 13 depicts the absolute lymphocyte count (ALC)/109 L vs. Cycle Day after administering a Btk inhibitor to individuals with mantle cell lymphoma. The Y Axis shows the Absolute Lymphocyte Counts (ALC) at each time point by cycle number and day in the X axis. Patients 32006, 38003, and 38004 were treated on schedule of 4 weeks on treatment followed by one week off. Thus, day1 of each cycle follows one week off drug for these patients. The other patients were treated with continuous daily dosing. Note that the patient with initial PD (32014) failed to show mobilization.

[0028] Figure 14 depicts the absolute lymphocyte count (ALC)/109 L vs. Cycle Day for after administering a Btk inhibitor to the individuals with mantle cell lymphoma shown in Figure 12. The axis has been changed, as compared to Fig. 12, to demonstrate low amplitude fluctuations. Note that all responding patients showed some degree of mobilization.

[0029] Figure 15 demonstrates that lymphocyte mobilization, specifically B Cell type, consistent with lymphoma cells, decreases as disease responds. Patient 32007, Cohort 4, had follicular lymphoma, grade 3, which gradually regressed from SD to CR. Although the changes of ALC in this case are not dramatic, the B cell fraction is undergoing characteristic cyclic increases in response to treatment with a Btk inhibitor. Also note the decreasing cycle by cycle magnitude of shifts consistent with cumulative disease control.

[0030] Figure 16 demonstrates that there is increased B Cell mobilization with disease progression. Patient 32004, Cohort 2, had follicular lymphoma, grade 1, which progressed from SD initially to PD following Cycle 6.

[0031] Figure 17 depicts early mobilization and eventual decrease of a $CD45^{DIM}$ B cell subpopulation in responding mantle cell lymphoma patient 200-005. This subpopulation has a typical MCL immunophenotype ($CD45^{DIM}$) and is different than that of normal lymphocytes.

[0032] Figure 18 depicts abnormal high light scatter $CD19^{+}$ cells mobilizing and then regressing in CR DLBCL Pt 324001. These $CD45^{+}$ cells with light scatter (SSC-H) in the upper panels were gated upon and their CD3 vs CD19 staining displayed in the lower panels. Here the putative malignant cells were "hidden" in the large MNC window normally defining monocytes. The sequence of mobilization followed by response is similar to other examples.

[0033] Figure 19 presents the cumulative best response in R/R MCL patients administered 560 mg/day PCI-32765 over successive cycles of treatment. CR= complete response. PR=partial response. SD=Stable disease. PD= Progressive disease.

[0034] Figure 20 presents the absolute lymphocyte count (ALC) (left) or sum of the product of the diameters (SPD) of the lymph nodes (LN) (right panel) of PCI-32756 alone or in combination with ofatumumab during the course of treatment with an irreversible Btk inhibitor (PCI-32765) in CLL/SLL patients administered 420 mg/day PCI-32765 for 28 days during cycle 1. Ofatumumab was administered at 300 mg on day 1 of cycle 2, followed by 2000 mg on days 8, 15, and 22 of cycle 2, days 1, 8, 15, and 22 of cycle 3, then on day 1 of cycles 5-8.

[0035] Figure 21 presents histological data showing lymphocyte mobilization following 12 cycles of PCI-32765 treatment at 420 mg/day in combination with ofatumumab in CLL/SLL patients as described in Fig. 20.

[0036] Figure 22 presents the absolute lymphocyte count (ALC) (left) or sum of the product of the diameters (SPD) of the lymph nodes (LN) (right panel) of PCI-32756 alone or in

combination with bendamustine during the course of treatment with an irreversible Btk inhibitor (PCI-32765) in CLL/SLL patients administered 420 mg/day PCI-32765 in 28 day cycles. Bendamustine was administered at 70 mg/m^2 (d1-2) and rituximab at 375 mg/m^2 (cycle 1) or 500 mg/m^2 (cycles 2-6) for 6 cycles.

[0037] Figure 23 presents data showing the results of a combination of a Btk inhibitor and Carboplatin or Velcade in DoHH2 cells (PCI-32765).

[0038] Figure 24 presents data showing the results of a combination of a Btk inhibitor(PCI-32765) and Dexamethasone or Lenalidomide in DoHH2 cells.

[0039] Figure 25 presents data showing the results of a combination of a Btk inhibitor(PCI-32765) and Temsirolimus or R406 in DoHH2 cells.

[0040] Figure 26 presents data showing the results of a combination of a Btk inhibitor(PCI-32765) and Gemcitabine or Doxorubicin in DoHH2 cells.

[0041] Figure 27 presents data showing the results of a combination of a Btk inhibitor(PCI-32765) and Cal-101 in TMD8 cells.

[0042] Figure 28 presents data showing the results of a combination of a Btk inhibitor(PCI-32765) and R406 in TMD8 cells.

[0043] Figure 29 presents data showing the results of a combination of a Btk inhibitor(PCI-32765) and vincristine in TMD8 cells.

[0044] Figure 30 presents data showing the results of a combination of a Btk inhibitor(PCI-32765) and doxorubicin in TMD8 cells.

[0045] Figure 31 presents data showing the results of a combination of a Btk inhibitor(PCI-32765) and lenolidomide in TMD8 cells.

[0046] Figure 32 presents data showing the results of a combination of a Btk inhibitor(PCI-32765) and velcade in TMD8 cells.

[0047] Figure 33 presents data showing the results of a combination of a Btk inhibitor(PCI-32765) and Fludarabine in TMD8 cells.

[0048] Figure 34 presents data showing the results of a combination of a Btk inhibitor(PCI-32765) and taxol in TMD8 cells.

[0049] Figure 35 presents data showing a flow plot of gated lymphocytes of PBMC samples from a representative MCL subject before and after PCI-32756 (ibrutinib) treatment (560 mg/day) for 7 days. PBMC was stained with CD3, CD19 and CD5. Note increase of $CD19^+CD3^-$ and $CD19^+CD5^+$ population after 7 days of drug treatment.

[0050] Figure 36 presents data showing that $CD19^+CD5^+$ cells have decreased CXCR4, CD38 and Ki67 following PCI-32765 (ibrutinib) Treatment (A) Significant reduction of surface CXCR4 expression in $CD19^+CD5^+$ cells following one week of ibrutinib treatment. (B)

Reduction of CD38 expression in $CD19^+CD5^+$ cells but not $CD19^+CD5^-$ cells during 4-weeks of treatment in 4 subjects treated with ibrutinib. (C) Surface CD38 expression ($p<0.01$) (left panel) and intracellular Ki67 ($p<0.05$) (right panel) is significantly reduced following one week of treatment. MFI of intracellular phospho-Erk (pT202/Y204/Erk1/2) of $CD20^+CD5+$ PBMC from healthy subjects or MCL patients treated with ibrutinib before treatment (D1) and after 1 week of treatment (D8) (lower panel). (D) CXCR4 and CD38 expression from lymph node biopsies and PBMC of three MCL lymphoma patients (subjects A, B, C) not treated with drug. (E) Percentage change in plasma chemokine and cytokine concentrations on Day 8 (left) or Day 29 (right) of ibrutinib treated MCL patients compared to pre-treatment (n=9).

[0051] Figure 37 presents data showing that PCI-32765 (ibrutinib) inhibits migration of MCL cells beneath stromal cells (pseudoemperipoliesis) and the formation of CXCL12 stimulated cortical actin. (A) Mino cells were pretreated with escalating doses of ibrutinib or vehicle for 30 min and then placed onto stromal cell populated plate. After 4 hrs, co-culture was washed several times, and migrated and adhered Mino cells were scored and counted in a flow cytometer with calibrated beads after staining with hCD19 and scoring for the $CD19^+$ population. Both pertussis toxin and ibrutinib dose-dependently inhibited migrated and adhered Mino cells (left panel). Mino cells stimulated with CXCL12 and treated with vehicle or drug were stained with Phalloidin and its intensity was determined using flow cytometry (right panel). (B) ibrutinib (100 nM) inhibited pseudoemperipoliesis of primary MCL ($hCD19^+$ cells) in co-culture with M2 stromal cells (left panel).

DETAILED DESCRIPTION OF THE INVENTION

[0052] There is currently a need for methods of treating (including, diagnosing) hematological malignancies, including relapsed and refractory B cell malignancies, and ABC-DLBCL. The present application is based, in part, on the unexpected discovery that Btk inhibitors induce mobilization (or, in some cases, lymphocytosis) of lymphoid cells in solid hematological malignancies. Mobilization of the lymphoid cells increases their exposure to additional cancer treatment s and their availability for biomarker screening.

[0053] 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. The method of claim 6, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, the hematological malignancy is a chronic lymphocytic leukemia (CLL), small lymphocytic

lymphoma (SLL), high risk CLL, or a non-CLL/SLL lymphoma. In some embodiments, the hematological malignancy is follicular lymphoma, diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma, Waldenstrom's macroglobulinemia, multiple myeloma, marginal zone lymphoma, Burkitt's lymphoma, non-Burkitt high grade B cell lymphoma, or extranodal marginal zone B cell lymphoma. In some embodiments, the hematological malignancy is acute or chronic myelogenous (or myeloid) leukemia, myelodysplastic syndrome, or acute lymphoblastic leukemia. In some embodiments, 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. In some embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second treatment comprises lenalidomide. In some embodiments, the second treatment comprises bortezomib. In some embodiments, the second treatment comprises sorafenib. In some embodiments, the second treatment comprises gemcitabine. In some embodiments, the second treatment comprises dexamethasone. In some embodiments, the second treatment comprises bendamustine. In some embodiments, the second treatment comprises R-406. In some embodiments, the second treatment comprises taxol. In some embodiments, the second treatment comprises vincristine. In some embodiments, the second treatment comprises doxorubicin. In some embodiments, the second treatment comprises temsirolimus. In some embodiments, the second treatment comprises carboplatin. In some embodiments, the second treatment comprises ofatumumab. In some embodiments, the second treatment comprises rituximab. In some embodiments, the second treatment comprises GA101. In some embodiments, the second treatment comprises R-ICE (ifosfamide, carboplatin, etoposide. In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual.

[0054] Disclosed herein, in certain embodiments, is a method for treating an indolent 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 indolent hematological 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 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, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second treatment comprises lenalidomide. In some embodiments, the second treatment comprises rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone

(R-CHOP). In some embodiments, the second treatment comprises temsirolimus. In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual.

[0055] Disclosed herein, in certain embodiments, is a method for treating a non-Hodgkin's lymphoma 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 non-Hodgkin's lymphoma; (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 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, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second treatment comprises bortezomib. In some embodiments, the second treatment comprises bendamustine and rituximab (BR). In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual.

[0056] Disclosed herein, in certain embodiments, is a method for treating a diffuse large b-cell lymphoma (DLBCL) 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 DLBCL; (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 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, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises

administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second treatment comprises bortezomib. In some embodiments, the second treatment comprises lenalidomide. In some embodiments, the second treatment comprises rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone (R-CHOP). In some embodiments, the second treatment comprises temsirolimus. In some embodiments, the DLBCL is DLBCL, ABC subtype (ABC-DLBCL). In some embodiments, the DLBCL is DLBCL, GCB subtype (GCB-DLBCL). In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual.

[0057] Disclosed herein, in certain embodiments, is a method for treating a follicular lymphoma (FL) 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 follicular lymphoma; (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 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, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second treatment comprises lenalidomide. In some embodiments, the second treatment comprises rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone (R-CHOP). In some embodiments, the second treatment comprises temsirolimus. In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual.

[0058] Disclosed herein, in certain embodiments, is a method for treating a CLL or SLL 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 CLL or SLL; (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 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, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined

length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second treatment comprises lenalidomide. In some embodiments, the second treatment comprises bendamustine and rituximab (BR). In some embodiments, the second treatment comprises fludarabine, cyclophosphamide, and rituximab (FCR). In some embodiments, the second treatment comprises ofatumumab. In some embodiments, the second treatment comprises rituximab. In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual.

[0059] Disclosed herein, in certain embodiments, is a method for treating a mantel cell lymphoma 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 mantel cell lymphoma; (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 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, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises

administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second treatment comprises temsirolimus. In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual.

[0060] Disclosed herein, in certain embodiments, is a method for treating a Waldenstrom's macroglobulinemia 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 mantel cell lymphoma; (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 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, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second

treatment comprises rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone (R-CHOP). In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual.

[0061] Disclosed herein, in certain embodiments, is a method for treating a multiple myeloma (MM) 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 MM; (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 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, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises preparing a biomarker profile for a

population of cells isolated from the plurality of cells, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile. In some embodiments, 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 (i.e. PCI-32765/ibrutinib). In some embodiments, the second treatment comprises lenalidomide. In some embodiments, the method comprises using an analytical instrument to analyze the mobilized plurality of cells in a sample obtained from the individual.

[0062] 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; and (b) preparing a biomarker profile for a population of cells isolated from the plurality of cells. 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 biomarker expression profile is used to diagnose, determine a prognosis, or create a predictive profile of a hematological malignancy. In some embodiments, 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. In some embodiments, the biomarker profile indicates if a hematological malignancy involves Btk signaling. In some embodiments, the biomarker profile indicates if survival of a hematological malignancy involves Btk signaling. In some embodiments, the biomarker profile indicates that a hematological malignancy does not involve Btk signaling. In some embodiments, the biomarker profile indicates that survival of a hematological malignancy does not involve Btk signaling. In some embodiments, the biomarker profile indicates if a hematological malignancy involves BCR signaling. In some embodiments, the biomarker profile indicates if survival of a hematological malignancy involves BCR signaling. In some embodiments, the biomarker profile indicates that a hematological malignancy does not involve BCR signaling. In some embodiments, the biomarker profile indicates that survival of a hematological malignancy does not involve BCR signaling. In some embodiments, 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. In some embodiments, 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. In some embodiments, the method further comprises providing the second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of the second treatment based on the biomarker profile.

Certain Terminology

[0063] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the claimed subject matter belongs. In the event that there are a plurality of definitions for terms herein, those in this section prevail. Where reference is made to a URL or other such identifier or address, it is understood that such identifiers can change and particular information on the internet can come and go, but equivalent information can be found by searching the internet. Reference thereto evidences the availability and public dissemination of such information.

[0064] It is to be understood that the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of any subject matter claimed. In this application, the use of the singular includes the plural unless specifically stated otherwise. It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. In this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, use of the term "including" as well as other forms, such as "include", "includes," and "included," is not limiting.

[0065] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in the application including, but not limited to, patents, patent applications, articles, books, manuals, and treatises are hereby expressly incorporated by reference in their entirety for any purpose.

[0066] Definition of standard chemistry terms may be found in reference works, including Carey and Sundberg "ADVANCED ORGANIC CHEMISTRY 4TH ED." Vols. A (2000) and B (2001), Plenum Press, New York. Unless otherwise indicated, conventional methods of mass spectroscopy, NMR, HPLC, protein chemistry, biochemistry, recombinant DNA techniques and

pharmacology, within the skill of the art are employed. Unless specific definitions are provided, the nomenclature employed in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those known in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients. Standard techniques can be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Reactions and purification techniques can be performed e.g., using kits of manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures can be generally performed of conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification.

[0067] It is to be understood that the methods and compositions described herein are not limited to the particular methodology, protocols, cell lines, constructs, and reagents described herein and as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the methods and compositions described herein, which will be limited only by the appended claims.

[0068] All publications and patents mentioned herein are incorporated herein by reference in their entirety for the purpose of describing and disclosing, for example, the constructs and methodologies that are described in the publications, which might be used in connection with the methods, compositions and compounds described herein. The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors described herein are not entitled to antedate such disclosure by virtue of prior invention or for any other reason.

[0069] An "alkyl" group refers to an aliphatic hydrocarbon group. The alkyl moiety may be a "saturated alkyl" group, which means that it does not contain any alkene or alkyne moieties. The alkyl moiety may also be an "unsaturated alkyl" moiety, which means that it contains at least one alkene or alkyne moiety. An "alkene" moiety refers to a group that has at least one carbon-carbon double bond, and an "alkyne" moiety refers to a group that has at least one carbon-carbon triple bond. The alkyl moiety, whether saturated or unsaturated, may be branched, straight chain, or cyclic. Depending on the structure, an alkyl group can be a monoradical or a diradical (i.e., an alkylene group). The alkyl group could also be a "lower alkyl" having 1 to 6 carbon atoms.

[0070] As used herein, C_1-C_x includes C_1-C_2, C_1-C_3.. C_1-C_x.

[0071] The "alkyl" moiety may have 1 to 10 carbon atoms (whenever it appears herein, a numerical range such as "1 to 10" refers to each integer in the given range; *e.g.*, "1 to 10 carbon atoms" means that the alkyl group may have 1 carbon atom, 2 carbon atoms, 3 carbon atoms, *etc.*, up to and including 10 carbon atoms, although the present definition also covers the occurrence of the term "alkyl" where no numerical range is designated). The alkyl group of the compounds described herein may be designated as "C_1-C_4 alkyl" or similar designations. By way of example only, "C_1-C_4 alkyl" indicates that there are one to four carbon atoms in the alkyl chain, *i.e.*, the alkyl chain is selected from among methyl, ethyl, propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, and t-butyl. Thus C_1-C_4 alkyl includes C_1-C_2 alkyl and C_1-C_3 alkyl. Alkyl groups can be substituted or unsubstituted. Typical alkyl groups include, but are in no way limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tertiary butyl, pentyl, hexyl, ethenyl, propenyl, butenyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like.

[0072] As used herein, the term "non-cyclic alkyl" refers to an alkyl that is not cyclic (i.e., a straight or branched chain containing at least one carbon atom). Non-cyclic alkyls can be fully saturated or can contain non-cyclic alkenes and/or alkynes. Non-cyclic alkyls can be optionally substituted.

[0073] The term "alkenyl" refers to a type of alkyl group in which the first two atoms of the alkyl group form a double bond that is not part of an aromatic group. That is, an alkenyl group begins with the atoms –C(R)=C(R)-R, wherein R refers to the remaining portions of the alkenyl group, which may be the same or different. The alkenyl moiety may be branched, straight chain, or cyclic (in which case, it would also be known as a "cycloalkenyl" group). Depending on the structure, an alkenyl group can be a monoradical or a diradical (i.e., an alkenylene group). Alkenyl groups can be optionally substituted. Non-limiting examples of an alkenyl group include –CH=CH_2, -C(CH_3)=CH_2, -CH=$CHCH_3$, –C(CH_3)=$CHCH_3$. Alkenylene groups include, but are not limited to, –CH=CH–, –C(CH_3)=CH–, –CH=$CHCH_2$–, –CH=$CHCH_2CH_2$– and –C(CH_3)=$CHCH_2$–. Alkenyl groups could have 2 to 10 carbons. The alkenyl group could also be a "lower alkenyl" having 2 to 6 carbon atoms.

[0074] The term "alkynyl" refers to a type of alkyl group in which the first two atoms of the alkyl group form a triple bond. That is, an alkynyl group begins with the atoms –C≡C-R, wherein R refers to the remaining portions of the alkynyl group, which may be the same or different. The "R" portion of the alkynyl moiety may be branched, straight chain, or cyclic. Depending on the structure, an alkynyl group can be a monoradical or a diradical (i.e., an alkynylene group). Alkynyl groups can be optionally substituted. Non-limiting examples of an alkynyl group include, but are not limited to, –C≡CH, -C≡CCH_3, –C≡CCH_2CH_3, –C≡C–, and –

$C{\equiv}CCH_2$–. Alkynyl groups can have 2 to 10 carbons. The alkynyl group could also be a "lower alkynyl" having 2 to 6 carbon atoms.

[0075] An "alkoxy" group refers to a (alkyl)O- group, where alkyl is as defined herein.

[0076] "Hydroxyalkyl" refers to an alkyl radical, as defined herein, substituted with at least one hydroxy group. Non-limiting examples of a hydroxyalkyl include, but are not limited to, hydroxymethyl, 2-hydroxyethyl, 2-hydroxypropyl, 3-hydroxypropyl, 1-(hydroxymethyl)-2-methylpropyl, 2-hydroxybutyl, 3-hydroxybutyl, 4-hydroxybutyl, 2,3-dihydroxypropyl, 1-(hydroxymethyl)-2-hydroxyethyl, 2,3-dihydroxybutyl, 3,4-dihydroxybutyl and 2-(hydroxymethyl)-3-hydroxypropyl.

[0077] "Alkoxyalkyl" refers to an alkyl radical, as defined herein, substituted with an alkoxy group, as defined herein.

[0078] An "alkenyloxy" group refers to a (alkenyl)O- group, where alkenyl is as defined herein.

[0079] The term "alkylamine" refers to the $-N(alkyl)_xH_y$ group, where x and y are selected from among x=1, y=1 and x=2, y=0. When x=2, the alkyl groups, taken together with the N atom to which they are attached, can optionally form a cyclic ring system.

[0080] "Alkylaminoalkyl" refers to an alkyl radical, as defined herein, substituted with an alkylamine, as defined herein.

[0081] An "amide" is a chemical moiety with the formula -C(O)NHR or -NHC(O)R, where R is selected from among alkyl, cycloalkyl, aryl, heteroaryl (bonded through a ring carbon) and heteroalicyclic (bonded through a ring carbon). An amide moiety may form a linkage between an amino acid or a peptide molecule and a compound described herein, thereby forming a prodrug. Any amine, or carboxyl side chain on the compounds described herein can be amidified. The procedures and specific groups to make such amides are known to those of skill in the art and can readily be found in reference sources such as Greene and Wuts, Protective Groups in Organic Synthesis, 3rd Ed., John Wiley & Sons, New York, NY, 1999, which is incorporated herein by reference in its entirety.

[0082] The term "ester" refers to a chemical moiety with formula -COOR, where R is selected from among alkyl, cycloalkyl, aryl, heteroaryl (bonded through a ring carbon) and heteroalicyclic (bonded through a ring carbon). Any hydroxy, or carboxyl side chain on the compounds described herein can be esterified. The procedures and specific groups to make such esters are known to those of skill in the art and can readily be found in reference sources such as Greene and Wuts, Protective Groups in Organic Synthesis, 3rd Ed., John Wiley & Sons, New York, NY, 1999, which is incorporated herein by reference in its entirety.

[0083] As used herein, the term "ring" refers to any covalently closed structure. Rings include, for example, carbocycles (e.g., aryls and cycloalkyls), heterocycles (e.g., heteroaryls and non-aromatic heterocycles), aromatics (e.g. aryls and heteroaryls), and non-aromatics (e.g., cycloalkyls and non-aromatic heterocycles). Rings can be optionally substituted. Rings can be monocyclic or polycyclic.

[0084] As used herein, the term "ring system" refers to one, or more than one ring.

[0085] The term "membered ring" can embrace any cyclic structure. The term "membered" is meant to denote the number of skeletal atoms that constitute the ring. Thus, for example, cyclohexyl, pyridine, pyran and thiopyran are 6-membered rings and cyclopentyl, pyrrole, furan, and thiophene are 5-membered rings.

[0086] The term "fused" refers to structures in which two or more rings share one or more bonds.

[0087] The term "carbocyclic" or "carbocycle" refers to a ring wherein each of the atoms forming the ring is a carbon atom. Carbocycle includes aryl and cycloalkyl. The term thus distinguishes carbocycle from heterocycle ("heterocyclic") in which the ring backbone contains at least one atom which is different from carbon (i.e. a heteroatom). Heterocycle includes heteroaryl and heterocycloalkyl. Carbocycles and heterocycles can be optionally substituted.

[0088] The term "aromatic" refers to a planar ring having a delocalized π-electron system containing 4n+2 π electrons, where n is an integer. Aromatic rings can be formed from five, six, seven, eight, nine, or more than nine atoms. Aromatics can be optionally substituted. The term "aromatic" includes both carbocyclic aryl (*e.g.*, phenyl) and heterocyclic aryl (or "heteroaryl" or "heteroaromatic") groups (*e.g.*, pyridine). The term includes monocyclic or fused-ring polycyclic (*i.e.*, rings which share adjacent pairs of carbon atoms) groups.

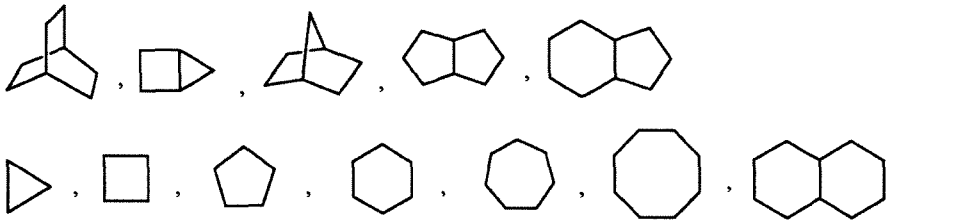
[0089] As used herein, the term "aryl" refers to an aromatic ring wherein each of the atoms forming the ring is a carbon atom. Aryl rings can be formed by five, six, seven, eight, nine, or more than nine carbon atoms. Aryl groups can be optionally substituted. Examples of aryl groups include, but are not limited to phenyl, naphthalenyl, phenanthrenyl, anthracenyl, fluorenyl, and indenyl. Depending on the structure, an aryl group can be a monoradical or a diradical (i.e., an arylene group).

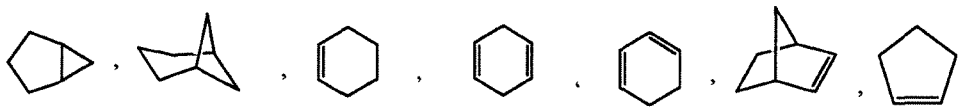
[0090] An "aryloxy" group refers to an (aryl)O- group, where aryl is as defined herein.

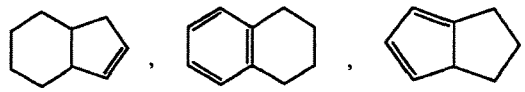
[0091] "Aralkyl" means an alkyl radical, as defined herein, substituted with an aryl group. Non-limiting aralkyl groups include, benzyl, phenethyl, and the like.

[0092] "Aralkenyl" means an alkenyl radical, as defined herein, substituted with an aryl group, as defined herein.

[0093] The term "cycloalkyl" refers to a monocyclic or polycyclic radical that contains only carbon and hydrogen, and may be saturated, partially unsaturated, or fully unsaturated. Cycloalkyl groups include groups having from 3 to 10 ring atoms. Illustrative examples of cycloalkyl groups include the following moieties:





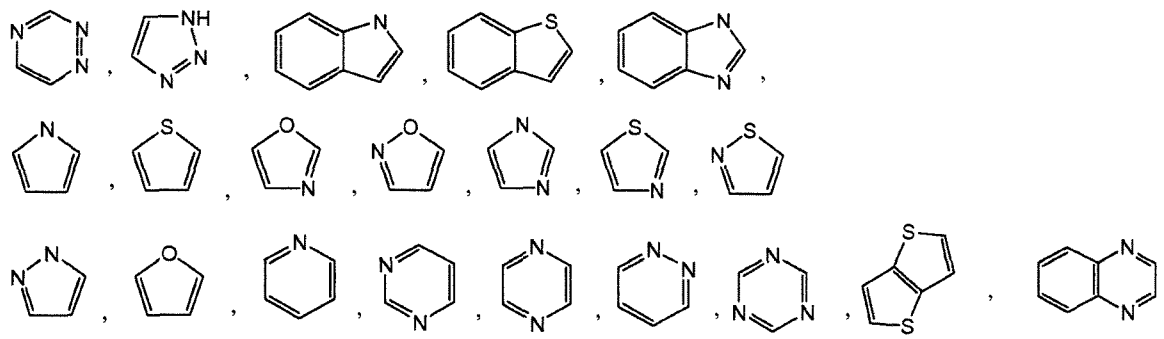
[0094] , and the like. Depending on the structure, a cycloalkyl group can be a monoradical or a diradical (e.g., an cycloalkylene group). The cycloalkyl group could also be a "lower cycloalkyl" having 3 to 8 carbon atoms.

[0095] "Cycloalkylalkyl" means an alkyl radical, as defined herein, substituted with a cycloalkyl group. Non-limiting cycloalkylalkyl groups include cyclopropylmethyl, cyclobutylmethyl, cyclopentylmethyl, cyclohexylmethyl, and the like.

[0096] The term "heterocycle" refers to heteroaromatic and heteroalicyclic groups containing one to four heteroatoms each selected from O, S and N, wherein each heterocyclic group has from 4 to 10 atoms in its ring system, and with the proviso that the ring of said group does not contain two adjacent O or S atoms. Herein, whenever the number of carbon atoms in a heterocycle is indicated (e.g., C_1-C_6 heterocycle), at least one other atom (the heteroatom) must be present in the ring. Designations such as "C_1-C_6 heterocycle" refer only to the number of carbon atoms in the ring and do not refer to the total number of atoms in the ring. It is understood that the heterocylic ring can have additional heteroatoms in the ring. Designations such as "4-6 membered heterocycle" refer to the total number of atoms that are contained in the ring (i.e., a four, five, or six membered ring, in which at least one atom is a carbon atom, at least one atom is a heteroatom and the remaining two to four atoms are either carbon atoms or heteroatoms). In heterocycles that have two or more heteroatoms, those two or more heteroatoms can be the same or different from one another. Heterocycles can be optionally substituted. Binding to a heterocycle can be at a heteroatom or via a carbon atom. Non-aromatic heterocyclic groups include groups having only 4 atoms in their ring system, but aromatic heterocyclic groups must have at least 5 atoms in their ring system. The heterocyclic groups include benzo-fused ring systems. An example of a 4-membered heterocyclic group is azetidinyl

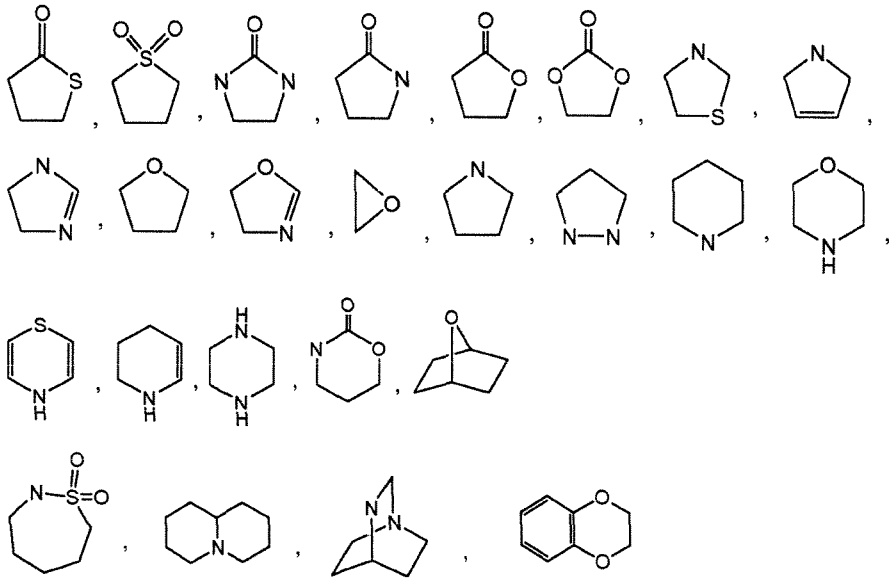
(derived from azetidine). An example of a 5-membered heterocyclic group is thiazolyl. An example of a 6-membered heterocyclic group is pyridyl, and an example of a 10-membered heterocyclic group is quinolinyl. Examples of non-aromatic heterocyclic groups are pyrrolidinyl, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothienyl, tetrahydropyranyl, dihydropyranyl, tetrahydrothiopyranyl, piperidino, morpholino, thiomorpholino, thioxanyl, piperazinyl, azetidinyl, oxetanyl, thietanyl, homopiperidinyl, oxepanyl, thiepanyl, oxazepinyl, diazepinyl, thiazepinyl, 1,2,3,6-tetrahydropyridinyl, 2-pyrrolinyl, 3-pyrrolinyl, indolinyl, 2H-pyranyl, 4H-pyranyl, dioxanyl, 1,3-dioxolanyl, pyrazolinyl, dithianyl, dithiolanyl, dihydropyranyl, dihydrothienyl, dihydrofuranyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, 3-azabicyclo[3.1.0]hexanyl, 3-azabicyclo[4.1.0]heptanyl, 3H-indolyl and quinolizinyl. Examples of aromatic heterocyclic groups are pyridinyl, imidazolyl, pyrimidinyl, pyrazolyl, triazolyl, pyrazinyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, oxazolyl, isothiazolyl, pyrrolyl, quinolinyl, isoquinolinyl, indolyl, benzimidazolyl, benzofuranyl, cinnolinyl, indazolyl, indolizinyl, phthalazinyl, pyridazinyl, triazinyl, isoindolyl, pteridinyl, purinyl, oxadiazolyl, thiadiazolyl, furazanyl, benzofurazanyl, benzothiophenyl, benzothiazolyl, benzoxazolyl, quinazolinyl, quinoxalinyl, naphthyridinyl, and furopyridinyl. The foregoing groups, as derived from the groups listed above, may be C-attached or *N*-attached where such is possible. For instance, a group derived from pyrrole may be pyrrol-1-yl (*N*-attached) or pyrrol-3-yl (C-attached). Further, a group derived from imidazole may be imidazol-1-yl or imidazol-3-yl (both *N*-attached) or imidazol-2-yl, imidazol-4-yl or imidazol-5-yl (all C-attached). The heterocyclic groups include benzo-fused ring systems and ring systems substituted with one or two oxo (=O) moieties such as pyrrolidin-2-one. Depending on the structure, a heterocycle group can be a monoradical or a diradical (i.e., a heterocyclene group).

[0097] The terms "heteroaryl" or, alternatively, "heteroaromatic" refers to an aryl group that includes one or more ring heteroatoms selected from nitrogen, oxygen and sulfur. An *N*-containing "heteroaromatic" or "heteroaryl" moiety refers to an aromatic group in which at least one of the skeletal atoms of the ring is a nitrogen atom. Illustrative examples of heteroaryl groups include the following moieties:



and the like. Depending on the structure, a heteroaryl group can be a monoradical or a diradical (i.e., a heteroarylene group).

[0098] As used herein, the term "non-aromatic heterocycle", "heterocycloalkyl" or "heteroalicyclic" refers to a non-aromatic ring wherein one or more atoms forming the ring is a heteroatom. A "non-aromatic heterocycle" or "heterocycloalkyl" group refers to a cycloalkyl group that includes at least one heteroatom selected from nitrogen, oxygen and sulfur. The radicals may be fused with an aryl or heteroaryl. Heterocycloalkyl rings can be formed by three, four, five, six, seven, eight, nine, or more than nine atoms. Heterocycloalkyl rings can be optionally substituted. In certain embodiments, non-aromatic heterocycles contain one or more carbonyl or thiocarbonyl groups such as, for example, oxo- and thio-containing groups. Examples of heterocycloalkyls include, but are not limited to, lactams, lactones, cyclic imides, cyclic thioimides, cyclic carbamates, tetrahydrothiopyran, 4H-pyran, tetrahydropyran, piperidine, 1,3-dioxin, 1,3-dioxane, 1,4-dioxin, 1,4-dioxane, piperazine, 1,3-oxathiane, 1,4-oxathiin, 1,4-oxathiane, tetrahydro-1,4-thiazine, 2H-1,2-oxazine, maleimide, succinimide, barbituric acid, thiobarbituric acid, dioxopiperazine, hydantoin, dihydrouracil, morpholine, trioxane, hexahydro-1,3,5-triazine, tetrahydrothiophene, tetrahydrofuran, pyrroline, pyrrolidine, pyrrolidone, pyrrolidione, pyrazoline, pyrazolidine, imidazoline, imidazolidine, 1,3-dioxole, 1,3-dioxolane, 1,3-dithiole, 1,3-dithiolane, isoxazoline, isoxazolidine, oxazoline, oxazolidine, oxazolidinone, thiazoline, thiazolidine, and 1,3-oxathiolane. Illustrative examples of heterocycloalkyl groups, also referred to as non-aromatic heterocycles, include:



and the like. The term heteroalicyclic also includes all ring forms of the carbohydrates, including but not limited to the monosaccharides, the disaccharides and the oligosaccharides. Depending on the structure, a heterocycloalkyl group can be a monoradical or a diradical (i.e., a heterocycloalkylene group).

[0099] The term "halo" or, alternatively, "halogen" or "halide" means fluoro, chloro, bromo and iodo.

[00100] The terms "haloalkyl," "haloalkenyl," "haloalkynyl" and "haloalkoxy" include alkyl, alkenyl, alkynyl and alkoxy structures in which at least one hydrogen is replaced with a halogen atom. In certain embodiments in which two or more hydrogen atoms are replaced with halogen atoms, the halogen atoms are all the same as one another. In other embodiments in which two or more hydrogen atoms are replaced with halogen atoms, the halogen atoms are not all the same as one another.

[00101] The term "fluoroalkyl," as used herein, refers to alkyl group in which at least one hydrogen is replaced with a fluorine atom. Examples of fluoroalkyl groups include, but are not limited to, $-CF_3$, $-CH_2CF_3$, $-CF_2CF_3$, $-CH_2CH_2CF_3$ and the like.

[00102] As used herein, the terms "heteroalkyl" "heteroalkenyl" and "heteroalkynyl" include optionally substituted alkyl, alkenyl and alkynyl radicals in which one or more skeletal chain atoms is a heteroatom, *e.g.*, oxygen, nitrogen, sulfur, silicon, phosphorus or combinations thereof. The heteroatom(s) may be placed at any interior position of the heteroalkyl group or at the position at which the heteroalkyl group is attached to the remainder of the molecule. Examples include, but are not limited to, $-CH_2-O-CH_3$, $-CH_2-CH_2-O-CH_3$, $-CH_2-NH-CH_3$, $-CH_2-CH_2-NH-CH_3$, $-CH_2-N(CH_3)-CH_3$, $-CH_2-CH_2-NH-CH_3$, $-CH_2-CH_2-N(CH_3)-CH_3$, $-CH_2-S-CH_2-CH_3$, $-CH_2-CH_2,-S(O)-CH_3$, $-CH_2-CH_2-S(O)_2-CH_3$, $-CH=CH-O-CH_3$, $-Si(CH_3)_3$, $-CH_2-CH=N-OCH_3$, and $-CH=CH-N(CH_3)-CH_3$. In addition, up to two heteroatoms may be consecutive, such as, by way of example, $-CH_2-NH-OCH_3$ and $-CH_2-O-Si(CH_3)_3$.

[00103] The term "heteroatom" refers to an atom other than carbon or hydrogen. Heteroatoms are typically independently selected from among oxygen, sulfur, nitrogen, silicon and phosphorus, but are not limited to these atoms. In embodiments in which two or more heteroatoms are present, the two or more heteroatoms can all be the same as one another, or some or all of the two or more heteroatoms can each be different from the others.

[00104] The term "bond" or "single bond" refers to a chemical bond between two atoms, or two moieties when the atoms joined by the bond are considered to be part of larger substructure.

[00105] An "isocyanato" group refers to a -NCO group.

[00106] An "isothiocyanato" group refers to a -NCS group.

[00107] The term "moiety" refers to a specific segment or functional group of a molecule. Chemical moieties are often recognized chemical entities embedded in or appended to a molecule.

[00108] A "sulfinyl" group refers to a -S(=O)-R.

[00109] A "sulfonyl" group refers to a $-S(=O)_2-R$.

[00110] A "thioalkoxy" or "alkylthio" group refers to a –S-alkyl group.

[00111] A "alkylthioalkyl" group refers to an alkyl group substituted with a –S-alkyl group.

[00112] As used herein, the term "O-carboxy" or "acyloxy" refers to a group of formula RC(=O)O-.

[00113] "Carboxy" means a -C(O)OH radical.

[00114] As used herein, the term "acetyl" refers to a group of formula $-C(=O)CH_3$.

[00115] "Acyl" refers to the group -C(O)R.

[00116] As used herein, the term "trihalomethanesulfonyl" refers to a group of formula $X_3CS(=O)_2-$ where X is a halogen.

[00117] As used herein, the term "cyano" refers to a group of formula -CN.

[00118] "Cyanoalkyl" means an alkyl radical, as defined herein, substituted with at least one cyano group.

[00119] As used herein, the term "N-sulfonamido" or "sulfonylamino" refers to a group of formula $RS(=O)_2NH-$.

[00120] As used herein, the term "O-carbamyl" refers to a group of formula $-OC(=O)NR_2$.

[00121] As used herein, the term "N-carbamyl" refers to a group of formula ROC(=O)NH-.

[00122] As used herein, the term "O-thiocarbamyl" refers to a group of formula -OC(=S)NR2.

[00123] As used herein, the term "N-thiocarbamyl" refers to a group of formula ROC(=S)NH-.

[00124] As used herein, the term "C-amido" refers to a group of formula -C(=O)NR2.

[00125] "Aminocarbonyl" refers to a -CONH2 radical.

[00126] As used herein, the term "N-amido" refers to a group of formula RC(=O)NH-.

[00127] As used herein, the substituent "R" appearing by itself and without a number designation refers to a substituent selected from among from alkyl, cycloalkyl, aryl, heteroaryl (bonded through a ring carbon) and non-aromatic heterocycle (bonded through a ring carbon).

[00128] The term "optionally substituted" or "substituted" means that the referenced group may be substituted with one or more additional group(s) individually and independently selected from alkyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, alkylthio, arylthio, alkylsulfoxide, arylsulfoxide, alkylsulfone, arylsulfone, cyano, halo, acyl, nitro, haloalkyl, fluoroalkyl, amino, including mono- and di-substituted amino groups, and the

protected derivatives thereof. By way of example an optional substituents may be L_sR_s, wherein each L_s is independently selected from a bond, -O-, -C(=O)-, -S-, -S(=O)-, -S(=O)$_2$-, -NH-, -NHC(O)-, -C(O)NH-, S(=O)$_2$NH-, -NHS(=O)$_2$, -OC(O)NH-, -NHC(O)O-, -(substituted or unsubstituted C_1-C_6 alkyl), or -(substituted or unsubstituted C_2-C_6 alkenyl); and each R_s is independently selected from H, (substituted or unsubstituted C_1-C_4alkyl), (substituted or unsubstituted C_3-C_6cycloalkyl), heteroaryl, or heteroalkyl. The protecting groups that may form the protective derivatives of the above substituents are known to those of skill in the art and may be found in references such as Greene and Wuts, above.

[00129] The term "Michael acceptor moiety" refers to a functional group that can participate in a Michael reaction, wherein a new covalent bond is formed between a portion of the Michael acceptor moiety and the donor moiety. The Michael acceptor moiety is an electrophile and the "donor moiety" is a nucleophile.

[00130] The term "nucleophile" or "nucleophilic" refers to an electron rich compound, or moiety thereof. An example of a nucleophile includes, but in no way is limited to, a cysteine residue of a molecule, such as, for example Cys 481 of Btk.

[00131] The term "electrophile", or "electrophilic" refers to an electron poor or electron deficient molecule, or moiety thereof. Examples of electrophiles include, but in no way are limited to, Michael acceptor moieties.

[00132] The term "acceptable" or "pharmaceutically acceptable", with respect to a formulation, composition or ingredient, as used herein, means having no persistent detrimental effect on the general health of the subject being treated or does not abrogate the biological activity or properties of the compound, and is relatively nontoxic.

[00133] "B-cell lymphoproliferative disorders (BCLD) biomarkers", as used herein, refer to any biological molecule (found either in blood, other body fluids, or tissues) or any chromosomal abnormality that is a sign of a BCLD-related condition or disease.

[00134] "Tumor," as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. "Neoplastic," as used herein, refers to any form of dysregulated or unregulated cell growth, whether malignant or benign, resulting in abnormal tissue growth. Thus, "neoplastic cells" include malignant and benign cells having dysregulated or unregulated cell growth.

[00135] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, B-cell lymphoproliferative disorders (BCLDs), such as lymphoma and leukemia, and solid tumors. By "B cell-related cancer" or "cancer of B-cell

lineage" is intended any type of cancer in which the dysregulated or unregulated cell growth is associated with B cells.

[00136] By "refractory" in the context of a cancer is intended the particular cancer is resistant to, or non-responsive to, therapy with a particular therapeutic agent. A cancer can be refractory to therapy with a particular therapeutic agent either from the onset of treatment with the particular therapeutic agent (i.e., non-responsive to initial exposure to the therapeutic agent), or as a result of developing resistance to the therapeutic agent, either over the course of a first treatment period with the therapeutic agent or during a subsequent treatment period with the therapeutic agent.

[00137] By "agonist activity" is intended that a substance functions as an agonist. An agonist combines with a receptor on a cell and initiates a reaction or activity that is similar to or the same as that initiated by the receptor's natural ligand.

[00138] By "antagonist activity" is intended that the substance functions as an antagonist. An antagonist of Btk prevents or reduces induction of any of the responses mediated by Btk.

[00139] By "significant" agonist activity is intended an agonist activity of at least 30%, 35%, 40%, 45%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% greater than the agonist activity induced by a neutral substance or negative control as measured in an assay of a B cell response. Preferably, "significant" agonist activity is an agonist activity that is at least 2-fold greater or at least 3-fold greater than the agonist activity induced by a neutral substance or negative control as measured in an assay of a B cell response. Thus, for example, where the B cell response of interest is B cell proliferation, "significant" agonist activity would be induction of a level of B cell proliferation that is at least 2-fold greater or at least 3-fold greater than the level of B cell proliferation induced by a neutral substance or negative control.

[00140] A substance "free of significant agonist activity" would exhibit an agonist activity of not more than about 25% greater than the agonist activity induced by a neutral substance or negative control, preferably not more than about 20% greater, 15% greater, 10% greater, 5% greater, 1% greater, 0.5% greater, or even not more than about 0.1% greater than the agonist activity induced by a neutral substance or negative control as measured in an assay of a B cell response.

[00141] In some embodiments, the Btk inhibitor therapeutic agent is an antagonist anti-Btk antibody. Such antibodies are free of significant agonist activity as noted above when bound to a Btk antigen in a human cell. In one embodiment of the invention, the antagonist anti-Btk antibody is free of significant agonist activity in one cellular response. In another embodiment of the invention, the antagonist anti-Btk antibody is free of significant agonist activity in assays of

more than one cellular response (e.g., proliferation and differentiation, or proliferation, differentiation, and, for B cells, antibody production).

[00142] By "Btk-mediated signaling" it is intended any of the biological activities that are dependent on, either directly or indirection, the activity of Btk. Examples of Btk-mediated signaling are signals that lead to proliferation and survival of Btk-expressing cells, and stimulation of one or more Btk-signaling pathways within Btk-expressing cells.

[00143] A Btk "signaling pathway" or "signal transduction pathway" is intended to mean at least one biochemical reaction, or a group of biochemical reactions, that results from the activity of Btk, and which generates a signal that, when transmitted through the signal pathway, leads to activation of one or more downstream molecules in the signaling cascade. Signal transduction pathways involve a number of signal transduction molecules that lead to transmission of a signal from the cell-surface across the plasma membrane of a cell, and through one or more in a series of signal transduction molecules, through the cytoplasm of the cell, and in some instances, into the cell's nucleus. Of particular interest to the present invention are Btk signal transduction pathways which ultimately regulate (either enhance or inhibit) the activation of NF-κB via the NF-κB signaling pathway.

[00144] The methods of the present invention are directed to methods for treating cancer that, in certain embodiments, utilize antibodies for determining the expression or presence of certain BCLD biomarkers in these methods. The following terms and definitions apply to such antibodies.

[00145] "Antibodies" and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. The terms are used synonymously. In some instances the antigen specificity of the immunoglobulin may be known.

[00146] The term "antibody" is used in the broadest sense and covers fully assembled antibodies, antibody fragments that can bind antigen (e.g., Fab, $F(ab')_2$, Fv, single chain antibodies, diabodies, antibody chimeras, hybrid antibodies, bispecific antibodies, humanized antibodies, and the like), and recombinant peptides comprising the forgoing.

[00147] The terms "monoclonal antibody" and "mAb" as used herein refer to an antibody obtained from a substantially homogeneous population of antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts.

[00148] Native antibodies" and "native immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different

immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy-chain variable domains.

[00149] The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies. Variable regions confer antigen-binding specificity. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity determining regions (CDRs) or hypervariable regions, both in the light chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are celled in the framework (FR) regions. The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β-pleated-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β-pleated-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see, Kabat et al. (1991) NIH PubL. No. 91-3242, Vol. I, pages 647-669). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as Fc receptor (FcR) binding, participation of the antibody in antibody-dependent cellular toxicity, initiation of complement dependent cytotoxicity, and mast cell degranulation.

[00150] The term "hypervariable region," when used herein, refers to the amino acid residues of an antibody that are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarily determining region" or "CDR" (i.e., residues 24-34 (L1), 50-56 (L2), and 89-97 (L3) in the light-chain variable domain and 31-35 (H1), 50-65 (H2), and 95-102 (H3) in the heavy-chain variable domain; Kabat et al. (1991) Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institute of Health, Bethesda, Md.) and/or those residues from a "hypervariable loop" (i.e., residues 26-32 (L1), 50-52 (L2), and 91-96 (L3) in the light-chain variable domain and (H1), 53-55 (H2), and 96-101 (13) in the heavy chain variable domain; Clothia and Lesk, (1987) J. Mol. Biol., 196:901-917). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues, as herein deemed.

[00151] "Antibody fragments" comprise a portion of an intact antibody, preferably the antigen-binding or variable region of the intact antibody. Examples of antibody fragments

include Fab, Fab, F(ab')2, and Fv fragments; diabodies; linear antibodies (Zapata et al. (1995) Protein Eng. 10:1057-1062); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')2 fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

[00152] "Fv" is the minimum antibody fragment that contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[00153] The Fab fragment also contains the constant domain of the light chain and the first constant domain (C_{H1}) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxy terminus of the heavy chain C_{H1} domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. Fab' fragments are produced by reducing the F(ab')2 fragment's heavy chain disulfide bridge. Other chemical couplings of antibody fragments are also known.

[00154] The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

[00155] Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of human immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. Different isotypes have different effector functions. For example, human IgG1 and IgG3 isotypes have ADCC (antibody dependent cell-mediated cytotoxicity) activity.

[00156] The word "label" when used herein refers to a detectable compound or composition that is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition that is detectable.

[00157] The term "acceptable" or "pharmaceutically acceptable", with respect to a formulation, composition or ingredient, as used herein, means having no persistent detrimental effect on the general health of the subject being treated or does not abrogate the biological activity or properties of the compound, and is relatively nontoxic.

[00158] As used herein, the term "agonist" refers to a compound, the presence of which results in a biological activity of a protein that is the same as the biological activity resulting from the presence of a naturally occurring ligand for the protein, such as, for example, Btk.

[00159] As used herein, the term "partial agonist" refers to a compound the presence of which results in a biological activity of a protein that is of the same type as that resulting from the presence of a naturally occurring ligand for the protein, but of a lower magnitude.

[00160] As used herein, the term "antagonist" refers to a compound, the presence of which results in a decrease in the magnitude of a biological activity of a protein. In certain embodiments, the presence of an antagonist results in complete inhibition of a biological activity of a protein, such as, for example, Btk. In certain embodiments, an antagonist is an inhibitor.

[00161] The term "Bruton's tyrosine kinase (Btk)," as used herein, refers to Bruton's tyrosine kinase from *Homo sapiens*, as disclosed in, e.g., U.S. Patent No. 6,326,469 (GenBank Accession No. NP_000052).

[00162] The term "Bruton's tyrosine kinase homolog," as used herein, refers to orthologs of Bruton's tyrosine kinase, e.g., the orthologs from mouse (GenBank Accession No. AAB47246), dog (GenBank Accession No. XP_549139.), rat (GenBank Accession No. NP_001007799), chicken (GenBank Accession No. NP_989564), or zebra fish (GenBank Accession No. XP_698117), and fusion proteins of any of the foregoing that exhibit kinase activity towards one or more substrates of Bruton's tyrosine kinase (e.g. a peptide substrate having the amino acid sequence "AVLESEEELYSSARQ").

[00163] The terms "co-administration" or "combination therapy" and the like, as used herein, are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment s in which the agents are administered by the same or different route of administration or at the same or different time.

[00164] The term "effective amount," as used herein, refers to a sufficient amount of a Btk inhibitory agent or a Btk inhibitor compound being administered which will result in an

increase or appearance in the blood of a subpopulation of lymphocytes (e.g., pharmaceutical debulking). For example, an "effective amount" for diagnostic and/or prognostic uses is the amount of the composition including a compound as disclosed herein required to provide a clinically significant decrease an increase or appearance in the blood of a subpopulation of lymphocytes without undue adverse side effects. An appropriate "effective amount" in any individual case may be determined using techniques, such as a dose escalation study.

[00165] The term "therapeutically effective amount," as used herein, refers to a sufficient amount of an agent or a compound being administered which will relieve to some extent one or more of the symptoms s B-cell lymphoproliferative disorder (BCLD). The result can be reduction and/or alleviation of the signs, symptoms, or causes of BCLD, or any other desired alteration of a biological system. The term "therapeutically effective amount" includes, for example, a prophylactically effective amount. An "effective amount" of a compound disclosed herein is an amount effective to achieve a desired pharmacologic effect or therapeutic improvement without undue adverse side effects. It is understood that "an effect amount" or "a therapeutically effective amount" can vary from subject to subject, due to variation in metabolism of the compound of any of Formula (A), Formula (B), Formula (C), or Formula (D), age, weight, general condition of the subject, the condition being treated, the severity of the condition being treated, and the judgment of the prescribing physician. By way of example only, therapeutically effective amounts may be determined by routine experimentation, including but not limited to a dose escalation clinical trial.

[00166] The terms "enhance" or "enhancing" means to increase or prolong either in potency or duration a desired effect. By way of example, "enhancing" the effect of therapeutic agents refers to the ability to increase or prolong, either in potency or duration, the effect of therapeutic agents on during treatment of a disease, disorder or condition. An "enhancing-effective amount," as used herein, refers to an amount adequate to enhance the effect of a therapeutic agent in the treatment of a disease, disorder or condition. When used in a patient, amounts effective for this use will depend on the severity and course of the disease, disorder or condition, previous therapy, the patient's health status and response to the drugs, and the judgment of the treating physician.

[00167] The term "homologous cysteine," as used herein refers to a cysteine residue found within a sequence position that is homologous to that of cysteine 481 of Bruton's tyrosine kinase, as defined herein. For example, cysteine 482 is the homologous cysteine of the rat ortholog of Bruton's tyrosine kinase; cysteine 479 is the homologous cysteine of the chicken ortholog; and cysteine 481 is the homologous cysteine in the zebra fish ortholog. In another example, the homologous cysteine of TXK, a Tec kinase family member related to Bruton's

tyrosine, is Cys 350. See also the sequence alignments of tyrosine kinases (TK) published on the world wide web at kinase.com/human/kinome/phylogeny.html.

[00168] The term "identical," as used herein, refers to two or more sequences or subsequences which are the same. In addition, the term "substantially identical," as used herein, refers to two or more sequences which have a percentage of sequential units which are the same when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using comparison algorithms or by manual alignment and visual inspection. By way of example only, two or more sequences may be "substantially identical" if the sequential units are about 60% identical, about 65% identical, about 70% identical, about 75% identical, about 80% identical, about 85% identical, about 90% identical, or about 95% identical over a specified region. Such percentages to describe the "percent identity" of two or more sequences. The identity of a sequence can exist over a region that is at least about 75-100 sequential units in length, over a region that is about 50 sequential units in length, or, where not specified, across the entire sequence. This definition also refers to the complement of a test sequence. By way of example only, two or more polypeptide sequences are identical when the amino acid residues are the same, while two or more polypeptide sequences are "substantially identical" if the amino acid residues are about 60% identical, about 65% identical, about 70% identical, about 75% identical, about 80% identical, about 85% identical, about 90% identical, or about 95% identical over a specified region. The identity can exist over a region that is at least about 75-100 amino acids in length, over a region that is about 50 amino acids in length, or, where not specified, across the entire sequence of a polypeptide sequence. In addition, by way of example only, two or more polynucleotide sequences are identical when the nucleic acid residues are the same, while two or more polynucleotide sequences are "substantially identical" if the nucleic acid residues are about 60% identical, about 65% identical, about 70% identical, about 75% identical, about 80% identical, about 85% identical, about 90% identical, or about 95% identical over a specified region. The identity can exist over a region that is at least about 75-100 nucleic acids in length, over a region that is about 50 nucleic acids in length, or, where not specified, across the entire sequence of a polynucleotide sequence.

[00169] The terms "inhibits", "inhibiting", or "inhibitor" of a kinase, as used herein, refer to inhibition of enzymatic phosphotransferase activity.

[00170] The term "irreversible inhibitor," as used herein, refers to a compound that, upon contact with a target protein (e.g., a kinase) causes the formation of a new covalent bond with or within the protein, whereby one or more of the target protein's biological activities (e.g., phosphotransferase activity) is diminished or abolished notwithstanding the subsequent presence or absence of the irreversible inhibitor.

[00171] The term "irreversible Btk inhibitor," as used herein, refers to an inhibitor of Btk that can form a covalent bond with an amino acid residue of Btk. In one embodiment, the irreversible inhibitor of Btk can form a covalent bond with a Cys residue of Btk; in particular embodiments, the irreversible inhibitor can form a covalent bond with a Cys 481 residue (or a homolog thereof) of Btk or a cysteine residue in the homologous corresponding position of another tyrosine kinase.

[00172] The term "isolated," as used herein, refers to separating and removing a component of interest from components not of interest. Isolated substances can be in either a dry or semi-dry state, or in solution, including but not limited to an aqueous solution. The isolated component can be in a homogeneous state or the isolated component can be a part of a pharmaceutical composition that comprises additional pharmaceutically acceptable carriers and/or excipients. By way of example only, nucleic acids or proteins are "isolated" when such nucleic acids or proteins are free of at least some of the cellular components with which it is associated in the natural state, or that the nucleic acid or protein has been concentrated to a level greater than the concentration of its in vivo or in vitro production. Also, by way of example, a gene is isolated when separated from open reading frames which flank the gene and encode a protein other than the gene of interest.

[00173] A "metabolite" of a compound disclosed herein is a derivative of that compound that is formed when the compound is metabolized. The term "active metabolite" refers to a biologically active derivative of a compound that is formed when the compound is metabolized. The term "metabolized," as used herein, refers to the sum of the processes (including, but not limited to, hydrolysis reactions and reactions catalyzed by enzymes, such as, oxidation reactions) by which a particular substance is changed by an organism. Thus, enzymes may produce specific structural alterations to a compound. For example, cytochrome P450 catalyzes a variety of oxidative and reductive reactions while uridine diphosphate glucuronyl transferases catalyze the transfer of an activated glucuronic-acid molecule to aromatic alcohols, aliphatic alcohols, carboxylic acids, amines and free sulfhydryl groups. Further information on metabolism may be obtained from The Pharmacological Basis of Therapeutics, 9th Edition, McGraw-Hill (1996). Metabolites of the compounds disclosed herein can be identified either by administration of compounds to a host and analysis of tissue samples from the host, or by incubation of compounds with hepatic cells *in vitro* and analysis of the resulting compounds. Both methods are well known in the art. In some embodiments, metabolites of a compound are formed by oxidative processes and correspond to the corresponding hydroxy-containing compound. In some embodiments, a compound is metabolized to pharmacologically active metabolites.

[00174] The term "modulate," as used herein, means to interact with a target either directly or indirectly so as to alter the activity of the target, including, by way of example only, to enhance the activity of the target, to inhibit the activity of the target, to limit the activity of the target, or to extend the activity of the target.

[00175] As used herein, the term "modulator" refers to a compound that alters an activity of a molecule. For example, a modulator can cause an increase or decrease in the magnitude of a certain activity of a molecule compared to the magnitude of the activity in the absence of the modulator. In certain embodiments, a modulator is an inhibitor, which decreases the magnitude of one or more activities of a molecule. In certain embodiments, an inhibitor completely prevents one or more activities of a molecule. In certain embodiments, a modulator is an activator, which increases the magnitude of at least one activity of a molecule. In certain embodiments the presence of a modulator results in an activity that does not occur in the absence of the modulator.

[00176] As used herein, the term "selective binding compound" refers to a compound that selectively binds to any portion of one or more target proteins.

[00177] As used herein, the term "selectively binds" refers to the ability of a selective binding compound to bind to a target protein, such as, for example, Btk, with greater affinity than it binds to a non-target protein. In certain embodiments, specific binding refers to binding to a target with an affinity that is at least 10, 50, 100, 250, 500, 1000 or more times greater than the affinity for a non-target.

[00178] As used herein, the term "selective modulator" refers to a compound that selectively modulates a target activity relative to a non-target activity. In certain embodiments, specific modulator refers to modulating a target activity at least 10, 50, 100, 250, 500, 1000 times more than a non-target activity.

[00179] The term "substantially purified," as used herein, refers to a component of interest that may be substantially or essentially free of other components which normally accompany or interact with the component of interest prior to purification. By way of example only, a component of interest may be "substantially purified" when the preparation of the component of interest contains less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% (by dry weight) of contaminating components. Thus, a "substantially purified" component of interest may have a purity level of about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or greater.

[00180] The term "subject" as used herein, refers to an animal which is the object of treatment, observation or experiment. By way of example only, a subject may be, but is not limited to, a mammal including, but not limited to, a human.

[00181] As used herein, the term "target activity" refers to a biological activity capable of being modulated by a selective modulator. Certain exemplary target activities include, but are not limited to, binding affinity, signal transduction, enzymatic activity, tumor growth, effects on particular biomarkers related to B-cell lymphoproliferative disorder pathology.

[00182] As used herein, the term "target protein" refers to a molecule or a portion of a protein capable of being bound by a selective binding compound. In certain embodiments, a target protein is Btk.

[00183] The terms "treat," "treating" or "treatment", as used herein, include alleviating, abating or ameliorating a disease or condition, or symptoms thereof; managing a disease or condition, or symptoms thereof; preventing additional symptoms; ameliorating or preventing the underlying metabolic causes of symptoms; inhibiting the disease or condition, e.g., arresting the development of the disease or condition; relieving the disease or condition; causing regression of the disease or condition, relieving a condition caused by the disease or condition; or stopping the symptoms of the disease or condition. The terms "treat," "treating" or "treatment", include, but are not limited to, prophylactic and/or therapeutic treatments.

[00184] As used herein, the IC_{50} refers to an amount, concentration or dosage of a particular test compound that achieves a 50% inhibition of a maximal response, such as inhibition of Btk, in an assay that measures such response.

[00185] As used herein, EC_{50} refers to a dosage, concentration or amount of a particular test compound that elicits a dose-dependent response at 50% of maximal expression of a particular response that is induced, provoked or potentiated by the particular test compound.

Hematological Malignancies

[00186] 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 hematological malignancy is a chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), high risk CLL, or a non-CLL/SLL lymphoma. In some embodiments, the hematological malignancy is follicular lymphoma, diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma, Waldenstrom's macroglobulinemia,

multiple myeloma, marginal zone lymphoma, Burkitt's lymphoma, non-Burkitt high grade B cell lymphoma, or extranodal marginal zone B cell lymphoma. In some embodiments, the hematological malignancy is acute or chronic myelogenous (or myeloid) leukemia, myelodysplastic syndrome, or acute lymphoblastic leukemia. In some embodiments, the hematological malignancy is non-Hodgkin's lymphoma (NHL). In some embodiments, the hematological malignancy is chronic lymphocytic leukemia (CLL). In some embodiments, the hematological malignancy is mantle cell lymphoma (MCL). In some embodiments, the hematological malignancy is diffuse large B-cell lymphoma (DLBCL). In some embodiments, the hematological malignancy is diffuse large B-cell lymphoma (DLBCL), ABC subtype. In some embodiments, the hematological malignancy is diffuse large B-cell lymphoma (DLBCL), GCB subtype. In some embodiments, the hematological malignancy is Waldenstrom's macroglobulinemia (WM). In some embodiments, the hematological malignancy is multiple myeloma. In some embodiments, the hematological malignancy is Burkitt's lymphoma. In some embodiments, the hematological malignancy is follicular lymphoma. In some embodiments, the hematological malignancy is transformed follicular lymphoma. In some embodiments, the hematological malignancy is marginal zone lymphoma.

[00187] 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 hematological malignancy is relapsed or refractory. In some embodiments, 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. 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time.

[00188] 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 hematological malignancy is a hematological malignancy that is classified as high-risk. In some embodiments, the hematological malignancy is high risk CLL or high risk SLL. 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the

number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time.

[00189] 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 hematological malignancy is an indolent hematological malignancy. 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral

blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, the second treatment is lenalidomide. In some embodiments, the second treatment is rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone (R-CHOP). In some embodiments, the second treatment is temsirolimus.

[00190] 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 hematological malignancy is a transformed hematological malignancy. 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as

compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time.

[00191] B-cell lymphoproliferative disorders (BCLDs) are neoplasms of the blood and encompass, inter alia, non-Hodgkin lymphoma, multiple myeloma, and leukemia. BCLDs can originate either in the lymphatic tissues (as in the case of lymphoma) or in the bone marrow (as in the case of leukemia and myeloma), and they all are involved with the uncontrolled growth of lymphocytes or white blood cells. There are many subtypes of BCLD, e.g., chronic lymphocytic leukemia (CLL) and non-Hodgkin lymphoma (NHL). The disease course and treatment of BCLD is dependent on the BCLD subtype; however, even within each subtype the clinical presentation, morphologic appearance, and response to therapy is heterogeneous.

[00192] Malignant lymphomas are neoplastic transformations of cells that reside predominantly within lymphoid tissues. Two groups of malignant lymphomas are Hodgkin's lymphoma and non-Hodgkin's lymphoma (NHL). Both types of lymphomas infiltrate reticuloendothelial tissues. However, they differ in the neoplastic cell of origin, site of disease, presence of systemic symptoms, and response to treatment (Freedman et al., "Non-Hodgkin's Lymphomas" Chapter 134, Cancer Medicine, (an approved publication of the American Cancer Society, B.C. Decker Inc., Hamilton, Ontario, 2003).

Non-Hodgkin's Lymphomas

[00193] Disclosed herein, in certain embodiments, is a method for treating a non-Hodgkin's lymphoma 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, the second treatment is bortezomib. In some embodiments, the second treatment is bendamustine and rituximab (BR).

[00194] Further disclosed herein, in certain embodiments, is a method for treating relapsed or refractory non-Hodgkin's lymphoma in an individual in need thereof, comprising: administering to the individual a therapeutically-effective amount of (R)-1-(3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one (i.e. PCI-32765/ibrutinib). In some embodiments, the non-Hodgkin's lymphoma is relapsed or refractory diffuse large B-cell lymphoma (DLBCL), relapsed or refractory mantle cell lymphoma, or relapsed or refractory follicular lymphoma.

[00195] Non-Hodgkin lymphomas (NHL) are a diverse group of malignancies that are predominately of B-cell origin. NHL may develop in any organs associated with lymphatic system such as spleen, lymph nodes or tonsils and can occur at any age. NHL is often marked by enlarged lymph nodes, fever, and weight loss. NHL is classified as either B-cell or T-cell NHL. Lymphomas related to lymphoproliferative disorders following bone marrow or stem cell transplantation are usually B-cell NHL. In the Working Formulation classification scheme, NHL has been divided into low-, intermediate-, and high-grade categories by virtue of their natural histories (see "The Non-Hodgkin's Lymphoma Pathologic Classification Project," Cancer 49(1982):2112-2135). The low-grade lymphomas are indolent, with a median survival of 5 to 10 years (Horning and Rosenberg (1984) N. Engl. J. Med. 311:1471-1475). Although chemotherapy can induce remissions in the majority of indolent lymphomas, cures are rare and most patients eventually relapse, requiring further therapy. The intermediate- and high-grade

lymphomas are more aggressive tumors, but they have a greater chance for cure with chemotherapy. However, a significant proportion of these patients will relapse and require further treatment.

[00196] A non-limiting list of the B-cell NHL includes Burkitt's lymphoma (e.g., Endemic Burkitt's Lymphoma and Sporadic Burkitt's Lymphoma), Cutaneous B-Cell Lymphoma, Cutaneous Marginal Zone Lymphoma (MZL), Diffuse Large Cell Lymphoma (DLBCL), Diffuse Mixed Small and Large Cell Lymphoma, Diffuse Small Cleaved Cell, Diffuse Small Lymphocytic Lymphoma, Extranodal Marginal Zone B-cell lymphoma, follicular lymphoma, Follicular Small Cleaved Cell (Grade 1), Follicular Mixed Small Cleaved and Large Cell (Grade 2), Follicular Large Cell (Grade 3), Intravascular Large B-Cell Lymphoma, Intravascular Lymphomatosis, Large Cell Immunoblastic Lymphoma, Large Cell Lymphoma (LCL), Lymphoblastic Lymphoma, MALT Lymphoma, Mantle Cell Lymphoma (MCL), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, mantle cell lymphoma, chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), extranodal marginal zone B-cell lymphoma-mucosa-associated lymphoid tissue (MALT) lymphoma, Mediastinal Large B-Cell Lymphoma, nodal marginal zone B-cell lymphoma, splenic marginal zone B-cell lymphoma, primary mediastinal B-cell lymphoma, lymphoplasmocytic lymphoma, hairy cell leukemia, Waldenstrom's Macroglobulinemia, and primary central nervous system (CNS) lymphoma. Additional non-Hodgkin's lymphomas are contemplated within the scope of the present invention and apparent to those of ordinary skill in the art.

DLBCL

[00197] Disclosed herein, in certain embodiments, is a method for treating a DLBCL 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the

mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, the second treatment is lenalidomide. In some embodiments, the second treatment is rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone (R-CHOP). In some embodiments, the second treatment is temsirolimus. In some embodiments, the second treatment is bortezomib.

[00198] As used herein, the term "Diffuse large B-cell lymphoma (DLBCL)" refers to a neoplasm of the germinal center B lymphocytes with a diffuse growth pattern and a high-intermediate proliferation index. DLBCLs represent approximately 30% of all lymphomas and may present with several morphological variants including the centroblastic, immunoblastic, T-cell/histiocyte rich, anaplastic and plasmoblastic subtypes. Genetic tests have shown that there are different subtypes of DLBCL. These subtypes seem to have different outlooks (prognoses) and responses to treatment. DLBCL can affect any age group but occurs mostly in older people (the average age is mid-60s).

[00199] Disclosed herein, in certain embodiments, is a method for treating (diffuse large b-cell lymphoma, ABC-subtype (ABC-DLBCL) 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, the second treatment is lenalidomide. In some embodiments, the second treatment is rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone (R-CHOP). In some embodiments, the second treatment is temsirolimus. In some embodiments, the second treatment is bortezomib.

[00200] The ABC subtype of diffuse large B-cell lymphoma (ABC-DLBCL) is thought to arise from post germinal center B cells that are arrested during plasmatic differentiation. The ABC subtype of DLBCL (ABC-DLBCL) accounts for approximately 30% total DLBCL diagnoses. It is considered the least curable of the DLBCL molecular subtypes and, as such, patients diagnosed with the ABC-DLBCL typically display significantly reduced survival rates compared with individuals with other types of DLCBL. ABC-DLBCL is most commonly associated with chromosomal translocations deregulating the germinal center master regulator

BCL6 and with mutations inactivating the PRDM1 gene, which encodes a transcriptional repressor required for plasma cell differentiation.

[00201] A particularly relevant signaling pathway in the pathogenesis of ABC-DLBCL is the one mediated by the nuclear factor (NF)-κB transcription complex. The NF-κB family comprises 5 members (p50, p52, p65, c-rel and RelB) that form homo- and heterodimers and function as transcriptional factors to mediate a variety of proliferation, apoptosis, inflammatory and immune responses and are critical for normal B-cell development and survival. NF-κB is widely used by eukaryotic cells as a regulator of genes that control cell proliferation and cell survival. As such, many different types of human tumors have misregulated NF-κB: that is, NF-κB is constitutively active. Active NF-κB turns on the expression of genes that keep the cell proliferating and protect the cell from conditions that would otherwise cause it to die via apoptosis.

[00202] The dependence of ABC DLBCLs on NF-κB depends on a signaling pathway upstream of IkB kinase comprised of CARD11, BCL10 and MALT1 (the CBM complex). Interference with the CBM pathway extinguishes NF-κB signaling in ABC DLBCL cells and induces apoptosis. The molecular basis for constitutive activity of the NF-κB pathway is a subject of current investigation but some somatic alterations to the genome of ABC DLBCLs clearly invoke this pathway. For example, somatic mutations of the coiled-coil domain of CARD11 in DLBCL render this signaling scaffold protein able to spontaneously nucleate protein-protein interaction with MALT1 and BCL10, causing IKK activity and NF-κB activation. Constitutive activity of the B cell receptor signaling pathway has been implicated in the activation of NF-κB in ABC DLBCLs with wild type CARD11, and this is associated with mutations within the cytoplasmic tails of the B cell receptor subunits CD79A and CD79B. Oncogenic activating mutations in the signaling adapter MYD88 activate NF-κB and synergize with B cell receptor signaling in sustaining the survival of ABC DLBCL cells. In addition, inactivating mutations in a negative regulator of the NF-κB pathway, A20, occur almost exclusively in ABC DLBCL.

[00203] Indeed, genetic alterations affecting multiple components of the NF-κB signaling pathway have been recently identified in more than 50% of ABC-DLBCL patients, where these lesions promote constitutive NF-κB activation, thereby contributing to lymphoma growth. These include mutations of CARD11 (~10% of the cases), a lymphocyte-specific cytoplasmic scaffolding protein that—together with MALT1 and BCL10—forms the BCR signalosome, which relays signals from antigen receptors to the downstream mediators of NF-κB activation. An even larger fraction of cases (~30%) carry biallelic genetic lesions inactivating the negative NF-κB regulator A20. Further, high levels of expression of NF-κB target genes have been

observed in ABC-DLBCL tumor samples. *See, e.g.*, U. Klein et al., (2008), *Nature Reviews Immunology* 8:22-23; R.E. Davis et al., (2001), *Journal of Experimental Medicine* 194:1861-1874; G. Lentz et al., (2008), *Science* 319:1676-1679; M. Compagno et al., (2009), *Nature* 459:712-721; and L. Srinivasan et al., (2009), *Cell* 139:573-586).

[00204] DLBCL cells of the ABC subtype, such as OCI-Ly10, have chronic active BCR signaling and are very sensitive to the Btk inhibitors described herein. The irreversible Btk inhibitors described herein potently and irreversibly inhibit the growth of OCI-Ly10 (EC50 continuous exposure = 10 nM, EC50 1 hour pulse = 50 nM). In addition, induction of apoptosis, as shown by caspase activation, Annexin-V flow cytometry and increase in sub-G0 fraction is observed in OCILy10. Both sensitive and resistant cells express Btk at similar levels, and the active site of Btk is fully occupied by the inhibitor in both as shown using a fluorescently labeled affinity probe. OCI-Ly10 cells are shown to have chronically active BCR signaling to NF-κB which is dose dependently inhibited by the Btk inhibitors described herein. The activity of Btk inhibitors in the cell lines studied herein are also characterized by comparing signal transduction profiles (Btk, PLCγ, ERK, NF-κB, AKT), cytokine secretion profiles and mRNA expression profiles, both with and without BCR stimulation, and observed significant differences in these profiles that lead to clinical biomarkers that identify the most sensitive patient populations to Btk inhibitor treatment. *See* U.S. Patent No. 7,711,492 and Staudt *et al.*, **Nature**, Vol. 463, Jan. 7, 2010, pp. 88-92, the contents of which are incorporated by reference in their entirety.

[00205] Disclosed herein, in certain embodiments, is a method for treating (diffuse large b-cell lymphoma, GCB-subtype (GCB-DLBCL) 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, the second treatment is lenalidomide. In some embodiments, the second treatment is rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone (R-CHOP). In some embodiments, the second treatment is temsirolimus. In some embodiments, the second treatment is bortezomib.

Follicular Lymphoma

[00206] Disclosed herein, in certain embodiments, is a method for treating a follicular lymphoma 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, 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, the second treatment is lenalidomide. In some embodiments, the second treatment is rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone (R-CHOP). In some embodiments, the second treatment is temsirolimus.

[00207] As used herein, the term "follicular lymphoma" refers to any of several types of non-Hodgkin's lymphoma in which the lymphomatous cells are clustered into nodules or follicles. The term follicular is used because the cells tend to grow in a circular, or nodular, pattern in lymph nodes. The average age for people with this lymphoma is about 60.

CLL/SLL

[00208] Disclosed herein, in certain embodiments, is a method for treating a CLL or SLL 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 CLL or SLL is high-risk. 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a

subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, the second treatment is bendamustine and rituximab (BR). In some embodiments, the second treatment is fludarabine, cyclophosphamide, and rituximab (FCR). In some embodiments, the second treatment is ofatumumab. In some embodiments, the second treatment is rituximab. In some embodiments, the second treatment is lenalidomide.

[00209] Chronic lymphocytic leukemia and small lymphocytic lymphoma (CLL/SLL) are commonly thought as the same disease with slightly different manifestations. Where the cancerous cells gather determines whether it is called CLL or SLL. When the cancer cells are primarily found in the lymph nodes, lima bean shaped structures of the lymphatic system (a system primarily of tiny vessels found in the body), it is called SLL. SLL accounts for about 5% to 10% of all lymphomas. When most of the cancer cells are in the bloodstream and the bone marrow, it is called CLL.

[00210] Both CLL and SLL are slow-growing diseases, although CLL, which is much more common, tends to grow slower. CLL and SLL are treated the same way. They are usually not considered curable with standard treatments, but depending on the stage and growth rate of the disease, most patients live longer than 10 years. Occasionally over time, these slow-growing lymphomas may transform into a more aggressive type of lymphoma.

[00211] Chronic lymphoid leukemia (CLL) is the most common type of leukemia. It is estimated that 100,760 people in the United States are living with or are in remission from CLL.

Most (>75%) people newly diagnosed with CLL are over the age of 50. Currently CLL treatment focuses on controlling the disease and its symptoms rather than on an outright cure. CLL is treated by chemotherapy, radiation therapy, biological therapy, or bone marrow transplantation. Symptoms are sometimes treated surgically (splenectomy removal of enlarged spleen) or by radiation therapy ("de-bulking" swollen lymph nodes). Though CLL progresses slowly in most cases, it is considered generally incurable. Certain CLLs are classified as high-risk. As used herein, "high risk CLL" means CLL characterized by at least one of the following 1) 17p13-; 2) 11q22-; 3) unmutated IgVH together with ZAP-70+ and/or CD38+; or 4) trisomy 12.

[00212] CLL treatment is typically administered when the patient's clinical symptoms or blood counts indicate that the disease has progressed to a point where it may affect the patient's quality of life.

[00213] Small lymphocytic leukemia (SLL) is very similar to CLL described supra, and is also a cancer of B-cells. In SLL the abnormal lymphocytes mainly affect the lymph nodes. However, in CLL the abnormal cells mainly affect the blood and the bone marrow. The spleen may be affected in both conditions. SLL accounts for about 1in 25 of all cases of non-Hodgkin lymphoma. It can occur at any time from young adulthood to old age, but is rare under the age of 50. SLL is considered an indolent lymphoma. This means that the disease progresses very slowly, and patients tend to live many years after diagnosis. However, most patients are diagnosed with advanced disease, and although SLL responds well to a variety of chemotherapy drugs, it is generally considered to be incurable. Although some cancers tend to occur more often in one gender or the other, cases and deaths due to SLL are evenly split between men and women. The average age at the time of diagnosis is 60 years.

[00214] Although SLL is indolent, it is persistently progressive. The usual pattern of this disease is one of high response rates to radiation therapy and/or chemotherapy, with a period of disease remission. This is followed months or years later by an inevitable relapse. Re-treatment leads to a response again, but again the disease will relapse. This means that although the short-term prognosis of SLL is quite good, over time, many patients develop fatal complications of recurrent disease. Considering the age of the individuals typically diagnosed with CLL and SLL, there is a need in the art for a simple and effective treatment of the disease with minimum side-effects that do not impede on the patient's quality of life. The instant invention fulfills this long standing need in the art.

Mantle Cell Lymphoma

[00215] Disclosed herein, in certain embodiments, is a method for treating a Mantle cell lymphoma 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, the second treatment is temsirolimus.

[00216] As used herein, the term, "Mantle cell lymphoma" refers to a subtype of B-cell lymphoma, due to CD5 positive antigen-naive pregerminal center B-cell within the mantle zone that surrounds normal germinal center follicles. MCL cells generally over-express cyclin D1 due to a t(11:14) chromosomal translocation in the DNA. More specifically, the translocation is at

t(11;14)(q13;q32). Only about 5% of lymphomas are of this type. The cells are small to medium in size. Men are affected most often. The average age of patients is in the early 60s. The lymphoma is usually widespread when it is diagnosed, involving lymph nodes, bone marrow, and, very often, the spleen. Mantle cell lymphoma is not a very fast growing lymphoma, but is difficult to treat.

Marginal Zone B-cell Lymphoma

[00217] Disclosed herein, in certain embodiments, is a method for treating a marginal zone B-cell lymphoma 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the

method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time.

[00218] As used herein, the term "marginal zone B-cell lymphoma" refers to a group of related B-cell neoplasms that involve the lymphoid tissues in the marginal zone, the patchy area outside the follicular mantle zone. Marginal zone lymphomas account for about 5% to 10% of lymphomas. The cells in these lymphomas look small under the microscope. There are 3 main types of marginal zone lymphomas including extranodal marginal zone B-cell lymphomas, nodal marginal zone B-cell lymphoma, and splenic marginal zone lymphoma.

MALT

[00219] Disclosed herein, in certain embodiments, is a method for treating a MALT 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before

administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time.

[00220] The term "mucosa-associated lymphoid tissue (MALT) lymphoma", as used herein, refers to extranodal manifestations of marginal-zone lymphomas. Most MALT lymphoma are a low grade, although a minority either manifest initially as intermediate-grade non-Hodgkin lymphoma (NHL) or evolve from the low-grade form. Most of the MALT lymphoma occur in the stomach, and roughly 70% of gastric MALT lymphoma are associated with Helicobacter pylori infection. Several cytogenetic abnormalities have been identified, the most common being trisomy 3 or t(11;18). Many of these other MALT lymphoma have also been linked to infections with bacteria or viruses. The average age of patients with MALT lymphoma is about 60.

Nodal Marginal Zone B-Cell Lymphoma

[00221] Disclosed herein, in certain embodiments, is a method for treating a nodal marginal zone B-cell lymphoma 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before

administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time.

[00222] The term "nodal marginal zone B-cell lymphoma" refers to an indolent B-cell lymphoma that is found mostly in the lymph nodes. The disease is rare and only accounts for 1% of all Non-Hodgkin's Lymphomas (NHL). It is most commonly diagnosed in older patients, with women more susceptible than men. The disease is classified as a marginal zone lymphoma because the mutation occurs in the marginal zone of the B-cells. Due to its confinement in the lymph nodes, this disease is also classified as nodal.

Splenic Marginal Zone B-Cell Lymphoma

[00223] Disclosed herein, in certain embodiments, is a method for treating a splenic marginal zone B-cell lymphoma 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized

plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time.

[00224] The term "splenic marginal zone B-cell lymphoma" refers to specific low-grade small B-cell lymphoma that is incorporated in the World Health Organization classification. Characteristic features are splenomegaly, moderate lymphocytosis with villous morphology, intrasinusoidal pattern of involvement of various organs, especially bone marrow, and relative indolent course. Tumor progression with increase of blastic forms and aggressive behavior are observed in a minority of patients. Molecular and cytogenetic studies have shown heterogeneous results probably because of the lack of standardized diagnostic criteria.

Burkitt Lymphoma

[00225] Disclosed herein, in certain embodiments, is a method for treating a Burkitt lymphoma 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments,

analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time.

[00226] The term "Burkitt lymphoma" refers to a type of Non-Hodgkin Lymphoma (NHL) that commonly affects children. It is a highly aggressive type of B-cell lymphoma that often starts and involves body parts other than lymph nodes. In spite of its fast-growing nature, Burkitt's lymphoma is often curable with modern intensive therapies. There are two broad types of Burkitt's lymphoma – the sporadic and the endemic varieties:

[00227] Endemic Burkitt's lymphoma: The disease involves children much more than adults, and is related to Epstein Barr Virus (EBV) infection in 95% cases. It occurs primarily is equatorial Africa, where about half of all childhood cancers are Burkitt's lymphoma. It characteristically has a high chance of involving the jawbone, a rather distinctive feature that is rare in sporadic Burkitt's. It also commonly involves the abdomen.

[00228] Sporadic Burkitt's lymphoma: The type of Burkitt's lymphoma that affects the rest of the world, including Europe and the Americas is the sporadic type. Here too, it's mainly a disease in children. The link between Epstein Barr Virus (EBV) is not as strong as with the endemic variety, though direct evidence of EBV infection is present in one out of five patients. More than the involvement of lymph nodes, it is the abdomen that is notably affected in more than 90% of the children. Bone marrow involvement is more common than in the sporadic variety.

Waldenström Macroglobulinemia

[00229] Disclosed herein, in certain embodiments, is a method for treating a Waldenström macroglobulinemia 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time. In some embodiments, the second treatment is rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone (R-CHOP).

[00230] The term "Waldenström macroglobulinemia", also known as lymphoplasmacytic lymphoma, is cancer involving a subtype of white blood cells called lymphocytes. It is characterized by an uncontrolled clonal proliferation of terminally differentiated B lymphocytes. It is also characterized by the lymphoma cells making an antibody called immunoglobulin M (IgM). The IgM antibodies circulate in the blood in large amounts, and cause the liquid part of the blood to thicken, like syrup. This can lead to decreased blood flow to many organs, which can cause problems with vision (because of poor circulation in blood vessels in the back of the eyes) and neurological problems (such as headache, dizziness, and confusion) caused by poor blood flow within the brain. Other symptoms can include feeling tired and weak, and a tendency

to bleed easily. The underlying etiology is not fully understood but a number of risk factors have been identified, including the locus 6p21.3 on chromosome 6. There is a 2- to 3-fold risk increase of developing WM in people with a personal history of autoimmune diseases with autoantibodies and particularly elevated risks associated with hepatitis, human immunodeficiency virus, and rickettsiosis.

Multiple Myeloma

[00231] Disclosed herein, in certain embodiments, is a method for treating a myeloma 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the

peripheral blood has increased for a predetermined length of time. In some embodiments, the second treatment is lenalidomide.

[00232] Multiple myeloma, also known as MM, myeloma, plasma cell myeloma, or as Kahler's disease (after Otto Kahler) is a cancer of the white blood cells known as plasma cells. A type of B cell, plasma cells are a crucial part of the immune system responsible for the production of antibodies in humans and other vertebrates. They are produced in the bone marrow and are transported through the lymphatic system.

Leukemia

[00233] Disclosed herein, in certain embodiments, is a method for treating a leukemia 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the concentration before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before

administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time.

[00234] Leukemia is a cancer of the blood or bone marrow characterized by an abnormal increase of blood cells, usually leukocytes (white blood cells). Leukemia is a broad term covering a spectrum of diseases. The first division is between its acute and chronic forms: (i) acute leukemia is characterized by the rapid increase of immature blood cells. This crowding makes the bone marrow unable to produce healthy blood cells. Immediate treatment is required in acute leukemia due to the rapid progression and accumulation of the malignant cells, which then spill over into the bloodstream and spread to other organs of the body. Acute forms of leukemia are the most common forms of leukemia in children; (ii) chronic leukemia is distinguished by the excessive build up of relatively mature, but still abnormal, white blood cells. Typically taking months or years to progress, the cells are produced at a much higher rate than normal cells, resulting in many abnormal white blood cells in the blood. Chronic leukemia mostly occurs in older people, but can theoretically occur in any age group. Additionally, the diseases are subdivided according to which kind of blood cell is affected. This split divides leukemias into lymphoblastic or lymphocytic leukemias and myeloid or myelogenous leukemias: (i) lymphoblastic or lymphocytic leukemias, the cancerous change takes place in a type of marrow cell that normally goes on to form lymphocytes, which are infection-fighting immune system cells; (ii) myeloid or myelogenous leukemias, the cancerous change takes place in a type of marrow cell that normally goes on to form red blood cells, some other types of white cells, and platelets.

[00235] Within these main categories, there are several subcategories including, but not limited to, Acute lymphoblastic leukemia (ALL), Acute myelogenous leukemia (AML), Chronic myelogenous leukemia (CML), and Hairy cell leukemia (HCL).

Btk inhibitors

[00236] Also presented herein are methods for treating a cancer such as by way of example only, a BCLD, in a subject wherein the subject has been treated with a dosing of a Btk inhibitor. In the following description of irreversible Btk compounds suitable for use in the methods described herein, definitions of referred-to standard chemistry terms may be found in reference works (if not otherwise defined herein), including Carey and Sundberg "Advanced Organic Chemistry 4th Ed." Vols. A (2000) and B (2001), Plenum Press, New York. Unless otherwise indicated, conventional methods of mass spectroscopy, NMR, HPLC, protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the ordinary

skill of the art are employed. In addition, nucleic acid and amino acid sequences for Btk (e.g., human Btk) are known in the art as disclosed in, e.g., U.S. Patent No. 6,326,469. Unless specific definitions are provided, the nomenclature employed in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those known in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[00237] The Btk inhibitor compounds described herein are selective for Btk and kinases having a cysteine residue in an amino acid sequence position of the tyrosine kinase that is homologous to the amino acid sequence position of cysteine 481 in Btk. Generally, an irreversible inhibitor compound of Btk used in the methods described herein is identified or characterized in an *in vitro* assay, e.g., an acellular biochemical assay or a cellular functional assay. Such assays are useful to determine an *in vitro* IC_{50} for an irreversible Btk inhibitor compound.

[00238] For example, an acellular kinase assay can be used to determine Btk activity after incubation of the kinase in the absence or presence of a range of concentrations of a candidate irreversible Btk inhibitor compound. If the candidate compound is in fact an irreversible Btk inhibitor, Btk kinase activity will not be recovered by repeat washing with inhibitor-free medium. See, e.g., J. B. Smaill, *et al.* (1999), *J. Med. Chem.*, 42(10):1803-1815. Further, covalent complex formation between Btk and a candidate irreversible Btk inhibitor is a useful indicator of irreversible inhibition of Btk that can be readily determined by a number of methods known in the art (e.g., mass spectrometry). For example, some irreversible Btk-inhibitor compounds can form a covalent bond with Cys 481 of Btk (e.g., via a Michael reaction).

[00239] Cellular functional assays for Btk inhibition include measuring one or more cellular endpoints in response to stimulating a Btk-mediated pathway in a cell line (e.g., BCR activation in Ramos cells) in the absence or presence of a range of concentrations of a candidate irreversible Btk inhibitor compound. Useful endpoints for determining a response to BCR activation include, e.g., autophosphorylation of Btk, phosphorylation of a Btk target protein (e.g., PLC-γ), and cytoplasmic calcium flux.

[00240] High throughput assays for many acellular biochemical assays (e.g., kinase assays) and cellular functional assays (e.g., calcium flux) are well known to those of ordinary skill in the art. In addition, high throughput screening systems are commercially available (see, e.g., Zymark Corp., Hopkinton, MA; Air Technical Industries, Mentor, OH; Beckman Instruments, Inc. Fullerton, CA; Precision Systems, Inc., Natick, MA, etc.). These systems typically automate entire procedures including all sample and reagent pipetting, liquid

dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate for the assay. Automated systems thereby allow the identification and characterization of a large number of irreversible Btk compounds without undue effort.

[00241] In some embodiments, the Btk inhibitor is selected from the group consisting of a small organic molecule, a macromolecule, a peptide or a non-peptide.

[00242] In some embodiments, the Btk inhibitor provided herein is a reversible or irreversible inhibitor. In certain embodiments, the Btk inhibitor is an irreversible inhibitor.

[00243] In some embodiments, the irreversible Btk inhibitor forms a covalent bond with a cysteine sidechain of a Bruton's tyrosine kinase, a Bruton's tyrosine kinase homolog, or a Btk tyrosine kinase cysteine homolog.

[00244] Irreversible Btk inhibitor compounds can use for the manufacture of a medicament for treating any of the foregoing conditions (e.g., autoimmune diseases, inflammatory diseases, allergy disorders, B-cell proliferative disorders, or thromboembolic disorders).

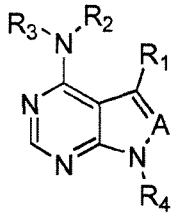
[00245] In some embodiments, the irreversible Btk inhibitor compound used for the methods described herein inhibits Btk or a Btk homolog kinase activity with an *in vitro* IC_{50} of less than 10 μM. (e.g., less than 1 μM, less than 0.5 μM, less than 0.4 μM, less than 0.3 μM, less than 0.1, less than 0.08 μM, less than 0.06 μM, less than 0.05 μM, less than 0.04 μM, less than 0.03 μM, less than less than 0.02 μM, less than 0.01, less than 0.008 μM, less than 0.006 μM, less than 0.005 μM, less than 0.004 μM, less than 0.003 μM, less than less than 0.002 μM, less than 0.001, less than 0.00099 μM, less than 0.00098 μM, less than 0.00097 μM, less than 0.00096 μM, less than 0.00095 μM, less than 0.00094 μM, less than 0.00093 μM, less than 0.00092, or less than 0.00090 μM).

[00246] In one embodiment, the irreversible Btk inhibitor compound selectively and irreversibly inhibits an activated form of its target tyrosine kinase (e.g., a phosphorylated form of the tyrosine kinase). For example, activated Btk is transphosphorylated at tyrosine 551. Thus, in these embodiments the irreversible Btk inhibitor inhibits the target kinase in cells only once the target kinase is activated by the signaling events.

[00247] In other embodiments, the Btk inhibitor used in the methods describe herein has the structure of any of Formula (A), Formula (B), Formula (C), Formula (D), Formula (E), or Formula (F). Also described herein are pharmaceutically acceptable salts, pharmaceutically acceptable solvates, pharmaceutically active metabolites, and pharmaceutically acceptable prodrugs of such compounds. Pharmaceutical compositions that include at least one such compound or a pharmaceutically acceptable salt, pharmaceutically acceptable solvate, pharmaceutically active metabolite or pharmaceutically acceptable prodrug of such compound,

are provided. In some embodiments, when compounds disclosed herein contain an oxidizable nitrogen atom, the nitrogen atom can be converted to an N-oxide by methods well known in the art. In certain embodiments, isomers and chemically protected forms of compounds having a structure represented by any of Formula (A), Formula (B), Formula (C), Formula (D), Formula (E), or Formula (F), are also provided.

[00248] Formula (A) is as follows:



Formula (A)

wherein:

A is independently selected from N or CR_5;

R_1 is H, L_2-(substituted or unsubstituted alkyl), L_2-(substituted or unsubstituted cycloalkyl), L_2-(substituted or unsubstituted alkenyl), L_2-(substituted or unsubstituted cycloalkenyl), L_2-(substituted or unsubstituted heterocycle), L_2-(substituted or unsubstituted heteroaryl), or L_2-(substituted or unsubstituted aryl), where L_2 is a bond, O, S, -S(=O), $-S(=O)_2$, C(=O), -(substituted or unsubstituted C_1-C_6 alkyl), or -(substituted or unsubstituted C_2-C_6 alkenyl);

R_2 and R_3 are independently selected from H, lower alkyl and substituted lower alkyl;

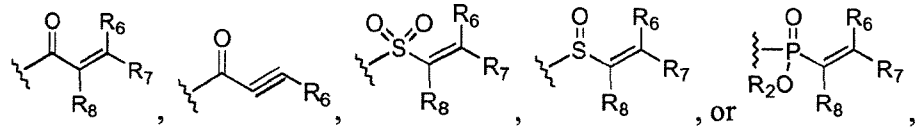
R_4 is L_3-X-L_4-G, wherein,

L_3 is optional, and when present is a bond, optionally substituted or unsubstituted alkyl, optionally substituted or unsubstituted cycloalkyl, optionally substituted or unsubstituted alkenyl, optionally substituted or unsubstituted alkynyl;

X is optional, and when present is a bond, O, -C(=O), S, -S(=O), $-S(=O)_2$, -NH, $-NR_9$, -NHC(O), -C(O)NH, $-NR_9C(O)$, $-C(O)NR_9$, $-S(=O)_2NH$, $-NHS(=O)_2$, $-S(=O)_2NR_9$-, $-NR_9S(=O)_2$, -OC(O)NH-, -NHC(O)O-, $-OC(O)NR_9$-, $-NR_9C(O)O$-, -CH=NO-, -ON=CH-, $-NR_{10}C(O)NR_{10}$-, heteroaryl, aryl, $-NR_{10}C(=NR_{11})NR_{10}$-, $-NR_{10}C(=NR_{11})$-, $-C(=NR_{11})NR_{10}$-, $-OC(=NR_{11})$-, or $-C(=NR_{11})O$-;

L_4 is optional, and when present is a bond, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycle;

or L_3, X and L_4 taken together form a nitrogen containing heterocyclic ring;

G is , wherein,

R_6, R_7 and R_8 are independently selected from among H, lower alkyl or substituted lower alkyl, lower heteroalkyl or substituted lower heteroalkyl, substituted or unsubstituted lower cycloalkyl, and substituted or unsubstituted lower heterocycloalkyl;

R_5 is H, halogen, -L_6-(substituted or unsubstituted C_1-C_3 alkyl), -L_6-(substituted or unsubstituted C_2-C_4 alkenyl), -L_6-(substituted or unsubstituted heteroaryl), or –L_6-(substituted or unsubstituted aryl), wherein L_6 is a bond, O, S, -S(=O), $S(=O)_2$, NH, C(O), -NHC(O)O, -OC(O)NH, -NHC(O), or -C(O)NH;

each R_9 is independently selected from among H, substituted or unsubstituted lower alkyl, and substituted or unsubstituted lower cycloalkyl;

each R_{10} is independently H, substituted or unsubstituted lower alkyl, or substituted or unsubstituted lower cycloalkyl; or

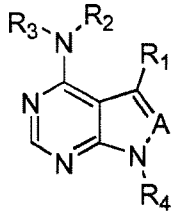
two R_{10} groups can together form a 5-, 6-, 7-, or 8-membered heterocyclic ring; or

R_9 and R_{10} can together form a 5-, 6-, 7-, or 8-membered heterocyclic ring; or

each R_{11} is independently selected from H, $–S(=O)_2R_8$, $–S(=O)_2NH_2$, -C(O)R_8, -CN, -NO_2, heteroaryl, or heteroalkyl; and

pharmaceutically active metabolites, pharmaceutically acceptable solvates, pharmaceutically acceptable salts, or pharmaceutically acceptable prodrugs thereof.

[00249] In one aspect are compounds having the structure of Formula (A1):



Formula (A1),

wherein

A is independently selected from N or CR_5;

R_1 is H, L_2-(substituted or unsubstituted alkyl), L_2-(substituted or unsubstituted cycloalkyl), L_2-(substituted or unsubstituted alkenyl), L_2-(substituted or unsubstituted cycloalkenyl), L_2-(substituted or unsubstituted heterocycle), L_2-(substituted or unsubstituted heteroaryl), or L_2-(substituted or unsubstituted aryl), where L_2 is a bond, O, S, -S(=O), -$S(=O)_2$, C(=O), -(substituted or unsubstituted C_1-C_6 alkyl), or -(substituted or unsubstituted C_2-C_6 alkenyl);

R_2 and R_3 are independently selected from H, lower alkyl and substituted lower alkyl;

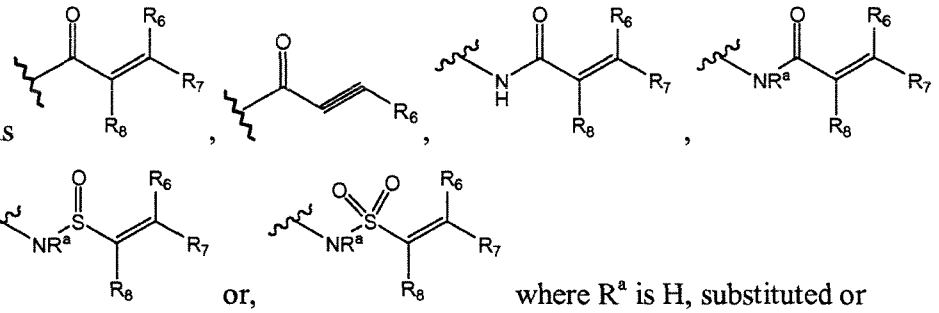
R_4 is L_3-X-L_4-G, wherein,

L_3 is optional, and when present is a bond, or an optionally substituted group selected from alkyl, heteroalkyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, or alkylheterocycloalkyl;

X is optional, and when present is a bond, O, -C(=O), S, -S(=O), -$S(=O)_2$, -NH, -NR_9, -NHC(O), -C(O)NH, -NR_9C(O), -C(O)NR_9, -$S(=O)_2$NH, -$NHS(=O)_2$, -$S(=O)_2NR_9$-, -$NR_9S(=O)_2$, -OC(O)NH-, -NHC(O)O-, -OC(O)NR_9-, -NR_9C(O)O-, -CH=NO-, -ON=CH-, -$NR_{10}C(O)NR_{10}$-, heteroaryl, aryl, -$NR_{10}C(=NR_{11})NR_{10}$-, -$NR_{10}C(=NR_{11})$-, -$C(=NR_{11})NR_{10}$-, -$OC(=NR_{11})$-, or -$C(=NR_{11})$O-;

L_4 is optional, and when present is a bond, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycle;

or L_3, X and L_4 taken together form a nitrogen containing heterocyclic ring, or an optionally substituted group selected from alkyl, heteroalkyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, or alkylheterocycloalkyl;

G is , where R^a is H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl; and either

R_7 and R_8 are H;

R_6 is H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_8alkylaminoalkyl, C_1-C_8hydroxyalkylaminoalkyl, C_1-C_8alkoxyalkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_1-C_8alkylC_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted C_2-C_8heterocycloalkyl, substituted or unsubstituted heteroaryl, C_1-C_4alkyl(aryl), C_1-C_4alkyl(heteroaryl), C_1-C_8alkylethers, C_1-C_8alkylamides, or C_1-C_4alkyl(C_2-C_8heterocycloalkyl);

R_6 and R_8 are H;

R_7 is H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_8alkylaminoalkyl, C_1-C_8hydroxyalkylaminoalkyl, C_1-

C_8alkoxyalkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_1-C_8alkylC_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted C_2-C_8heterocycloalkyl, substituted or unsubstituted heteroaryl, C_1-C_4alkyl(aryl), C_1-C_4alkyl(heteroaryl), C_1-C_8alkylethers, C_1-C_8alkylamides, or C_1-C_4alkyl(C_2-C_8heterocycloalkyl); or

R_6 and R_8 taken together form a bond;

R_7 is H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_8alkylaminoalkyl, C_1-C_8hydroxyalkylaminoalkyl, C_1-C_8alkoxyalkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_1-C_8alkylC_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted C_2-C_8heterocycloalkyl, substituted or unsubstituted heteroaryl, C_1-C_4alkyl(aryl), C_1-C_4alkyl(heteroaryl), C_1-C_8alkylethers, C_1-C_8alkylamides, or C_1-C_4alkyl(C_2-C_8heterocycloalkyl); or

R_5 is H, halogen, -L_6-(substituted or unsubstituted C_1-C_3 alkyl), -L_6-(substituted or unsubstituted C_2-C_4 alkenyl), -L_6-(substituted or unsubstituted heteroaryl), or –L_6-(substituted or unsubstituted aryl), wherein L_6 is a bond, O, S, -S(=O), $S(=O)_2$, NH, C(O), -NHC(O)O, -OC(O)NH, -NHC(O), or -C(O)NH;

each R_9 is independently selected from among H, substituted or unsubstituted lower alkyl, and substituted or unsubstituted lower cycloalkyl;

each R_{10} is independently H, substituted or unsubstituted lower alkyl, or substituted or unsubstituted lower cycloalkyl; or

two R_{10} groups can together form a 5-, 6-, 7-, or 8-membered heterocyclic ring; or

R_9 and R_{10} can together form a 5-, 6-, 7-, or 8-membered heterocyclic ring; or

each R_{11} is independently selected from H, $–S(=O)_2R_8$, $–S(=O)_2NH_2$, -C(O)R_8, -CN, -NO_2, heteroaryl, or heteroalkyl; and pharmaceutically active metabolites, pharmaceutically acceptable solvates, pharmaceutically acceptable salts, or pharmaceutically acceptable prodrugs thereof.

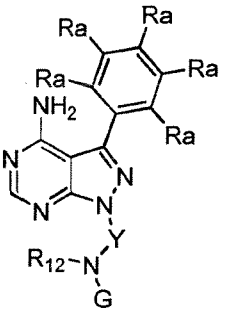
[00250] In another embodiment are provided pharmaceutically acceptable salts of compounds of Formula (A1). By way of example only, are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid. Further salts include those in which the counterion is an anion, such as adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate,

gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, and valerate. Further salts include those in which the counterion is an cation, such as sodium, lithium, potassium, calcium, magnesium, ammonium, and quaternary ammonium (substituted with at least one organic moiety) cations.

[00251] In another embodiment are pharmaceutically acceptable esters of compounds of Formula (A1), including those in which the ester group is selected from a formate, acetate, propionate, butyrate, acrylate and ethylsuccinate.

[00252] In another embodiment are pharmaceutically acceptable carbamates of compounds of Formula (A1). In another embodiment are pharmaceutically acceptable N-acyl derivatives of compounds of Formula (A1). Examples of N-acyl groups include N-acetyl and N-ethoxycarbonyl groups.

[00253] In a further embodiment, the compound of Formula (A) has the following structure of Formula (B):

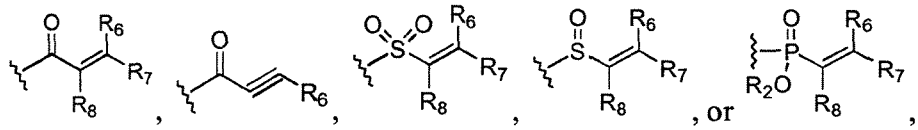


Formula (B)

wherein:

Y is alkyl or substituted alkyl, or a 4-, 5-, or 6-membered cycloalkyl ring;

each R_a is independently H, halogen, $-CF_3$, -CN, $-NO_2$, OH, NH_2, $-L_a$-(substituted or unsubstituted alkyl), $-L_a$-(substituted or unsubstituted alkenyl), $-L_a$-(substituted or unsubstituted heteroaryl), or $-L_a$-(substituted or unsubstituted aryl), wherein L_a is a bond, O, S, -S(=O), $-S(=O)_2$, NH, C(O), CH_2, -NHC(O)O, -NHC(O), or -C(O)NH;

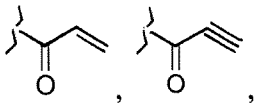
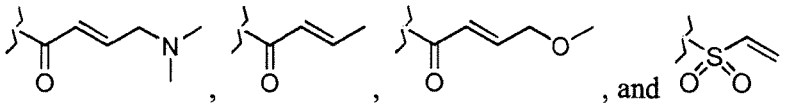
G is , wherein,

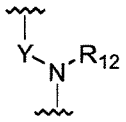
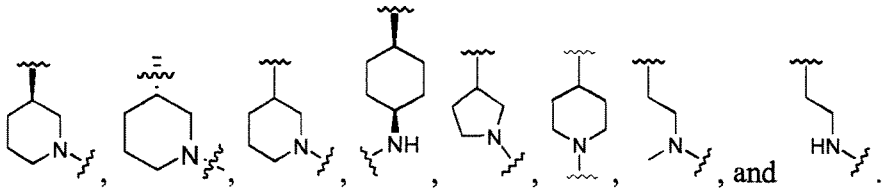
R_6, R_7 and R_8 are independently selected from among H, lower alkyl or substituted lower alkyl, lower heteroalkyl or substituted lower heteroalkyl, substituted or unsubstituted lower cycloalkyl, and substituted or unsubstituted lower heterocycloalkyl;

R_{12} is H or lower alkyl; or

Y and R_{12} taken together form a 4-, 5-, or 6-membered heterocyclic ring; and

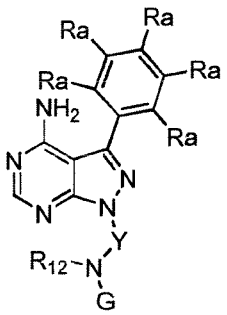
pharmaceutically acceptable active metabolites, pharmaceutically acceptable solvates, pharmaceutically acceptable salts, or pharmaceutically acceptable prodrugs thereof.

[00254] In further embodiments, G is selected from among , , and .

[00255] In further embodiments,  is selected from among .

[00256]

[00257] In a further embodiment, the compound of Formula (A1) has the following structure of Formula (B1):

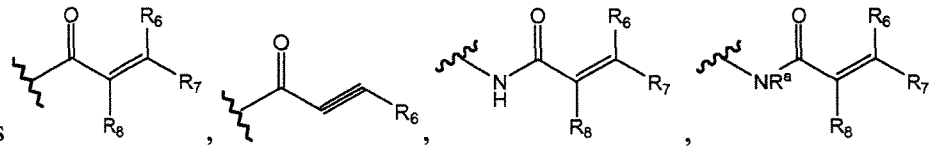


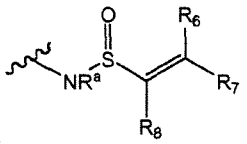
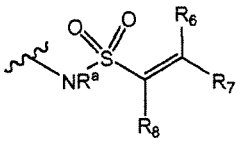
Formula (B1),

wherein:

Y is an optionally substituted group selected from among alkylene, heteroalkylene, arylene, heteroarylene, alkylenearylene, alkyleneheteroarylene, and alkyleneheterocycloalkylene;

each R_a is independently H, halogen, $-CF_3$, -CN, $-NO_2$, OH, NH_2, $-L_a$-(substituted or unsubstituted alkyl), $-L_a$-(substituted or unsubstituted alkenyl), $-L_a$-(substituted or unsubstituted heteroaryl), or $-L_a$-(substituted or unsubstituted aryl), wherein L_a is a bond, O, S, -S(=O), $-S(=O)_2$, NH, C(O), CH_2, -NHC(O)O, -NHC(O), or -C(O)NH;

G is .


, or,  where R^a is H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl; and either

R_7 and R_8 are H;

R_6 is H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_8alkylaminoalkyl, C_1-C_8hydroxyalkylaminoalkyl, C_1-C_8alkoxyalkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_1-C_8alkylC_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted C_2-C_8heterocycloalkyl, substituted or unsubstituted heteroaryl, C_1-C_4alkyl(aryl), C_1-C_4alkyl(heteroaryl), C_1-C_8alkylethers, C_1-C_8alkylamides, or C_1-C_4alkyl(C_2-C_8heterocycloalkyl);

R_6 and R_8 are H;

R_7 is H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_8alkylaminoalkyl, C_1-C_8hydroxyalkylaminoalkyl, C_1-C_8alkoxyalkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_1-C_8alkylC_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted C_2-C_8heterocycloalkyl, substituted or unsubstituted heteroaryl, C_1-C_4alkyl(aryl), C_1-C_4alkyl(heteroaryl), C_1-C_8alkylethers, C_1-C_8alkylamides, or C_1-C_4alkyl(C_2-C_8heterocycloalkyl); or

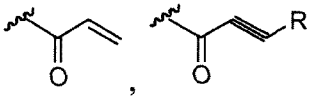
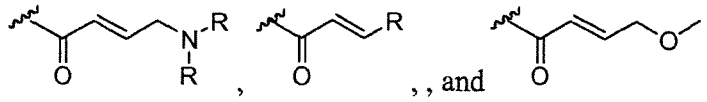
R_6 and R_8 taken together form a bond;

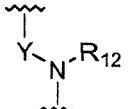
R_7 is H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_8alkylaminoalkyl, C_1-C_8hydroxyalkylaminoalkyl, C_1-C_8alkoxyalkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_1-C_8alkylC_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted C_2-C_8heterocycloalkyl, substituted or unsubstituted heteroaryl, C_1-C_4alkyl(aryl), C_1-C_4alkyl(heteroaryl), C_1-C_8alkylethers, C_1-C_8alkylamides, or C_1-C_4alkyl(C_2-C_8heterocycloalkyl);

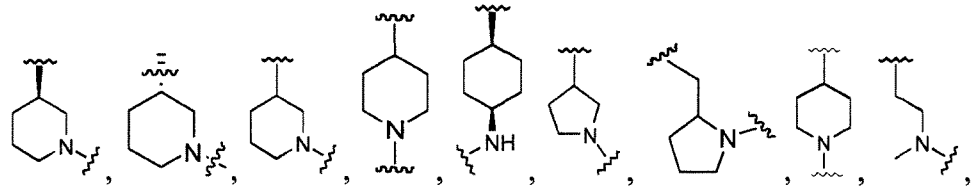
R_{12} is H or lower alkyl; or

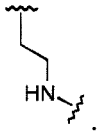
Y and R_{12} taken together form a 4-, 5-, or 6-membered heterocyclic ring; and

pharmaceutically acceptable active metabolites, pharmaceutically acceptable solvates, pharmaceutically acceptable salts, or pharmaceutically acceptable prodrugs thereof.

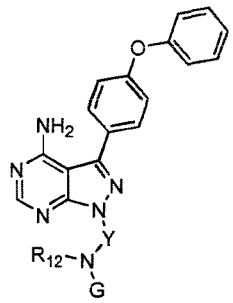
[00258] In further embodiments, G is selected from among , , , , and ., where R is H, alkyl, alkylhydroxy, heterocycloalkyl, heteroaryl, alkylalkoxy, alkylalkoxyalkyl.

[00259] In further embodiments,  is selected from among

[00260] 

and .

[00261] In a further embodiment, the compound of Formula (B) has the following structure of Formula (C):

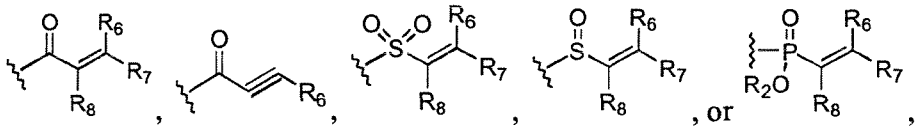


Formula (C)

Y is alkyl or substituted alkyl, or a 4-, 5-, or 6-membered cycloalkyl ring;

R_{12} is H or lower alkyl; or

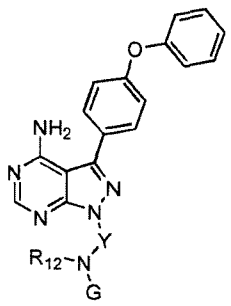
Y and R_{12} taken together form a 4-, 5-, or 6-membered heterocyclic ring;

G is , wherein,

R_6, R_7 and R_8 are independently selected from among H, lower alkyl or substituted lower alkyl, lower heteroalkyl or substituted lower heteroalkyl, substituted or unsubstituted lower cycloalkyl, and substituted or unsubstituted lower heterocycloalkyl; and

pharmaceutically acceptable active metabolites, pharmaceutically acceptable solvates, pharmaceutically acceptable salts, or pharmaceutically acceptable prodrugs thereof.

[00262] In further embodiment, the compound of Formula (B1) has the following structure of Formula (C1):

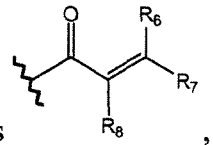
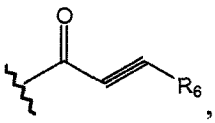
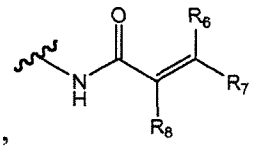
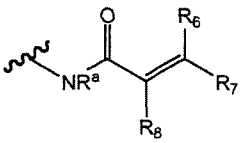
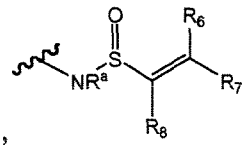
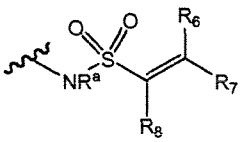


Formula (C1),

Y is an optionally substituted group selected from among alkyl, heteroalkyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, and alkylheterocycloalkyl;

R_{12} is H or lower alkyl; or

Y and R_{12} taken together form a 4-, 5-, or 6-membered heterocyclic ring;

G is , , , , , , where R^a is H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl; and either

R_7 and R_8 are H;

R_6 is H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_8alkylaminoalkyl, C_1-C_8hydroxyalkylaminoalkyl, C_1-C_8alkoxyalkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_1-C_8alkylC_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted C_2-C_8heterocycloalkyl, substituted or unsubstituted heteroaryl, C_1-C_4alkyl(aryl), C_1-C_4alkyl(heteroaryl), C_1-C_8alkylethers, C_1-C_8alkylamides, or C_1-C_4alkyl(C_2-C_8heterocycloalkyl);

R_6 and R_8 are H;

R_7 is H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_8alkylaminoalkyl, C_1-C_8hydroxyalkylaminoalkyl, C_1-C_8alkoxyalkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_1-C_8alkylC_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted C_2-C_8heterocycloalkyl, substituted or unsubstituted heteroaryl, C_1-

C_4alkyl(aryl), C_1-C_4alkyl(heteroaryl), C_1-C_8alkylethers, C_1-C_8alkylamides, or C_1-C_4alkyl(C_2-C_8heterocycloalkyl); or

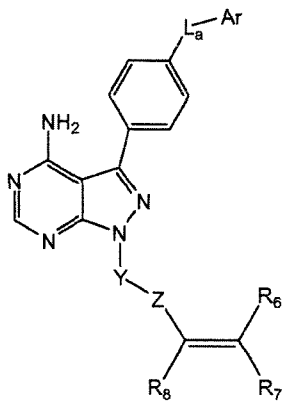
R_6 and R_8 taken together form a bond;

R_7 is H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_8alkylaminoalkyl, C_1-C_8hydroxyalkylaminoalkyl, C_1-C_8alkoxyalkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_1-C_8alkylC_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted C_2-C_8heterocycloalkyl, substituted or unsubstituted heteroaryl, C_1-C_4alkyl(aryl), C_1-C_4alkyl(heteroaryl), C_1-C_8alkylethers, C_1-C_8alkylamides, or C_1-C_4alkyl(C_2-C_8heterocycloalkyl); and

pharmaceutically acceptable active metabolites, pharmaceutically acceptable solvates, pharmaceutically acceptable salts, or pharmaceutically acceptable prodrugs thereof.

[00263] In a further or alternative embodiment, the "G" group of any of Formula (A1), Formula (B1), or Formula (C1) is any group that is used to tailor the physical and biological properties of the molecule. Such tailoring/modifications are achieved using groups which modulate Michael acceptor chemical reactivity, acidity, basicity, lipophilicity, solubility and other physical properties of the molecule. The physical and biological properties modulated by such modifications to G include, by way of example only, enhancing chemical reactivity of Michael acceptor group, solubility, *in vivo* absorption, and *in vivo* metabolism. In addition, *in vivo* metabolism includes, by way of example only, controlling *in vivo* PK properties, off-target activities, potential toxicities associated with cypP450 interactions, drug-drug interactions, and the like. Further, modifications to G allow for the tailoring of the *in vivo* efficacy of the compound through the modulation of, by way of example, specific and non-specific protein binding to plasma proteins and lipids and tissue distribution *in vivo*.

[00264] In another embodiment, provided herein is a compound of Formula (D). Formula (D) is as follows:



Formula (D)

wherein:

L_a is CH_2, O, NH or S;

Ar is a substituted or unsubstituted aryl, or a substituted or unsubstituted heteroaryl;

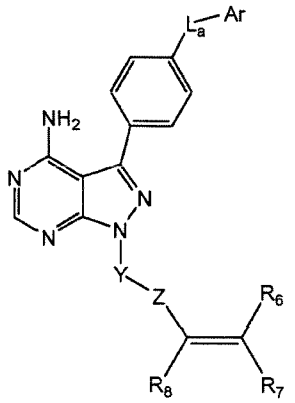
Y is an optionally substituted group selected from among alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl;

Z is C(=O), OC(=O), NHC(=O), C(=S), $S(=O)_x$, $OS(=O)_x$, $NHS(=O)_x$, where x is 1 or 2;

R_6, R_7, and R_8 are each independently selected from among H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_2-C_6heterocycloalkyl, C_1-C_6alkoxyalkyl, C_1-C_8alkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted C_1-C_4alkyl(aryl), substituted or unsubstituted C_1-C_4alkyl(heteroaryl), substituted or unsubstituted C_1-C_4alkyl(C_3-C_8cycloalkyl), or substituted or unsubstituted C_1-C_4alkyl(C_2-C_8heterocycloalkyl); or

R_7 and R_8 taken together form a bond; and pharmaceutically active metabolites, or pharmaceutically acceptable solvates, pharmaceutically acceptable salts, or pharmaceutically acceptable prodrugs thereof.

[00265] In one embodiment are compounds having the structure of Formula (D1):



Formula (D1)

wherein

L_a is CH_2, O, NH or S;

Ar is an optionally substituted aromatic carbocycle or an aromatic heterocycle;

Y is an optionally substituted group selected from among alkylene, heteroalkylene, arylene, heteroarylene, alkylenearylene, alkyleneheteroarylene, and alkyleneheterocycloalkylene, or combination thereof;

Z is C(=O), NHC(=O), $NR^aC(=O)$, $NR^aS(=O)_x$, where x is 1 or 2, and R^a is H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl; and either

R_7 and R_8 are H;

R_6 is H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_8alkylaminoalkyl, C_1-C_8hydroxyalkylaminoalkyl, C_1-C_8alkoxyalkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_1-C_8alkylC_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted C_2-C_8heterocycloalkyl, substituted or unsubstituted heteroaryl, C_1-C_4alkyl(aryl), C_1-C_4alkyl(heteroaryl), C_1-C_8alkylethers, C_1-C_8alkylamides, or C_1-C_4alkyl(C_2-C_8heterocycloalkyl);

R_6 and R_8 are H;

R_7 is H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_8alkylaminoalkyl, C_1-C_8hydroxyalkylaminoalkyl, C_1-C_8alkoxyalkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_1-C_8alkylC_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted C_2-C_8heterocycloalkyl, substituted or unsubstituted heteroaryl, C_1-C_4alkyl(aryl), C_1-C_4alkyl(heteroaryl), C_1-C_8alkylethers, C_1-C_8alkylamides, or C_1-C_4alkyl(C_2-C_8heterocycloalkyl); or

R_6 and R_8 taken together form a bond;

R_7 is H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_8alkylaminoalkyl, C_1-C_8hydroxyalkylaminoalkyl, C_1-C_8alkoxyalkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_1-C_8alkylC_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted C_2-C_8heterocycloalkyl, substituted or unsubstituted heteroaryl, C_1-C_4alkyl(aryl), C_1-C_4alkyl(heteroaryl), C_1-C_8alkylethers, C_1-C_8alkylamides, or C_1-C_4alkyl(C_2-C_8heterocycloalkyl);

or combinations thereof; and

pharmaceutically active metabolites, or pharmaceutically acceptable solvates, pharmaceutically acceptable salts, or pharmaceutically acceptable prodrugs thereof.

[00266] In another embodiment are provided pharmaceutically acceptable salts of compounds of Formula (D1). By way of example only, are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid. Further salts include those in which the counterion is an anion, such as adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate,

lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, and valerate. Further salts include those in which the counterion is an cation, such as sodium, lithium, potassium, calcium, magnesium, ammonium, and quaternary ammonium (substituted with at least one organic moiety) cations.

[00267] In another embodiment are pharmaceutically acceptable esters of compounds of Formula (D1), including those in which the ester group is selected from a formate, acetate, propionate, butyrate, acrylate and ethylsuccinate.

[00268] In another embodiment are pharmaceutically acceptable carbamates of compounds of Formula (D1). In another embodiment are pharmaceutically acceptable N-acyl derivatives of compounds of Formula (D1). Examples of N-acyl groups include N-acetyl and N-ethoxycarbonyl groups.

[00269] In a further embodiment, L_a is O.

[00270] In a further embodiment, Ar is phenyl.

[00271] In a further embodiment, Z is C(=O), NHC(=O), or $NCH_3C(=O)$.

[00272] In a further embodiment, each of R_1, R_2, and R_3 is H.

[00273] In one embodiment is a compound of Formula (D1) wherein R_6, R_7, and R_8 are all H. In another embodiment, R_6, R_7, and R_8 are not all H.

[00274] For any and all of the embodiments, substituents can be selected from among from a subset of the listed alternatives. For example, in some embodiments, L_a is CH_2, O, or NH. In other embodiments, L_a is O or NH. In yet other embodiments, L_a is O.

[00275] In some embodiments, Ar is a substituted or unsubstituted aryl. In yet other embodiments, Ar is a 6-membered aryl. In some other embodiments, Ar is phenyl.

[00276] In some embodiments, x is 2. In yet other embodiments, Z is C(=O), OC(=O), NHC(=O), $S(=O)_x$, $OS(=O)_x$, or $NHS(=O)_x$. In some other embodiments, Z is C(=O), NHC(=O), or $S(=O)_2$.

[00277] In some embodiments, R_7 and R_8 are independently selected from among H, unsubstituted C_1-C_4 alkyl, substituted C_1-C_4alkyl, unsubstituted C_1-C_4heteroalkyl, and substituted C_1-C_4heteroalkyl; or R_7 and R_8 taken together form a bond. In yet other embodiments, each of R_7 and R_8 is H; or R_7 and R_8 taken together form a bond.

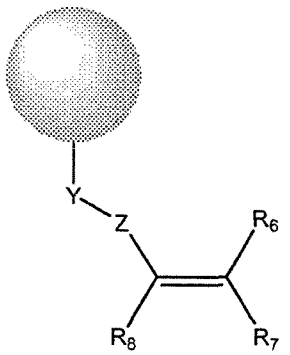
[00278] In some embodiments, R_6 is H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_6alkoxyalkyl, C_1-C_2alkyl-$N(C_1$-C_3alkyl$)_2$, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, C_1-C_4alkyl(aryl), C_1-

C_4alkyl(heteroaryl), C_1-C_4alkyl(C_3-C_8cycloalkyl), or C_1-C_4alkyl(C_2-C_8heterocycloalkyl). In some other embodiments, R_6 is H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_6alkoxyalkyl, C_1-C_2alkyl-N(C_1-C_3alkyl)$_2$, C_1-C_4alkyl(aryl), C_1-C_4alkyl(heteroaryl), C_1-C_4alkyl(C_3-C_8cycloalkyl), or C_1-C_4alkyl(C_2-C_8heterocycloalkyl). In yet other embodiments, R_6 is H, substituted or unsubstituted C_1-C_4alkyl, -CH_2-O-(C_1-C_3alkyl), -CH_2-N(C_1-C_3alkyl)$_2$, C_1-C_4alkyl(phenyl), or C_1-C_4alkyl(5- or 6-membered heteroaryl). In some embodiments, R_6 is H, substituted or unsubstituted C_1-C_4alkyl, -CH_2-O-(C_1-C_3alkyl), -CH_2-N(C_1-C_3alkyl)$_2$, C_1-C_4alkyl(phenyl), or C_1-C_4alkyl(5- or 6-membered heteroaryl containing 1 or 2 N atoms), or C_1-C_4alkyl(5- or 6-membered heterocycloalkyl containing 1 or 2 N atoms).

[00279] In some embodiments, Y is an optionally substituted group selected from among alkyl, heteroalkyl, cycloalkyl, and heterocycloalkyl. In other embodiments, Y is an optionally substituted group selected from among C_1-C_6alkyl, C_1-C_6heteroalkyl, 4-, 5-, 6- or 7-membered cycloalkyl, and 4-, 5-, 6- or 7-membered heterocycloalkyl. In yet other embodiments, Y is an optionally substituted group selected from among C_1-C_6alkyl, C_1-C_6heteroalkyl, 5-, or 6-membered cycloalkyl, and 5-, or 6-membered heterocycloalkyl containing 1 or 2 N atoms. In some other embodiments, Y is a 5-, or 6-membered cycloalkyl, or a 5-, or 6-membered heterocycloalkyl containing 1 or 2 N atoms.

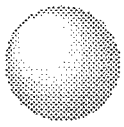
[00280] Any combination of the groups described above for the various variables is contemplated herein. It is understood that substituents and substitution patterns on the compounds provided herein can be selected by one of ordinary skill in the art to provide compounds that are chemically stable and that can be synthesized by techniques known in the art, as well as those set forth herein.

[00281] In one embodiment the irreversible inhibitor of a kinase has the structure of Formula (E):



Formula (E)

wherein:

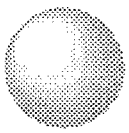
wherein  is a moiety that binds to the active site of a kinase, including a tyrosine kinase, further including a Btk kinase cysteine homolog;

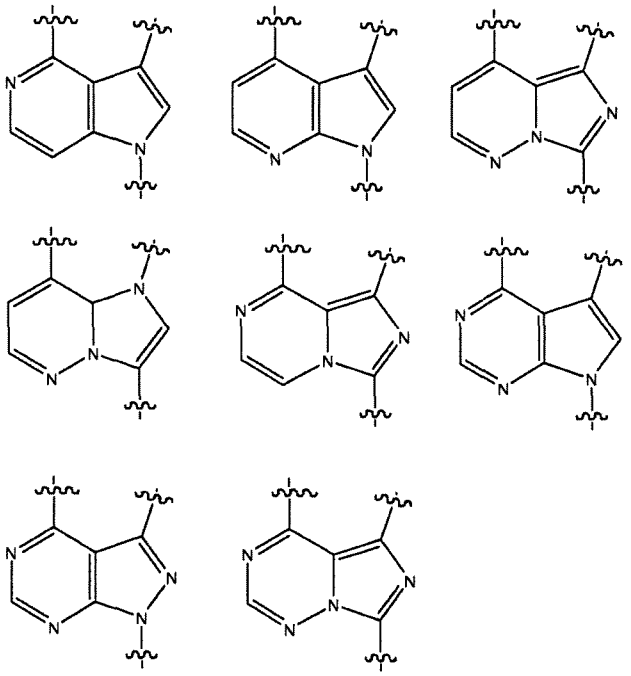
Y is an optionally substituted group selected from among alkylene, heteroalkylene, arylene, heteroarylene, heterocycloalkylene, cycloalkylene, alkylenearylene, alkyleneheteroarylene, alkylenecycloalkylene, and alkyleneheterocycloalkylene;

Z is C(=O), OC(=O), NHC(=O), $NCH_3C(=O)$, C(=S), $S(=O)_x$, $OS(=O)_x$, $NHS(=O)_x$, where x is 1 or 2;

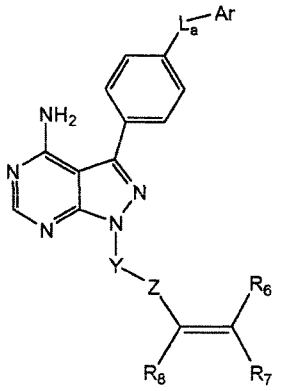
R_6, R_7, and R_8 are each independently selected from among H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_2-C_6heterocycloalkyl, C_1-C_6alkoxyalkyl, C_1-C_8alkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted C_1-C_4alkyl(aryl), substituted or unsubstituted C_1-C_4alkyl(heteroaryl), substituted or unsubstituted C_1-C_4alkyl(C_3-C_8cycloalkyl), or substituted or unsubstituted C_1-C_4alkyl(C_2-C_8heterocycloalkyl); or

R_7 and R_8 taken together form a bond; and pharmaceutically active metabolites, or pharmaceutically acceptable solvates, pharmaceutically acceptable salts, or pharmaceutically acceptable prodrugs thereof.

[00282] In some embodiments,  is a substituted fused biaryl moiety selected from



[00283] In one aspect, provided herein are compounds of Formula (F). Formula (F) is as follows:



Formula (F)

wherein

L_a is CH_2, O, NH or S;

Ar is a substituted or unsubstituted aryl, or a substituted or unsubstituted heteroaryl; and either

(a) Y is an optionally substituted group selected from among alkylene, heteroalkylene, arylene, heteroarylene, alkylenearylene, alkyleneheteroarylene, alkylenecycloalkylene and alkyleneheterocycloalkylene;

Z is C(=O), NHC(=O), $NR^aC(=O)$, $NR^aS(=O)_x$, where x is 1 or 2, and R^a is H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl; and either

(i) R_6, R_7, and R_8 are each independently selected from among H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_2-C_6heterocycloalkyl, C_1-

C_6alkoxyalkyl, C_1-C_8alkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted C_1-C_4alkyl(aryl), substituted or unsubstituted C_1-C_4alkyl(heteroaryl), substituted or unsubstituted C_1-C_4alkyl(C_3-C_8cycloalkyl), or substituted or unsubstituted C_1-C_4alkyl(C_2-C_8heterocycloalkyl);

(ii) R_6 and R_8 are H;

R_7 is H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_8alkylaminoalkyl, C_1-C_8 hydroxyalkylaminoalkyl, C_1-C_8 alkoxyalkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_1-C_8alkylC_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted C_2-C_8heterocycloalkyl, substituted or unsubstituted heteroaryl, C_1-C_4alkyl(aryl), C_1-C_4alkyl(heteroaryl), C_1-C_8alkylethers, C_1-C_8alkylamides, or C_1-C_4alkyl(C_2-C_8heterocycloalkyl); or

(iii) R_7 and R_8 taken together form a bond;

R_6 is selected from among H, substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_2-C_6heterocycloalkyl, C_1-C_6alkoxyalkyl, C_1-C_8alkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted C_1-C_4alkyl(aryl), substituted or unsubstituted C_1-C_4alkyl(heteroaryl), substituted or unsubstituted C_1-C_4alkyl(C_3-C_8cycloalkyl), or substituted or unsubstituted C_1-C_4alkyl(C_2-C_8heterocycloalkyl) or

(b) Y is an optionally substituted group selected from cycloalkylene or heterocycloalkylene;

Z is C(=O), NHC(=O), $NR^aC(=O)$, $NR^aS(=O)_x$, where x is 1 or 2, and R^a is H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl; and either

(i) R_7 and R_8 are H;

R_6 is substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_8alkylaminoalkyl, C_1-C_8 hydroxyalkylaminoalkyl, C_1-C_8 alkoxyalkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_1-C_8alkylC_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted C_2-C_8heterocycloalkyl, substituted or unsubstituted heteroaryl, C_1-C_4alkyl(aryl), C_1-C_4alkyl(heteroaryl), C_1-C_8alkylethers, C_1-C_8alkylamides, or C_1-C_4alkyl(C_2-C_8heterocycloalkyl);

(ii) R_6 and R_8 are H;

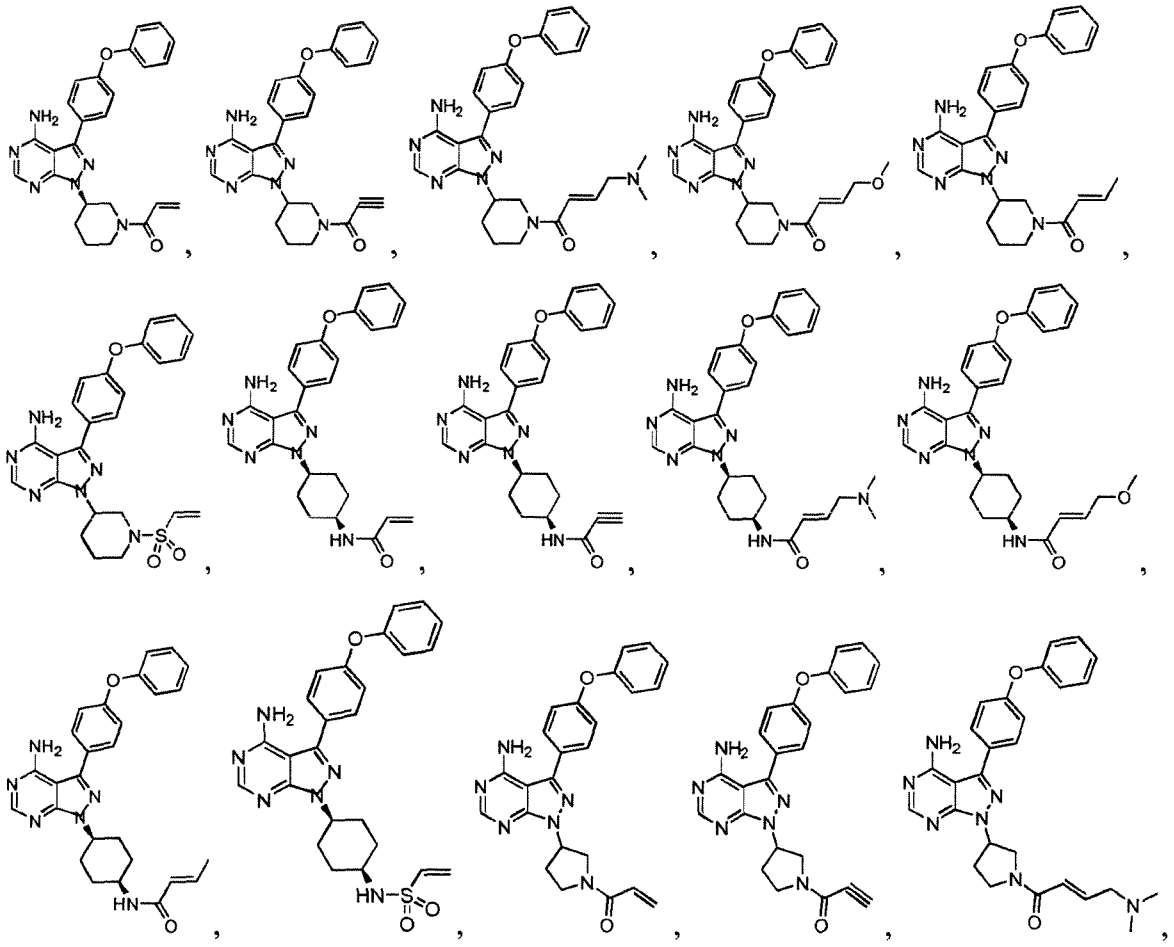
R_7 is substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_8alkylaminoalkyl, C_1-C_8 hydroxyalkylaminoalkyl, C_1-C_8 alkoxyalkylaminoalkyl,

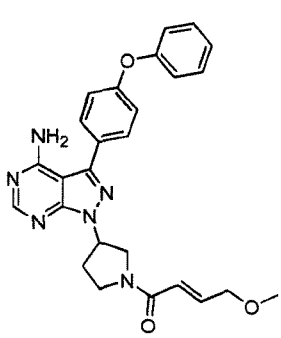
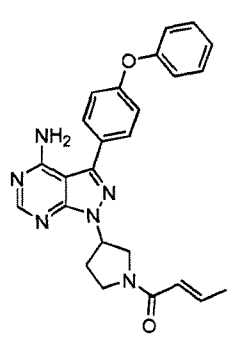
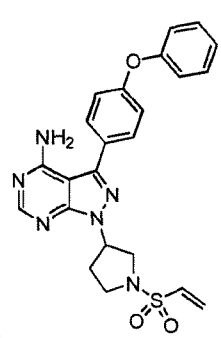
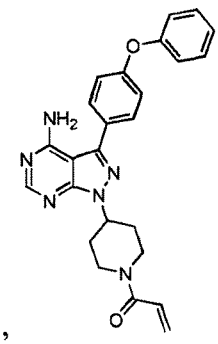
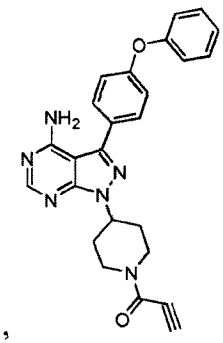
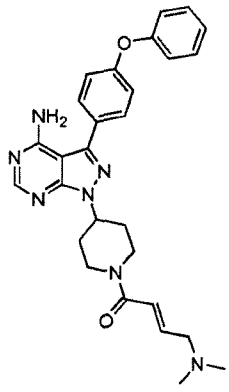
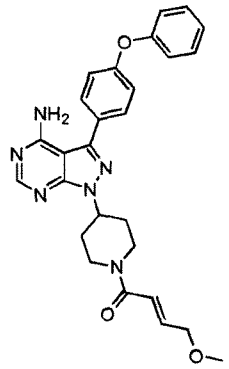
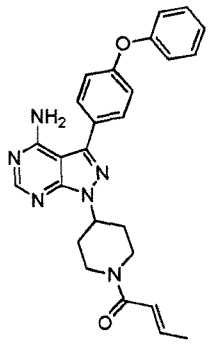
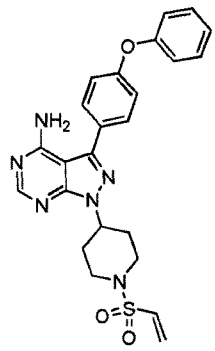
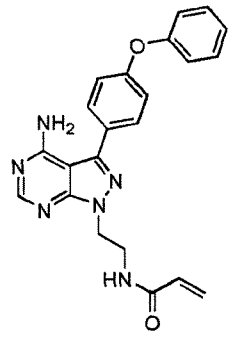
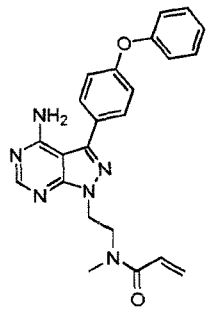
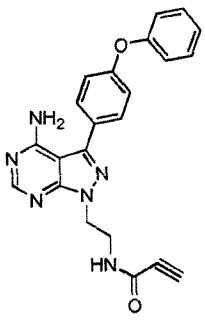
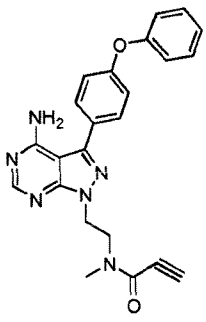
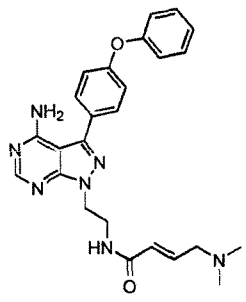
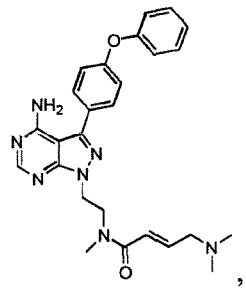
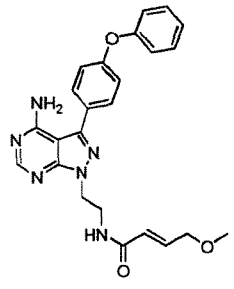
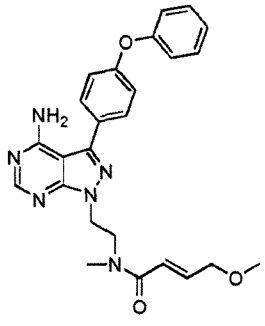
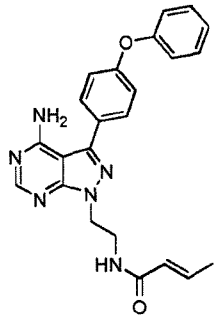
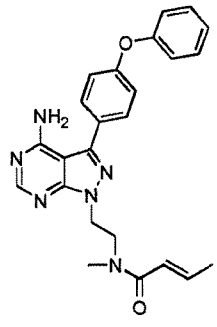
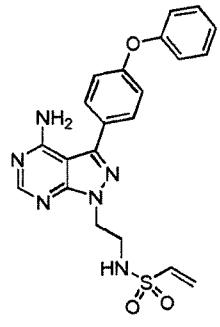
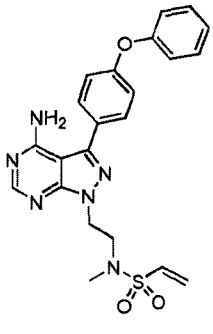
substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_1-C_8alkylC_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted C_2-C_8heterocycloalkyl, substituted or unsubstituted heteroaryl, C_1-C_4alkyl(aryl), C_1-C_4alkyl(heteroaryl), C_1-C_8alkylethers, C_1-C_8alkylamides, or C_1-C_4alkyl(C_2-C_8heterocycloalkyl); or

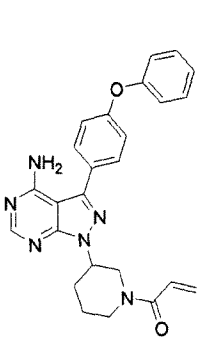
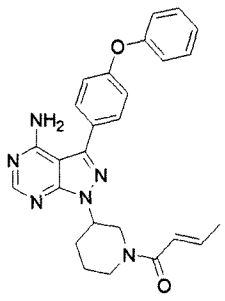
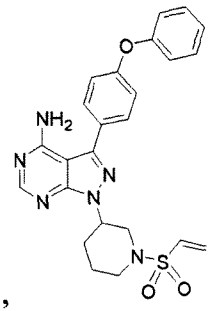
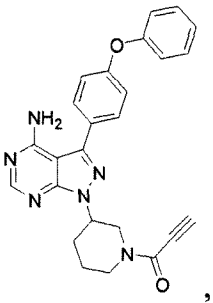
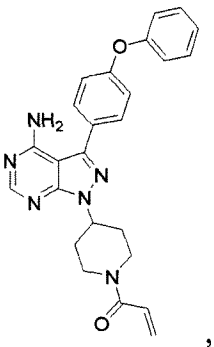
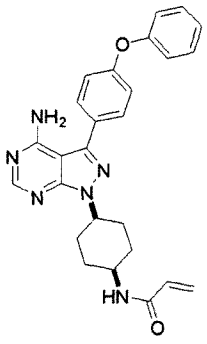
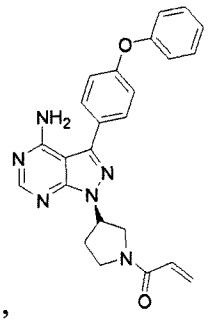
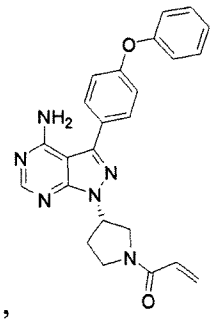
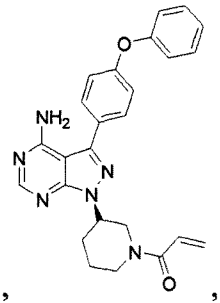
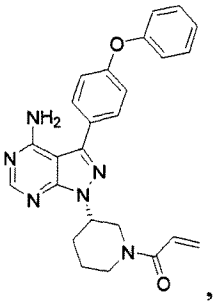
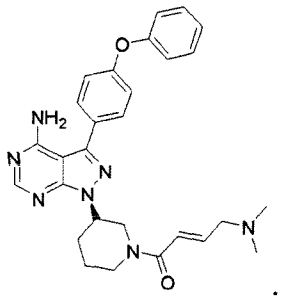
(iii) R_7 and R_8 taken together form a bond;

R_6 is substituted or unsubstituted C_1-C_4alkyl, substituted or unsubstituted C_1-C_4heteroalkyl, C_1-C_8alkylaminoalkyl, C_1-C_8hydroxyalkylaminoalkyl, C_1-C_8alkoxyalkylaminoalkyl, substituted or unsubstituted C_3-C_6cycloalkyl, substituted or unsubstituted C_1-C_8alkylC_3-C_6cycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted C_2-C_8heterocycloalkyl, substituted or unsubstituted heteroaryl, C_1-C_4alkyl(aryl), C_1-C_4alkyl(heteroaryl), C_1-C_8alkylethers, C_1-C_8alkylamides, or C_1-C_4alkyl(C_2-C_8heterocycloalkyl); and pharmaceutically active metabolites, or pharmaceutically acceptable solvates, pharmaceutically acceptable salts, or pharmaceutically acceptable prodrugs thereof

[00284] Further embodiments of compounds of Formula (A), Formula (B), Formula (C), Formula (D), include, but are not limited to, compounds selected from the group consisting of:



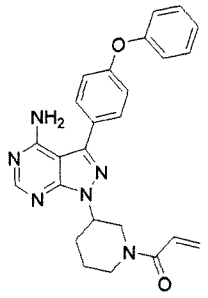

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and .

[00285] In still another embodiment, compounds provided herein are selected from among: , , , , , , , , , , and .

[00286] In one aspect, provided herein is a compound selected from among: 1-(3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one (Compound **4**); (E)-1-(3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)but-2-en-1-one (Compound **5**); 1-(3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)sulfonylethene (Compound **6**); 1-(3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)prop-2-yn-1-one (Compound **8**); 1-(4-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one (Compound **9**); N-((1s,4s)-4-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)cyclohexyl)acrylamide (Compound **10**); 1-((R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)pyrrolidin-1-yl)prop-2-en-1-one (Compound **11**); 1-((S)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)pyrrolidin-1-yl)prop-2-en-1-one (Compound **12**); 1-((R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one (Compound **13**); 1-((S)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one

(Compound **14**); and (E)-1-(3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)-4-(dimethylamino)but-2-en-1-one (Compound **15**).

[00287] In some embodiments, the Btk inhibitor has the structure:

[00288] 

[00289] In some embodiments, the 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 (i.e. PCI-32765/ibrutinib).

[00290] In one embodiment, the Btk inhibitor is α-cyano-β-hydroxy-β- methyl-*N*-(2,5-dibromophenyl)propenamide (LFM-A13), AVL-101, 4-tert-butyl-N-(3-(8-(phenylamino)imidazo[1,2-a]pyrazin-6-yl)phenyl)benzamide, 5-(3-amino-2-methylphenyl)-1-methyl-3-(4-(morpholine-4-carbonyl)phenylamino)pyrazin-2(1H)-one, N-(2-methyl-3-(4-methyl-6-(4-(morpholine-4-carbonyl)phenylamino)-5-oxo-4,5-dihydropyrazin-2-yl)phenyl)acetamide, 4-tert-butyl-N-(2-methyl-3-(4-methyl-6-(4-(morpholine-4-carbonyl)phenylamino)-5-oxo-4,5-dihydropyrazin-2-yl)phenyl)benzamide, 5-(3-(4-tert-butylbenzylamino)-2-methylphenyl)-1-methyl-3-(4-(morpholine-4-carbonyl)phenylamino)pyrazin-2(1H)-one, 5-(3-(3-tert-butylbenzylamino)-2-methylphenyl)-1-methyl-3-(4-(morpholine-4-carbonyl)phenylamino)pyrazin-2(1H)-one, 3-tert-butyl-N-(2-methyl-3-(4-methyl-6-(4-(morpholine-4-carbonyl)phenylamino)-5-oxo-4,5-dihydropyrazin-2-yl)phenyl)benzamide, 6-tert-butyl-N-(2-methyl-3-(4-methyl-6-(4-(morpholine-4-carbonyl)phenylamino)-5-oxo-4,5-dihydropyrazin-2-yl)phenyl)nicotinamide, and terreic acid.

[00291] Throughout the specification, groups and substituents thereof can be chosen by one skilled in the field to provide stable moieties and compounds.

Preparation of Compounds

[00292] Compounds of Formula D may be synthesized using standard synthetic techniques known to those of skill in the art or using methods known in the art in combination with methods described herein. In additions, solvents, temperatures and other reaction conditions presented herein may vary according to those of skill in the art. As a further guide the following synthetic methods may also be utilized.

[00293] The reactions can be employed in a linear sequence to provide the compounds described herein or they may be used to synthesize fragments which are subsequently joined by the methods described herein and/or known in the art.

Formation of Covalent Linkages by Reaction of an Electrophile with a Nucleophile

[00294] The compounds described herein can be modified using various electrophiles or nucleophiles to form new functional groups or substituents. Table 1 entitled "Examples of Covalent Linkages and Precursors Thereof" lists selected examples of covalent linkages and precursor functional groups which yield and can be used as guidance toward the variety of electrophiles and nucleophiles combinations available. Precursor functional groups are shown as electrophilic groups and nucleophilic groups.

Table 1: Examples of Covalent Linkages and Precursors Thereof

Covalent Linkage Product	Electrophile	Nucleophile
Carboxamides	Activated esters	amines/anilines
Carboxamides	acyl azides	amines/anilines
Carboxamides	acyl halides	amines/anilines
Esters	acyl halides	alcohols/phenols
Esters	acyl nitriles	alcohols/phenols
Carboxamides	acyl nitriles	amines/anilines
Imines	Aldehydes	amines/anilines
Hydrazones	aldehydes or ketones	Hydrazines
Oximes	aldehydes or ketones	Hydroxylamines
Alkyl amines	alkyl halides	amines/anilines
Esters	alkyl halides	carboxylic acids
Thioethers	alkyl halides	Thiols
Ethers	alkyl halides	alcohols/phenols
Thioethers	alkyl sulfonates	Thiols
Esters	alkyl sulfonates	carboxylic acids
Ethers	alkyl sulfonates	alcohols/phenols
Esters	Anhydrides	alcohols/phenols
Carboxamides	Anhydrides	amines/anilines
Thiophenols	aryl halides	Thiols
Aryl amines	aryl halides	Amines
Thioethers	Azindines	Thiols
Boronate esters	Boronates	Glycols
Carboxamides	carboxylic acids	amines/anilines
Esters	carboxylic acids	Alcohols
hydrazines	Hydrazides	carboxylic acids

N-acylureas or Anhydrides	carbodiimides	carboxylic acids
Esters	diazoalkanes	carboxylic acids
Thioethers	Epoxides	Thiols
Thioethers	haloacetamides	Thiols
Ammotriazines	halotriazines	amines/anilines
Triazinyl ethers	halotriazines	alcohols/phenols
Amidines	imido esters	amines/anilines
Ureas	Isocyanates	amines/anilines
Urethanes	Isocyanates	alcohols/phenols
Thioureas	isothiocyanates	amines/anilines
Thioethers	Maleimides	Thiols
Phosphite esters	phosphoramidites	Alcohols
Silyl ethers	silyl halides	Alcohols
Alkyl amines	sulfonate esters	amines/anilines
Thioethers	sulfonate esters	Thiols
Esters	sulfonate esters	carboxylic acids
Ethers	sulfonate esters	Alcohols
Sulfonamides	sulfonyl halides	amines/anilines
Sulfonate esters	sulfonyl halides	phenols/alcohols
Alkyl thiol	α,β-unsaturated ester	thiols
Alkyl ethers	α,β-unsaturated ester	alcohols
Alkyl amines	α,β-unsaturated ester	amines
Alkyl thiol	Vinyl sulfone	thiols
Alkyl ethers	Vinyl sulfone	alcohols
Alkyl amines	Vinyl sulfone	amines
Vinyl sulfide	Propargyl amide	thiol

Use of Protecting Groups

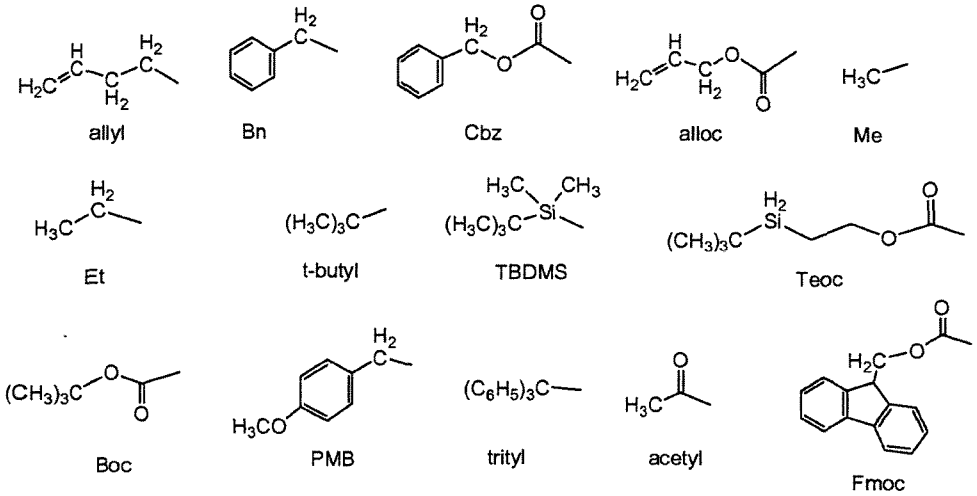
[00295] In the reactions described, it may be necessary to protect reactive functional groups, for example hydroxy, amino, imino, thio or carboxy groups, where these are desired in the final product, to avoid their unwanted participation in the reactions. Protecting groups are used to block some or all reactive moieties and prevent such groups from participating in chemical reactions until the protective group is removed. In one embodiment, each protective group be removable by a different means. Protective groups that are cleaved under totally disparate reaction conditions fulfill the requirement of differential removal. Protective groups

can be removed by acid, base, and hydrogenolysis. Groups such as trityl, dimethoxytrityl, acetal and t-butyldimethylsilyl are acid labile and may be used to protect carboxy and hydroxy reactive moieties in the presence of amino groups protected with Cbz groups, which are removable by hydrogenolysis, and Fmoc groups, which are base labile. Carboxylic acid and hydroxy reactive moieties may be blocked with base labile groups such as, but not limited to, methyl, ethyl, and acetyl in the presence of amines blocked with acid labile groups such as t-butyl carbamate or with carbamates that are both acid and base stable but hydrolytically removable.

[00296] Carboxylic acid and hydroxy reactive moieties may also be blocked with hydrolytically removable protective groups such as the benzyl group, while amine groups capable of hydrogen bonding with acids may be blocked with base labile groups such as Fmoc. Carboxylic acid reactive moieties may be protected by conversion to simple ester compounds as exemplified herein, or they may be blocked with oxidatively-removable protective groups such as 2,4-dimethoxybenzyl, while co-existing amino groups may be blocked with fluoride labile silyl carbamates.

[00297] Allyl blocking groups are useful in the presence of acid- and base- protecting groups since the former are stable and can be subsequently removed by metal or pi-acid catalysts. For example, an allyl-blocked carboxylic acid can be deprotected with a Pd^0-catalyzed reaction in the presence of acid labile t-butyl carbamate or base-labile acetate amine protecting groups. Yet another form of protecting group is a resin to which a compound or intermediate may be attached. As long as the residue is attached to the resin, that functional group is blocked and cannot react. Once released from the resin, the functional group is available to react.

[00298] Typically blocking/protecting groups may be selected from:



[00299] Other protecting groups, plus a detailed description of techniques applicable to the creation of protecting groups and their removal are described in Greene and Wuts, Protective Groups in Organic Synthesis, 3rd Ed., John Wiley & Sons, New York, NY, 1999, and

Kocienski, Protective Groups, Thieme Verlag, New York, NY, 1994, which are incorporated herein by reference in their entirety.

Further Forms of Compounds

[00300] The compounds described herein may possess one or more stereocenters and each center may exist in the R or S configuration. The compounds presented herein include all diastereomeric, enantiomeric, and epimeric forms as well as the appropriate mixtures thereof. Stereoisomers may be obtained, if desired, by methods known in the art as, for example, the separation of stereoisomers by chiral chromatographic columns.

[00301] Diasteromeric mixtures can be separated into their individual diastereomers on the basis of their physical chemical differences by methods known, for example, by chromatography and/or fractional crystallization. In one embodiment, enantiomers can be separated by chiral chromatographic columns. In other embodiments, enantiomers can be separated by converting the enantiomeric mixture into a diastereomeric mixture by reaction with an appropriate optically active compound (e.g., alcohol), separating the diastereomers and converting (e.g., hydrolyzing) the individual diastereomers to the corresponding pure enantiomers. All such isomers, including diastereomers, enantiomers, and mixtures thereof are considered as part of the compositions described herein.

[00302] The methods and formulations described herein include the use of *N*-oxides, crystalline forms (also known as polymorphs), or pharmaceutically acceptable salts of compounds described herein, as well as active metabolites of these compounds having the same type of activity. In some situations, compounds may exist as tautomers. All tautomers are included within the scope of the compounds presented herein. In addition, the compounds described herein can exist in unsolvated as well as solvated forms with pharmaceutically acceptable solvents such as water, ethanol, and the like. The solvated forms of the compounds presented herein are also considered to be disclosed herein.

[00303] Compounds of Formula D in unoxidized form can be prepared from N-oxides of compounds of Formula D by treating with a reducing agent, such as, but not limited to, sulfur, sulfur dioxide, triphenyl phosphine, lithium borohydride, sodium borohydride, phosphorus trichloride, tribromide, or the like in a suitable inert organic solvent, such as, but not limited to, acetonitrile, ethanol, aqueous dioxane, or the like at 0 to 80°C.

[00304] In some embodiments, compounds described herein are prepared as prodrugs. A "prodrug" refers to an agent that is converted into the parent drug *in vivo*. Prodrugs are often useful because, in some situations, they may be easier to administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent is not. The prodrug may also have improved solubility in pharmaceutical compositions over the parent drug. An

example, without limitation, of a prodrug would be a compound described herein, which is administered as an ester (the "prodrug") to facilitate transmittal across a cell membrane where water solubility is detrimental to mobility but which then is metabolically hydrolyzed to the carboxylic acid, the active entity, once inside the cell where water-solubility is beneficial. A further example of a prodrug might be a short peptide (polyaminoacid) bonded to an acid group where the peptide is metabolized to reveal the active moiety. In certain embodiments, upon *in vivo* administration, a prodrug is chemically converted to the biologically, pharmaceutically or therapeutically active form of the compound. In certain embodiments, a prodrug is enzymatically metabolized by one or more steps or processes to the biologically, pharmaceutically or therapeutically active form of the compound. To produce a prodrug, a pharmaceutically active compound is modified such that the active compound will be regenerated upon *in vivo* administration. The prodrug can be designed to alter the metabolic stability or the transport characteristics of a drug, to mask side effects or toxicity, to improve the flavor of a drug or to alter other characteristics or properties of a drug. By virtue of knowledge of pharmacodynamic processes and drug metabolism *in vivo*, those of skill in this art, once a pharmaceutically active compound is known, can design prodrugs of the compound. (see, for example, Nogrady (1985) *Medicinal Chemistry A Biochemical Approach*, Oxford University Press, New York, pages 388-392; Silverman (1992), The Organic Chemistry of Drug Design and Drug Action, Academic Press, Inc., San Diego, pages 352-401, Saulnier *et al.*, (1994), *Bioorganic and Medicinal Chemistry Letters*, Vol. 4, p. 1985).

[00305] Prodrug forms of the herein described compounds, wherein the prodrug is metabolized *in vivo* to produce a derivative as set forth herein are included within the scope of the claims. In some cases, some of the herein-described compounds may be a prodrug for another derivative or active compound.

[00306] Prodrugs are often useful because, in some situations, they may be easier to administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent is not. The prodrug may also have improved solubility in pharmaceutical compositions over the parent drug. Prodrugs may be designed as reversible drug derivatives, for use as modifiers to enhance drug transport to site-specific tissues. In some embodiments, the design of a prodrug increases the effective water solubility. See, e.g., Fedorak et al., *Am. J. Physiol.*, 269:G210-218 (1995); McLoed et al., *Gastroenterol*, 106:405-413 (1994); Hochhaus et al., *Biomed. Chrom.*, 6:283-286 (1992); J. Larsen and H. Bundgaard, *Int. J. Pharmaceutics*, 37, 87 (1987); J. Larsen et al., *Int. J. Pharmaceutics*, 47, 103 (1988); Sinkula et al., *J. Pharm. Sci.*, 64:181-210 (1975); T. Higuchi and V. Stella, *Pro-drugs as Novel Delivery Systems*, Vol. 14 of the A.C.S. Symposium Series; and Edward B. Roche, *Bioreversible Carriers in Drug Design*,

American Pharmaceutical Association and Pergamon Press, 1987, all incorporated herein in their entirety.

[00307] Sites on the aromatic ring portion of compounds of Formula D can be susceptible to various metabolic reactions, therefore incorporation of appropriate substituents on the aromatic ring structures, such as, by way of example only, halogens can reduce, minimize or eliminate this metabolic pathway.

[00308] Compounds described herein include isotopically-labeled compounds, which are identical to those recited in the various formulas and structures presented herein, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into the present compounds include isotopes of hydrogen, carbon, nitrogen, oxygen, fluorine and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{35}S, ^{18}F, ^{36}Cl, respectively. Certain isotopically-labeled compounds described herein, for example those into which radioactive isotopes such as ^{3}H and ^{14}C are incorporated, are useful in drug and/or substrate tissue distribution assays. Further, substitution with isotopes such as deuterium, i.e., ^{2}H, can afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements.

[00309] In additional or further embodiments, the compounds described herein are metabolized upon administration to an organism in need to produce a metabolite that is then used to produce a desired effect, including a desired therapeutic effect.

[00310] Compounds described herein may be formed as, and/or used as, pharmaceutically acceptable salts. The type of pharmaceutical acceptable salts, include, but are not limited to: (1) acid addition salts, formed) by reacting the free base form of the compound with a pharmaceutically acceptable: inorganic acid such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, metaphosphoric acid, and the like; or with an organic acid such as acetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, trifluoroacetic acid, tartaric acid, citric acid, benzoic acid, 3-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, toluenesulfonic acid, 2-naphthalenesulfonic acid, 4-methylbicyclo-[2.2.2]oct-2-ene-1-carboxylic acid, glucoheptonic acid, 4,4'-methylenebis-(3-hydroxy-2-ene-1 -carboxylic acid), 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid, and the like; (2) salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, e.g., an

alkali metal ion (e.g. lithium, sodium, potassium), an alkaline earth ion (e.g. magnesium, or calcium), or an aluminum ion; or coordinates with an organic base. Acceptable organic bases include ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like. Acceptable inorganic bases include aluminum hydroxide, calcium hydroxide, potassium hydroxide, sodium carbonate, sodium hydroxide, and the like.

[00311] The corresponding counterions of the pharmaceutically acceptable salts may be analyzed and identified using various methods including, but not limited to, ion exchange chromatography, ion chromatography, capillary electrophoresis, inductively coupled plasma, atomic absorption spectroscopy, mass spectrometry, or any combination thereof.

[00312] The salts are recovered by using at least one of the following techniques: filtration, precipitation with a non-solvent followed by filtration, evaporation of the solvent, or, in the case of aqueous solutions, lyophilization.

[00313] It should be understood that a reference to a pharmaceutically acceptable salt includes the solvent addition forms or crystal forms thereof, particularly solvates or polymorphs. Solvates contain either stoichiometric or non-stoichiometric amounts of a solvent, and may be formed during the process of crystallization with pharmaceutically acceptable solvents such as water, ethanol, and the like. Hydrates are formed when the solvent is water, or alcoholates are formed when the solvent is alcohol. Solvates of compounds described herein can be conveniently prepared or formed during the processes described herein. In addition, the compounds provided herein can exist in unsolvated as well as solvated forms. In general, the solvated forms are considered equivalent to the unsolvated forms for the purposes of the compounds and methods provided herein.

[00314] It should be understood that a reference to a salt includes the solvent addition forms or crystal forms thereof, particularly solvates or polymorphs. Solvates contain either stoichiometric or non-stoichiometric amounts of a solvent, and are often formed during the process of crystallization with pharmaceutically acceptable solvents such as water, ethanol, and the like. Hydrates are formed when the solvent is water, or alcoholates are formed when the solvent is alcohol. Polymorphs include the different crystal packing arrangements of the same elemental composition of a compound. Polymorphs usually have different X-ray diffraction patterns, infrared spectra, melting points, density, hardness, crystal shape, optical and electrical properties, stability, and solubility. Various factors such as the recrystallization solvent, rate of crystallization, and storage temperature may cause a single crystal form to dominate.

[00315] Compounds described herein may be in various forms, including but not limited to, amorphous forms, milled forms and nano-particulate forms. In addition, compounds described herein include crystalline forms, also known as polymorphs. Polymorphs include the

different crystal packing arrangements of the same elemental composition of a compound. Polymorphs usually have different X-ray diffraction patterns, infrared spectra, melting points, density, hardness, crystal shape, optical and electrical properties, stability, and solubility. Various factors such as the recrystallization solvent, rate of crystallization, and storage temperature may cause a single crystal form to dominate.

[00316] The screening and characterization of the pharmaceutically acceptable salts, polymorphs and/or solvates may be accomplished using a variety of techniques including, but not limited to, thermal analysis, x-ray diffraction, spectroscopy, vapor sorption, and microscopy. Thermal analysis methods address thermo chemical degradation or thermo physical processes including, but not limited to, polymorphic transitions, and such methods are used to analyze the relationships between polymorphic forms, determine weight loss, to find the glass transition temperature, or for excipient compatibility studies. Such methods include, but are not limited to, Differential scanning calorimetry (DSC), Modulated Differential Scanning Calorimetry (MDCS), Thermogravimetric analysis (TGA), and Thermogravimetric and Infrared analysis (TG/IR). X-ray diffraction methods include, but are not limited to, single crystal and powder diffractometers and synchrotron sources. The various spectroscopic techniques used include, but are not limited to, Raman, FTIR, UVIS, and NMR (liquid and solid state). The various microscopy techniques include, but are not limited to, polarized light microscopy, Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray Analysis (EDX), Environmental Scanning Electron Microscopy with EDX (in gas or water vapor atmosphere), IR microscopy, and Raman microscopy.

[00317] Throughout the specification, groups and substituents thereof can be chosen by one skilled in the field to provide stable moieties and compounds.

Pharmacokinetics

[00318] Disclosed herein, in certain embodiments, is a method for treating a hematological malignancy in an individual in need thereof, comprising: 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. In some embodiments, the method further comprises administering a second treatment to the individual.

[00319] In some embodiments, the Btk inhibitor has a day 1 C_{max} between 40 mg/mL and 400 ng/mL. In some embodiments, the Btk inhibitor has a day 1 C_{max} between 45 mg/mL and 390 ng/mL. In some embodiments, the Btk inhibitor has a day 1 C_{max} between 48.7 ng/mL and 383ng/mL. In some embodiments, the Btk inhibitor has a day 1 C_{max} of between 40 and 50 ng/mL. In some embodiments, the Btk inhibitor has a day 1 C_{max} of between 80 and 90 ng/mL.

In some embodiments, the Btk inhibitor has a day 1 C_{max} of between 90 and 100 ng/mL. In some embodiments, the Btk inhibitor has a day 1 C_{max} of 100 and 110 ng/mL. In some embodiments, the Btk inhibitor has a day 1 C_{max} of 110 and 120 ng/mL. In some embodiments, the Btk inhibitor has a day 1 C_{max} of 120 and 130 ng/mL. In some embodiments, the Btk inhibitor has a day 1 C_{max} of between 130 and 140 ng/mL. In some embodiments, the Btk inhibitor has a day 1 C_{max} of between 140 and 150 ng/mL. In some embodiments, the Btk inhibitor has a day 1 C_{max} of between 150 and 160 ng/mL. In some embodiments, the Btk inhibitor has a day 1 C_{max} of between 160 and 170 ng/mL. In some embodiments, the Btk inhibitor has a day 1 C_{max} of between 170 and 180 ng/mL. In some embodiments, the Btk inhibitor has a day 1 C_{max} of between 180 and 190 ng/mL. In some embodiments, the Btk inhibitor has a day 1 C_{max} of between 190 and 200 ng/mL. In some embodiments, the Btk inhibitor has a day 1 C_{max} of between 200 and 300 ng/mL. In some embodiments, the Btk inhibitor has a day 1 C_{max} of between 300 and 400 ng/mL.

[00320] In some embodiments, the Btk inhibitor has a day 1 C_{max} between 40 mg/mL and 400 ng/mL. In some embodiments, the Btk inhibitor has a day 1 C_{max} between 48.7 ng/mL and 383ng/mL. In some embodiments, a dose of 1.25 mg/kg of the Btk inhibitor has a day 1 C_{max} of 48.7 ng/mL. In some embodiments, a dose of 2.5 mg/kg of the Btk inhibitor has a day 1 C_{max} of 90.4 ng/mL. In some embodiments, a dose of 5 mg/kg of the Btk inhibitor has a day 1 C_{max} of 86.1 ng/mL. In some embodiments, a dose of 8.3 mg/kg of the Btk inhibitor has a day 1 C_{max} of 135 ng/mL. In some embodiments, a dose of 12.5 mg/kg of the Btk inhibitor has a day 1 C_{max} of 383 ng/mL. In some embodiments, a dose of 560 mg/day of the Btk inhibitor has a day 1 C_{max} of 156 ng/mL.

[00321] In some embodiments, the Btk inhibitor has a steady state C_{max} between 20 mg/mL and 300 ng/mL. In some embodiments, the Btk inhibitor has a steady state C_{max} between 20 mg/mL and 30 ng/mL. In some embodiments, the Btk inhibitor has a steady state C_{max} between 30 mg/mL and 50 ng/mL. In some embodiments, the Btk inhibitor has a steady state C_{max} between 50 mg/mL and 70 ng/mL. In some embodiments, the Btk inhibitor has a steady state C_{max} between 70 mg/mL and 90 ng/mL. In some embodiments, the Btk inhibitor has a steady state C_{max} between 90 mg/mL and 100 ng/mL. In some embodiments, the Btk inhibitor has a steady state C_{max} between 100 mg/mL and 110 ng/mL. In some embodiments, the Btk inhibitor has a steady state C_{max} between 110 mg/mL and 120 ng/mL. In some embodiments, the Btk inhibitor has a steady state C_{max} between 120 mg/mL and 130 ng/mL. In some embodiments, the Btk inhibitor has a steady state C_{max} between 130 mg/mL and 140 ng/mL. In some embodiments, the Btk inhibitor has a steady state C_{max} between 140 mg/mL and 150 ng/mL. In some embodiments, the Btk inhibitor has a steady state C_{max} between 150 mg/mL and

160 ng/mL. In some embodiments, the Btk inhibitor has a steady state C_{max} between 160 mg/mL and 170 ng/mL. In some embodiments, the Btk inhibitor has a steady state C_{max} between 170 mg/mL and 180 ng/mL. In some embodiments, the Btk inhibitor has a steady state C_{max} between 180 mg/mL and 190 ng/mL. In some embodiments, the Btk inhibitor has a steady state C_{max} between 200 mg/mL and 240 ng/mL.

[00322] In some embodiments, the Btk inhibitor has a steady state C_{max} between 27 ng/mL and 236 ng/mL. In some embodiments, a dose of 1.25 mg/kg of the Btk inhibitor has a steady state C_{max} of 27 ng/mL. In some embodiments, a dose of 2.5 mg/kg of the Btk inhibitor has a steady state C_{max} of 114 ng/mL. In some embodiments, a dose of 5 mg/kg of the Btk inhibitor has a steady state C_{max} of 112 ng/mL. In some embodiments, a dose of 8.3 mg/kg of the Btk inhibitor has a steady state C_{max} of 183 ng/mL. In some embodiments, a dose of 12.5 mg/kg of the Btk inhibitor has a steady state C_{max} of 236 ng/mL. In some embodiments, a dose of 560 mg/day of the Btk inhibitor has a steady state C_{max} of 122 ng/mL.

[00323] In some embodiments, the Btk inhibitor has a T_{max} between 1 and 2.5 hours. In some embodiments, the Btk inhibitor has a Tmax of between 1.5 and 2.3 hours. In some embodiments, the Btk inhibitor has a T_{max} of between 1.7 and 2.3 hours. In some embodiments, the Btk inhibitor has a T_{max} of between 1.8 and 2.2 hours..

[00324] In some embodiments, a dose of 1.25 mg/kg of the Btk inhibitor has a T_{max} of 1 hour. In some embodiments, a dose of 2.5 mg/kg of the Btk inhibitor has a T_{max} of 2.1 hours. In some embodiments, a dose of 5 mg/kg of the Btk inhibitor has a T_{max} of 2.3 hours. In some embodiments, a dose of 8.3 mg/kg of the Btk inhibitor has a T_{max} of 1.8 hours. In some embodiments, a dose of 12.5 mg/kg of the Btk inhibitor has a T_{max} of 1.7 hours. In some embodiments, a dose of 560 mg/day of the Btk inhibitor has a T_{max} of 1.8 hours.

[00325] In some embodiments, the mean half-life of the Btk inhibitor post-T_{max} is between 1.5 and 3 hours. In some embodiments, the Btk inhibitor has a mean half-life post-T_{max} of between 1.5 and 2.7hours. In some embodiments, the Btk inhibitor has a mean half-life post-T_{max} of between 1.5 and 2.5 hours. In some embodiments, the Btk inhibitor has a mean half-life post-T_{max} of between 1.5 and 2.2 hours. In some embodiments, the Btk inhibitor has a mean half-life post-T_{max} of between 1.5 and 1.7 hours. In some embodiments, the Btk inhibitor has a mean half-life post-T_{max} of between 2 and 3 hours. In some embodiments, the Btk inhibitor has a mean half-life post-T_{max} of between 2.5 and 3 hours. In some embodiments, the Btk inhibitor has a mean half-life post-T_{max} of between 2.5 and 2.9 hours. In some embodiments, the Btk inhibitor has a mean half-life post-T_{max} of between 2.5 and 2.8 hours. In some embodiments, the Btk inhibitor has a mean half-life post-T_{max} of between 2.5 and 2.7 hours.

[00326] In some embodiments, a dose of 1.25 mg/kg of the Btk inhibitor has a mean half-life post-T_{max} of 1.7 hours. In some embodiments, a dose of 2.5 mg/kg of the Btk inhibitor has a mean half-life post-T_{max} of 1.5 hours. In some embodiments, a dose of 5 mg/kg of the Btk inhibitor has a mean half-life post-T_{max} of 2.5 hours. In some embodiments, a dose of 8.3 mg/kg of the Btk inhibitor has a mean half-life post-T_{max} of 2.1 hours. In some embodiments, a dose of 12.5 mg/kg of the Btk inhibitor has a mean half-life post-T_{max} of 1.5 hours. In some embodiments, a 560 mg dose of the Btk inhibitor has a mean half-life post-T_{max} of 2.65 hours.

[00327] In some embodiments, the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of between 100 and 2000 ng•h/mL. In some embodiments, the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of between 150 and 1600 ng•h/mL. In some embodiments, the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of between 150 and 1100 ng•h/mL. In some embodiments, the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of between 150 and 1000 ng•h/mL. In some embodiments, the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of between 150 and 750 ng•h/mL. In some embodiments, the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of between 150 and 500 ng•h/mL. In some embodiments, the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of between 100 and 200 ng•h/mL. In some embodiments, the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of between 400 and 500 ng•h/mL. In some embodiments, the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of between 400 and 800 ng•h/mL. In some embodiments, the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of between 400 and 1000 ng•h/mL. In some embodiments, the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of between 700 and 1000 ng•h/mL. In some embodiments, the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of between 700 and 800 ng•h/mL.

[00328] In some embodiments, a 1.25 mg/kg dose of the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of 181 ng•h/mL. In some embodiments, a 2.5 mg/kg dose of the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of 494 ng•h/mL. In some embodiments, a 5 mg/kg dose of the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of 419 ng•h/mL. In some embodiments, a 8.3 mg/kg dose of the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of 923 ng•h/mL. In some embodiments, a 12.5 mg/kg dose of the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of 1550 ng•h/mL. In some embodiments, a 560 mg dose of the Btk inhibitor has a Day 1 $AUC_{0-\infty}$ of 749 ng•h/mL.

[00329] In some embodiments, body weight normalized dosing (mg/kg/day) of a Btk inhibitor results in variable Day 1 $AUC_{0-\infty}$ and steady-state AUC_{0-24}.

[00330] In some embodiments, the Btk inhibitor has a steady state AUC_{0-24} of between 300 and 3000 ng•h/mL. In some embodiments, the Btk inhibitor has a steady state AUC_{0-24} of between 300 and 2500 ng•h/mL. In some embodiments, the Btk inhibitor has a steady state AUC_{0-24} of between 300 and 2000 ng•h/mL. In some embodiments, the Btk inhibitor has a steady state AUC_{0-24} of between 300 and 1600 ng•h/mL. In some embodiments, the Btk inhibitor has a steady state AUC_{0-24} of between 1500 and 2500 ng•h/mL. In some embodiments, the Btk

inhibitor has a steady state AUC_{0-24} of between 1500 and 2000 ng•h/mL. In some embodiments, the Btk inhibitor has a steady state AUC_{0-24} of between 1500 and 1900 ng•h/mL. In some embodiments, the Btk inhibitor has a steady state AUC_{0-24} of between 1500 and 1600 ng•h/mL.

[00331] In some embodiments, a 1.25 mg/kg dose of the Btk inhibitor has a steady state AUC_{0-24} of 301 ng•h/mL. In some embodiments, a 2.5 mg/kg dose of the Btk inhibitor has a steady state AUC_{0-24} of 1840 ng•h/mL. In some embodiments, a 5 mg/kg dose of the Btk inhibitor has a steady state AUC_{0-24} of 1580 ng•h/mL. In some embodiments, a 8.3 mg/kg dose of the Btk inhibitor has a steady state AUC_{0-24} of 2330 ng•h/mL. In some embodiments, a 12.5 mg/kg dose of the Btk inhibitor has a steady state AUC_{0-24} of 2936 ng•h/mL. In some embodiments, a 560 mg dose of the Btk inhibitor has a steady state AUC_{0-24} of 1553 ng•h/mL.

[00332] In some embodiments, the unbound fraction of the Btk inhibitor is between 1% and 5%. In some embodiments, the unbound fraction of the Btk inhibitor is between 1.5% and 4%. In some embodiments, the unbound fraction of the Btk inhibitor is between 2% and 3%. In some embodiments, the unbound fraction of the Btk inhibitor is 2.5%.

Second Treatments

[00333] 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; and (b) administering a second treatment to the individual. Further 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, analyzing the mobilized plurality of cells comprises measuring the peripheral blood concentration of the mobilized plurality of cells. In some embodiments, the method further comprises 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. In some embodiments, administering the second treatment occurs after a subsequent decrease in peripheral blood concentration of the mobilized plurality of cells. In some embodiments, 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. In some embodiments, the method further comprises administering the second

treatment after the peripheral blood concentration of the mobilized plurality of cells has increased for a predetermined length of time. In some embodiments, analyzing the mobilized plurality of cells comprises counting the number of mobilized plurality of cells in the peripheral blood. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood increases as compared to the number before administration of the Btk inhibitor. In some embodiments, administering the second treatment occurs after a subsequent decrease in the number of mobilized plurality of cells in the peripheral blood. In some embodiments, analyzing the mobilized plurality of cells comprises measuring the duration of an increase in the number of mobilized plurality of cells in the peripheral blood as compared to the number before administration of the Btk inhibitor. In some embodiments, the method further comprises administering the second treatment after the number of mobilized plurality of cells in the peripheral blood has increased for a predetermined length of time.

[00334] In some embodiments, administering a Btk inhibitor before the second treatment reduces immune-mediated reactions to the second treatment. In some embodiments, administering a Btk inhibitor before ofatumumab reduces immune-mediated reactions to ofatumumab.

[00335] In some embodiments, the second treatment comprises a chemotherapeutic agent, a steroid, an immunotherapeutic agent, a targeted therapy, or a combination thereof. In some embodiments, the second treatment comprises a B cell receptor pathway inhibitor. In some embodiments, the B cell receptor pathway inhibitor is a CD79A inhibitor, a CD79B inhibitor, a CD19 inhibitor, a Lyn inhibitor, a Syk inhibitor, a PI3K inhibitor, a Blnk inhibitor, a PLCγ inhibitor, a PKCβ inhibitor, or a combination thereof. In some embodiments, the second treatment comprises an antibody, B cell receptor signaling inhibitor, a PI3K inhibitor, an IAP inhibitor, an mTOR inhibitor, a radioimmunotherapeutic, a DNA damaging agent, a proteosome inhibitor, a histone deacytlase inhibitor, a protein kinase inhibitor, a hedgehog inhibitor, an Hsp90 inhibitor, a telomerase inhibitor, a Jak1/2 inhibitor, a protease inhibitor, a PKC inhibitor, a PARP inhibitor, or a combination thereof.

[00336] In some embodiments, the second treatment comprises chlorambucil, ifosphamide, doxorubicin, mesalazine, thalidomide, lenalidomide, temsirolimus, everolimus, fludarabine, fostamatinib, paclitaxel, docetaxel, ofatumumab, rituximab, dexamethasone, prednisone, CAL-101, ibritumomab, tositumomab, bortezomib, pentostatin, endostatin, or a combination thereof.

[00337] In some embodiments, the second treatment comprises lenalidomide.

[00338] In some embodiments, the second treatment comprises bortezomib.

[00339] In some embodiments, the second treatment comprises sorafenib.

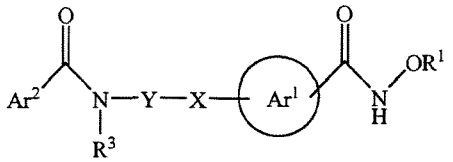
[00340] In some embodiments, the second treatment comprises gemcitabine.

[00341] In some embodiments, the second treatment comprises dexamethasone.

[00342] In some embodiments, the second treatment comprises bendamustine.

[00343] In some embodiments, the second treatment comprises R-406.

[00344] In some embodiments, the second treatment comprises an HDAC inhibitor. In some embodiments, the HDAC inhibitor has the structure of Formula (I):



Formula (I)

wherein:

R^1 is hydrogen or alkyl;

X is -O-, $-NR^2-$, or $-S(O)_n$ where n is 0-2 and R^2 is hydrogen or alkyl;

Y is alkylene optionally substituted with cycloalkyl, optionally substituted phenyl, alkylthio, alkylsulfinyl, alkysulfonyl, optionally substituted phenylalkylthio, optionally substituted phenylalkylsulfonyl, hydroxy, or optionally substituted phenoxy;

Ar^1 is phenylene or heteroarylene wherein said Ar^1 is optionally substituted with one or two groups independently selected from alkyl, halo, hydroxy, alkoxy, haloalkoxy, or haloalkyl;

R^3 is hydrogen, alkyl, hydroxyalkyl, or optionally substituted phenyl; and

Ar^2 is aryl, aralkyl, aralkenyl, heteroaryl, heteroaralkyl, heteroaralkenyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, or heterocycloalkylalkyl;

and individual stereoisomers, individual geometric isomers, or mixtures thereof; or a pharmaceutically acceptable salt thereof.

In some embodiments, the histone deacetylase inhibitor is 3-((dimethylamino)methyl)-N-(2-(4-(hydroxycarbamoyl)phenoxy)ethyl)benzofuran-2-carboxamide.

[00345] In some embodiments, the second treatment comprises taxol.

[00346] In some embodiments, the second treatment comprises vincristine.

[00347] In some embodiments, the second treatment comprises doxorubicin.

[00348] In some embodiments, the second treatment comprises temsirolimus.

[00349] In some embodiments, the second treatment comprises carboplatin.

[00350] In some embodiments, the second treatment comprises ofatumumab.

[00351] In some embodiments, the second treatment comprises rituximab.

[00352] In some embodiments, the second treatment comprises cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone, and optionally, rituximab.

[00353] In some embodiments, the second treatment comprises bendamustine, and rituximab.

[00354] In some embodiments, the second treatment comprises fludarabine, cyclophosphamide, and rituximab.

[00355] In some embodiments, the second treatment comprises cyclophosphamide, vincristine, and prednisone, and optionally, rituximab.

[00356] In some embodiments, the second treatment comprises etoposide, doxorubicin, vincristine, cyclophosphamide, prednisolone, and optionally, rituximab.

[00357] In some embodiments, the second treatment comprises dexamethasone and lenalidomide.

[00358] Additional cancer treatment s include Nitrogen Mustards such as for example, bendamustine, chlorambucil, chlormethine, cyclophosphamide, ifosfamide, melphalan, prednimustine, trofosfamide; Alkyl Sulfonates like busulfan, mannosulfan, treosulfan; Ethylene Imines like carboquone, thiotepa, triaziquone; Nitrosoureas like carmustine, fotemustine, lomustine, nimustine, ranimustine, semustine, streptozocin; Epoxides such as for example, etoglucid; Other Alkylating Agents such as for example dacarbazine, mitobronitol, pipobroman, temozolomide; Folic Acid Analogues such as for example methotrexate, permetrexed, pralatrexate, raltitrexed; Purine Analogs such as for example cladribine, clofarabine, fludarabine, mercaptopurine, nelarabine, tioguanine; Pyrimidine Analogs such as for example azacitidine, capecitabine, carmofur, cytarabine, decitabine, fluorouracil, gemcitabine, tegafur; Vinca Alkaloids such as for example vinblastine, vincristine, vindesine, vinflunine, vinorelbine; Podophyllotoxin Derivatives such as for example etoposide, teniposide; Colchicine derivatives such as for example demecolcine; Taxanes such as for example docetaxel, paclitaxel, paclitaxel poliglumex; Other Plant Alkaloids and Natural Products such as for example trabectedin; Actinomycines such as for example dactinomycin; Antracyclines such as for example aclarubicin, daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, pirarubicin, valrubicin, zorubincin; Other Cytotoxic Antibiotics such as for example bleomycin, ixabepilone, mitomycin, plicamycin; Platinum Compounds such as for example carboplatin, cisplatin, oxaliplatin, satraplatin; Methylhydrazines such as for example procarbazine; Sensitizers such as for example aminolevulinic acid, efaproxiral, methyl aminolevulinate, porfimer sodium, temoporfin; Protein Kinase Inhibitors such as for example dasatinib, erlotinib, everolimus, gefitinib, imatinib, lapatinib, nilotinib, pazonanib, sorafenib, sunitinib, temsirolimus; Other Antineoplastic Agents such as for example alitretinoin, altretamine, amzacrine, anagrelide, arsenic trioxide, asparaginase, bexarotene, bortezomib, celecoxib, denileukin diftitox, estramustine, hydroxycarbamide, irinotecan, lonidamine, masoprocol, miltefosein, mitoguazone,

mitotane, oblimersen, pegaspargase, pentostatin, romidepsin, sitimagene ceradenovec, tiazofurine, topotecan, tretinoin, vorinostat; Estrogens such as for example diethylstilbenol, ethinylestradiol, fosfestrol, polyestradiol phosphate; Progestogens such as for example gestonorone, medroxyprogesterone, megestrol; Gonadotropin Releasing Hormone Analogs such as for example buserelin, goserelin, leuprorelin, triptorelin; Anti-Estrogens such as for example fulvestrant, tamoxifen, toremifene; Anti-Androgens such as for example bicalutamide, flutamide, nilutamide, , Enzyme Inhibitors, aminoglutethimide, anastrozole, exemestane, formestane, letrozole, vorozole; Other Hormone Antagonists such as for example abarelix, degarelix; Immunostimulants such as for example histamine dihydrochloride, mifamurtide, pidotimod, plerixafor, roquinimex, thymopentin; Immunosuppressants such as for example everolimus, gusperimus, leflunomide, mycophenolic acid, sirolimus; Calcineurin Inhibitors such as for example ciclosporin, tacrolimus; Other Immunosuppressants such as for example azathioprine, lenalidomide, methotrexate, thalidomide; and Radiopharmaceuticals such as for example, iobenguane.

[00359] Additional cancer treatment s include interferons, interleukins, Tumor Necrosis Factors, Growth Factors, or the like.

[00360] Additional cancer treatment s include Immunostimulants such as for example ancestim, filgrastim, lenograstim, molgramostim, pegfilgrastim, sargramostim; Interferons such as for example interferon alfa natural, interferon alfa-2a, interferon alfa-2b, interferon alfacon-1, interferon alfa-n1, interferon beta natural, interferon beta-1a, interferon beta-1b, interferon gamma, peginterferon alfa-2a, peginterferon alfa-2b; Interleukins such as for example aldesleukin, oprelvekin; Other Immunostimulants such as for example BCG vaccine, glatiramer acetate, histamine dihydrochloride, immunocyanin, lentinan, melanoma vaccine, mifamurtide, pegademase, pidotimod, plerixafor, poly I:C, poly ICLC, roquinimex, tasonermin, thymopentin; Immunosuppressants such as for example abatacept, abetimus, alefacept, antilymphocyte immunoglobulin (horse), antithymocyte immunoglobulin (rabbit), eculizumab, efalizumab, everolimus, gusperimus, leflunomide, muromab-CD3, mycophenolic acid, natalizumab, sirolimus; TNF alpha Inhibitors such as for example adalimumab, afelimomab, certolizumab pegol, etanercept, golimumab, infliximab; Interleukin Inhibitors such as for example anakinra, basiliximab, canakinumab, daclizumab, mepolizumab, rilonacept, tocilizumab, ustekinumab; Calcineurin Inhibitors such as for example ciclosporin, tacrolimus; Other Immunosuppressants such as for example azathioprine, lenalidomide, methotrexate, thalidomide.

[00361] Additional cancer treatment s include Adalimumab, Alemtuzumab, Basiliximab, Bevacizumab, Cetuximab, Certolizumab pegol, Daclizumab, Eculizumab, Efalizumab, Gemtuzumab, Ibritumomab tiuxetan, Infliximab, Muromonab-CD3, Natalizumab,

Panitumumab, Ranibizumab, Rituximab, Tositumomab, Trastuzumab, or the like, or a combination thereof.

[00362] Additional cancer treatment s include Monoclonal Antibodies such as for example alemtuzumab, bevacizumab, catumaxomab, cetuximab, edrecolomab, gemtuzumab, ofatumumab, panitumumab, rituximab, trastuzumab, , Immunosuppressants, eculizumab, efalizumab, muromab-CD3, natalizumab; TNF alpha Inhibitors such as for example adalimumab, afelimomab, certolizumab pegol, golimumab, infliximab, , Interleukin Inhibitors, basiliximab, canakinumab, daclizumab, mepolizumab, tocilizumab, ustekinumab, , Radiopharmaceuticals, ibritumomab tiuxetan, tositumomab; Others Monoclonal Antibodies such as for example abagovomab, adecatumumab, alemtuzumab, anti-CD30 monoclonal antibody Xmab2513, anti-MET monoclonal antibody MetMab, apolizumab, apomab, arcitumomab, basiliximab, bispecific antibody 2B1, blinatumomab, brentuximab vedotin, capromab pendetide, cixutumumab, claudiximab, conatumumab, dacetuzumab, denosumab, eculizumab, epratuzumab, epratuzumab, ertumaxomab, etaracizumab, figitumumab, fresolimumab, galiximab, ganitumab, gemtuzumab ozogamicin, glembatumumab, ibritumomab, inotuzumab ozogamicin, ipilimumab, lexatumumab, lintuzumab, lintuzumab, lucatumumab, mapatumumab, matuzumab, milatuzumab, monoclonal antibody CC49, necitumumab, nimotuzumab, ofatumumab, oregovomab, pertuzumab, ramacurimab, ranibizumab, siplizumab, sonepcizumab, tanezumab, tositumomab, trastuzumab, tremelimumab, tucotuzumab celmoleukin, veltuzumab, visilizumab, volociximab, zalutumumab.

[00363] Additional cancer treatment s include agents that affect the tumor micro-environment such as cellular signaling network (e.g. phosphatidylinositol 3-kinase (PI3K) signaling pathway, signaling from the B-cell receptor and the IgE receptor). In some embodiments, the second agent is a PI3K signaling inhibitor or a syc kinase inhibitor. In one embodiment, the syk inhibitor is R788. In another embodiment is a PKCγ inhibitor such as by way of example only, enzastaurin.

[00364] Examples of agents that affect the tumor micro-environment include PI3K signaling inhibitor, syc kinase inhibitor, Protein Kinase Inhibitors such as for example dasatinib, erlotinib, everolimus, gefitinib, imatinib, lapatinib, nilotinib, pazonanib, sorafenib, sunitinib, temsirolimus; Other Angiogenesis Inhibitors such as for example GT-111, JI-101, R1530; Other Kinase Inhibitors such as for example AC220, AC480, ACE-041, AMG 900, AP24534, Arry-614, AT7519, AT9283, AV-951, axitinib, AZD1152, AZD7762, AZD8055, AZD8931, bafetinib, BAY 73-4506, BGJ398, BGT226, BI 811283, BI6727, BIBF 1120, BIBW 2992, BMS-690154, BMS-777607, BMS-863233, BSK-461364, CAL-101, CEP-11981, CYC116, DCC-2036, dinaciclib, dovitinib lactate, E7050, EMD 1214063, ENMD-2076, fostamatinib

disodium, GSK2256098, GSK690693, INCB18424, INNO-406, JNJ-26483327, JX-594, KX2-391, linifanib, LY2603618, MGCD265, MK-0457, MK1496, MLN8054, MLN8237, MP470, NMS-1116354, NMS-1286937, ON 01919.Na, OSI-027, OSI-930, Btk inhibitor, PF-00562271, PF-02341066, PF-03814735, PF-04217903, PF-04554878, PF-04691502, PF-3758309, PHA-739358, PLC3397, progenipoietin, R547, R763, ramucirumab, regorafenib, RO5185426, SAR103168, S3333333CH 727965, SGI-1176, SGX523, SNS-314, TAK-593, TAK-901, TKI258, TLN-232, TTP607, XL147, XL228, XL281RO5126766, XL418, XL765.

[00365] Further examples of anti-cancer agents for use in combination with a Btk inhibitor compound include inhibitors of mitogen-activated protein kinase signaling, e.g., U0126, PD98059, PD184352, PD0325901, ARRY-142886, SB239063, SP600125, BAY 43-9006, wortmannin, or LY294002; Syk inhibitors; mTOR inhibitors; and antibodies (e.g., rituxan).

[00366] Other anti-cancer agents that can be employed in combination with a Btk inhibitor compound include Adriamycin, Dactinomycin, Bleomycin, Vinblastine, Cisplatin, acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; flurocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; iimofosine; interleukin Il (including recombinant interleukin II, or rlL2), interferon alfa-2a; interferon alfa-2b; interferon alfa-nl; interferon alfa-n3; interferon beta-l a; interferon gamma-l b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide;

mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazoie; nogalamycin; ormaplatin; oxisuran; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride.

[00367] Other anti-cancer agents that can be employed in combination with a Btk inhibitor compound include: 20-epi-1, 25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorlns; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor;

cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; 9- dioxamycin; diphenyl spiromustine; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-such as for example growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1 -based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol;

phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylerie conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen-binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

[00368] Yet other anticancer agents that can be employed in combination with a Btk inhibitor compound include alkylating agents, antimetabolites, natural products, or hormones, e.g., nitrogen mustards (e.g., mechloroethamine, cyclophosphamide, chlorambucil, etc.), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., carmustine, lomusitne, ete.), or triazenes (decarbazine, etc.). Examples of antimetabolites include but are not limited to folic acid analog (e.g., methotrexate), or pyrimidine analogs (e.g., Cytarabine), purine analogs (e.g., mercaptopurine, thioguanine, pentostatin).

[00369] Examples of alkylating agents that can be employed in combination a Btk inhibitor compound include, but are not limited to, nitrogen mustards (e.g., mechloroethamine, cyclophosphamide, chlorambucil, meiphalan, etc.), ethylenimine and methylmelamines (e.g., hexamethlymelamine, thiotepa), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., carmustine, lomusitne, semustine, streptozocin, etc.), or triazenes (decarbazine, ete.). Examples of antimetabolites include, but are not limited to folic acid analog (e.g., methotrexate), or pyrimidine analogs (e.g., fluorouracil, floxouridine, Cytarabine), purine analogs (e.g., mercaptopurine, thioguanine, pentostatin.

[00370] Examples of anti-cancer agents which act by arresting cells in the G2-M phases due to stabilized microtubules and which can be used in combination with a Btk inhibitor compound include without limitation the following marketed drugs and drugs in development: Erbulozole (also known as R-55104), Dolastatin 10 (also known as DLS-10 and NSC-376128), Mivobulin isethionate (also known as CI-980), Vincristine, NSC-639829, Discodermolide (also known as NVP-XX-A-296), ABT-751 (Abbott, also known as E-7010), Altorhyrtins (such as Altorhyrtin A and Altorhyrtin C), Spongistatins (such as Spongistatin 1, Spongistatin 2, Spongistatin 3, Spongistatin 4, Spongistatin 5, Spongistatin 6, Spongistatin 7, Spongistatin 8, and Spongistatin 9), Cemadotin hydrochloride (also known as LU-103793 and NSC-D-669356), Epothilones (such as Epothilone A, Epothilone B, Epothilone C (also known as desoxyepothilone A or dEpoA), Epothilone D (also referred to as KOS-862, dEpoB, and desoxyepothilone B), Epothilone E, Epothilone F, Epothilone B N-oxide, Epothilone A N-oxide, 16-aza-epothilone B, 21-aminoepothilone B (also known as BMS-310705), 21-hydroxyepothilone D (also known as Desoxyepothilone F and dEpoF), 26-fluoroepothilone), Auristatin PE (also known as NSC-654663), Soblidotin (also known as TZT-1027), LS-4559-P (Pharmacia, also known as LS-4577), LS-4578 (Pharmacia, also known as LS-477-P), LS-4477 (Pharmacia), LS-4559 (Pharmacia), RPR-112378 (Aventis), Vincristine sulfate, DZ-3358 (Daiichi), FR-182877 (Fujisawa, also known as WS-9885B), GS-164 (Takeda), GS-198 (Takeda), KAR-2 (Hungarian Academy of Sciences), BSF-223651 (BASF, also known as ILX-651 and LU-223651), SAH-49960 (Lilly/Novartis), SDZ-268970 (Lilly/Novartis), AM-97 (Armad/Kyowa Hakko), AM-132 (Armad), AM-138 (Armad/Kyowa Hakko), IDN-5005 (Indena), Cryptophycin 52 (also known as LY-355703), AC-7739 (Ajinomoto, also known as AVE-8063A and CS-39.HCI), AC-7700 (Ajinomoto, also known as AVE-8062, AVE-8062A, CS-39-L-Ser.HCI, and RPR-258062A), Vitilevuamide, Tubulysin A, Canadensol, Centaureidin (also known as NSC-106969), T-138067 (Tularik, also known as T-67, TL-138067 and TI-138067), COBRA-1 (Parker Hughes Institute, also known as DDE-261 and WHI-261), H10 (Kansas State University), H16 (Kansas State University), Oncocidin A1 (also known as BTO-

956 and DIME), DDE-313 (Parker Hughes Institute), Fijianolide B, Laulimalide, SPA-2 (Parker Hughes Institute), SPA-1 (Parker Hughes Institute, also known as SPIKET-P), 3-IAABU (Cytoskeleton/Mt. Sinai School of Medicine, also known as MF-569), Narcosine (also known as NSC-5366), Nascapine, D-24851 (Asta Medica), A-105972 (Abbott), Hemiasterlin, 3-BAABU (Cytoskeleton/Mt. Sinai School of Medicine, also known as MF-191), TMPN (Arizona State University), Vanadocene acetylacetonate, T-138026 (Tularik), Monsatrol, lnanocine (also known as NSC-698666), 3-lAABE (Cytoskeleton/Mt. Sinai School of Medicine), A-204197 (Abbott), T-607 (Tuiarik, also known as T-900607), RPR- 115781 (Aventis), Eleutherobins (such as Desmethyleleutherobin, Desaetyleleutherobin, lsoeleutherobin A, and Z-Eleutherobin), Caribaeoside, Caribaeolin, Halichondrin B, D-64131 (Asta Medica), D-68144 (Asta Medica), Diazonamide A, A-293620 (Abbott), NPI-2350 (Nereus), Taccalonolide A, TUB-245 (Aventis), A-259754 (Abbott), Diozostatin, (-)-Phenylahistin (also known as NSCL-96F037), D-68838 (Asta Medica), D-68836 (Asta Medica), Myoseverin B, D-43411 (Zentaris, also known as D-81862), A-289099 (Abbott), A-318315 (Abbott), HTI-286 (also known as SPA-110, trifluoroacetate salt) (Wyeth), D-82317 (Zentaris), D-82318 (Zentaris), SC-12983 (NCI), Resverastatin phosphate sodium, BPR-OY-007 (National Health Research Institutes), and SSR-250411 (Sanofi).

Biomarkers

[00371] 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; and (b) preparing a biomarker profile for a population of cells isolated from the plurality of cells. In some embodiments, the amount of the irreversible Btk inhibitor is sufficient to induce lymphocytosis of a plurality of cells from the malignancy.

[00372] In some embodiments, the biomarker expression profile is used to diagnose, determine a prognosis, or create a predictive profile of a hematological malignancy. In some embodiments, 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.

[00373] In some embodiments, the biomarker profile indicates if a hematological malignancy involves Btk signaling. In some embodiments, the biomarker profile indicates if survival of a hematological malignancy involves Btk signaling.

[00374] In some embodiments, the biomarker profile indicates that a hematological malignancy does not involve Btk signaling. In some embodiments, the biomarker profile indicates that survival of a hematological malignancy does not involve Btk signaling.

[00375] In some embodiments, the biomarker profile indicates if a hematological malignancy involves BCR signaling. In some embodiments, the biomarker profile indicates if survival of a hematological malignancy involves BCR signaling.

[00376] In some embodiments, the biomarker profile indicates that a hematological malignancy does not involve BCR signaling. In some embodiments, the biomarker profile indicates that survival of a hematological malignancy does not involve BCR signaling.

[00377] In some embodiments, the hematological malignancy is CLL. In some embodiments, the hematological malignancy is diffuse large b-cell lymphoma (DLBCL). In some embodiments, the hematological malignancy is diffuse large b-cell lymphoma, ABC-subtype (ABC-DLBCL). In some embodiments, the hematological malignancy is mantle cell lymphoma (MCL). In some embodiments, the hematological malignancy is follicular lymphoma (FL).

[00378] In some embodiments, the biomarker is any cytogenetic, cell surface molecular or protein or RNA expression marker. In some embodiments, 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.

[00379] In some embodiments, the method further comprises providing a second treatment to the individual based on the biomarker profile. In some embodiments, the method further comprises not administering an irreversible Btk inhibitor based on the biomarker profile. In some embodiments, the method further comprises not administering second treatment based on the biomarker profile. In some embodiments, the method further comprises predicting the efficacy of a treatment based on the biomarker profile.

[00380] In certain embodiments, the methods comprise diagnosing, determining a prognosis, or creating a predictive profile of a hematological malignancy based upon the expression or presence of certain biomarkers. In other embodiments, the methods further comprise stratifying patient populations based upon the expression or presence of certain biomarkers in the affected lymphocytes. In still other embodiments, the methods further comprise determining a therapeutic for the subject based upon the expression or presence of certain biomarkers in the affected lymphocytes. In yet other embodiments, the methods further comprise predicting a response to therapy in a subject based upon the expression or presence of certain biomarkers in the affected lymphocytes.

[00381] In certain aspects, provided herein are methods of diagnosing, determining a prognosis, or creating a predictive profile of a hematological malignancy in a subject comprising: (a) administering a Btk inhibitor to the subject sufficient to result in an increase or

appearance in the blood of a subpopulation of lymphocytes; and (b) determining the expression or presence of one or more biomarkers from one or more subpopulation of lymphocytes; wherein the expression or presence of one or more biomarkers is used to diagnose the hematological malignancy, determine the prognosis of the hematological malignancy, or create a predictive profile of the hematological malignancy. In one embodiment, the increase or appearance in the blood of a subpopulation of lymphocytes is determined by immunophenotyping. In another embodiment, the increase or appearance in the blood of a subpopulation of lymphocytes is determined by fluorescent activated cell sorting (FACS).

[00382] In other aspects, provided herein are methods of stratifying a patient population having a hematological malignancy comprising: (a) administering a Btk inhibitor to the subject sufficient to result in an increase or appearance in the blood of a subpopulation of lymphocytes; and (b) determining the expression or presence of one or more biomarkers from one or more subpopulation of lymphocytes; wherein the expression or presence of one or more biomarkers is used to stratify patients for treatment of the hematological malignancy. In one embodiment, the increase or appearance in the blood of a subpopulation of lymphocytes is determined by immunophenotyping. In another embodiment, the increase or appearance in the blood of a subpopulation of lymphocytes is determined by fluorescent activated cell sorting (FACS).

[00383] In still other aspects, provided herein are methods of determining a therapeutic in a subject having a hematological malignancy comprising: (a) administering a Btk inhibitor to the subject sufficient to result in an increase or appearance in the blood of a subpopulation of lymphocytes; and (b) determining the expression or presence of one or more biomarkers from one or more subpopulation of lymphocytes; wherein the expression or presence of one or more biomarkers is used to determine the therapeutic for the treatment of the hematological malignancy. In one embodiment, the increase or appearance in the blood of a subpopulation of lymphocytes is determined by immunophenotyping. In another embodiment, the increase or appearance in the blood of a subpopulation of lymphocytes is determined by fluorescent activated cell sorting (FACS).

[00384] In yet other aspects, provided herein are methods of predicting a response to therapy in a subject having a hematological malignancy comprising: (a) administering a Btk inhibitor to the subject sufficient to result in an increase or appearance in the blood of a subpopulation of lymphocytes; and (b) determining the expression or presence of one or more biomarkers from one or more subpopulation of lymphocytes; wherein the expression or presence of one or more biomarkers is used to predict the subject's response to therapy for the hematological malignancy. In one embodiment, the increase or appearance in the blood of a subpopulation of lymphocytes is determined by immunophenotyping. In another embodiment,

the increase or appearance in the blood of a subpopulation of lymphocytes is determined by fluorescent activated cell sorting (FACS).

[00385] In certain aspects, provided herein are methods of diagnosing, determining a prognosis, or creating a predictive profile of a hematological malignancy in a subject comprising determining the expression or presence of one or more biomarkers from one or more subpopulation of lymphocytes in a subject that has received a dose of a Btk inhibitor wherein the expression or presence of one or more biomarkers is used to diagnose the hematological malignancy, determine the prognosis of the hematological malignancy, or create a predictive profile of the hematological malignancy. In one embodiment, the dose of Btk inhibitor is sufficient to result in an increase or appearance in the blood of a subpopulation of lymphocytes defined by immunophenotyping. In another embodiment, the determining the expression or presence of one or more biomarkers from one or more subpopulation of lymphocytes further comprises isolating, detecting or measuring one or more type of lymphocyte. In still another embodiment, the Btk inhibitor is a reversible or irreversible inhibitor.

[00386] In other aspects, provided herein are methods of stratifying a patient population having a hematological malignancy comprising determining the expression or presence of one or more biomarkers from one or more subpopulation of lymphocytes in a subject that has received a dose of a Btk inhibitor wherein the expression or presence of one or more biomarkers is used to stratify patients for treatment of the hematological malignancy. In one embodiment, the dose of Btk inhibitor is sufficient to result in an increase or appearance in the blood of a subpopulation of lymphocytes defined by immunophenotyping. In another embodiment, the determining the expression or presence of one or more biomarkers from one or more subpopulation of lymphocytes further comprises isolating, detecting or measuring one or more type of lymphocyte. In still another embodiment, the Btk inhibitor is a reversible or irreversible inhibitor.

[00387] In still other aspects, provided herein are methods of determining the therapeutic in a subject having a hematological malignancy comprising determining the expression or presence of one or more biomarkers from one or more subpopulation of lymphocytes in a subject that has received a dose of a Btk inhibitor wherein the expression or presence of one or more biomarkers is used to determine the therapeutic for the treatment of the hematological malignancy. In one embodiment, the dose of Btk inhibitor is sufficient to result in an increase or appearance in the blood of a subpopulation of lymphocytes defined by immunophenotyping. In another embodiment, the determining the expression or presence of one or more biomarkers from one or more subpopulation of lymphocytes further comprises isolating, detecting or

measuring one or more type of lymphocyte. In still another embodiment, the Btk inhibitor is a reversible or irreversible inhibitor.

[00388] In yet other aspects, provided herein are methods of predicting a response to therapy in a subject having a hematological malignancy comprising determining the expression or presence of one or more biomarkers from one or more circulating lymphocytes in a subject that has received a dose of a Btk inhibitor wherein the expression or presence of one or more biomarkers is used to predict the subject's response to therapy for the hematological malignancy. In one embodiment, the dose of Btk inhibitor is sufficient to result in an increase or appearance in the blood of a subpopulation of lymphocytes defined by immunophenotyping. In another embodiment, the determining the expression or presence of one or more biomarkers from one or more subpopulation of lymphocytes further comprises isolating, detecting or measuring one or more type of lymphocyte. In still another embodiment, the Btk inhibitor is a reversible or irreversible inhibitor.

[00389] As contemplated herein, any biomarker related to hematological malignancies are in some embodiments utilized in the present methods. These biomarkers include any biological molecule (found either in blood, other body fluids, or tissues) or any chromosomal abnormality that is a sign of a hematological malignancy. In certain embodiments, the biomarkers include, but are not limited to, TdT, CD5, CD11c, CD19, CD20, CD22, CD79a, CD15, CD30, CD38, CD138, CD103, CD25, ZAP-70, p53 mutational status, ATM mutational status, mutational status of IgV_H, chromosome 17 deletions (del 17p), chromosome 6 deletions (del 6q), chromosome 7 deletions (del 7q), chromosome 11 deletions (del 11q), trisomy 12, chromosome 13 deletions (del 13 q), t(11:14) chromosomal translocation, t(14:18) chromosomal translocation, CD10, CD23, beta-2 microglobulin, bcl-2 expression, CD9, presence of *Helicobacter pylori*, CD154/CD40, Akt, NF-κB,WNT, Mtor, ERK, MAPK, and Src tyrosine kinase expression. In certain embodiments, the biomarkers include ZAP-70, CD5, t(14;18), CD38, β-2 microglobulin, p53 mutational status, ATM mutational status, chromosome 17p deletion, chromosome 11q deletion, surface or cytoplasmic immunoglobulin, CD138, CD25, 6q deletion, CD19, CD20, CD22, CD11c, CD 103, chromosome 7q deletion, V_H mutational status, or a combination thereof.

[00390] In certain embodiments, subpopulations of patients having a hematological malignancy cancer or pre- that would benefit from a known treatment are identified by screening candidate subjects for one or more clinically useful biomarkers known in the art. Any clinically useful prognostic marker known to those of skill in the art can be used. In some embodiments, the subpopulation includes patients having chronic lymphocytic leukemia (CLL), and the clinically useful prognostic markers of particular interest include, but are not limited to, ZAP-70,

CD38,.beta.2 microglobulin, and cytogenetic markers, for example, p53 mutational status, ATM mutational status, chromosome deletions, such as the chromosome 17p deletion and the chromosome 11q deletion, all of which are clinically useful prognostic markers for this disease.

[00391] ZAP-70 is a tyrosine kinase that associates with the zeta subunit of the T cell antigen receptor (TCR) and plays a pivotal role in T cell activation and development (Chan et al. (1992) Cell 71:649-662). ZAP-70 undergoes tyrosine phosphorylation and is essential in mediating signal transduction following TCR stimulation. Overexpression or constitutive activation of tyrosine kinases has been demonstrated to be involved in a number of malignancies including leukemias and several types of solid tumors. For example, increased ZAP-70 RNA expression levels are a prognostic marker of chronic lymphocytic leukemia (CLL) (Rosenwald et al. (2001) J. Exp. Med. 194:1639-1647). ZAP-70 is expressed in T-cells and natural killer cells, but is not known to be expressed in normal B-cells. However, ZAP-70 is expressed at high levels in the B-cells of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) patients, and more particularly in the subset of CLL patients who tend to have the more aggressive clinical course that is found in CLL/SLL patients with unmutated Ig genes (Wiestner et al. (2003) Blood 101: 4944-4951; U.S. Patent Application Publication No. 20030203416). Because of the correlation between ZAP-70 expression levels and Ig gene mutation status, ZAP-70 can be used as a prognostic indicator to identify those patients likely to have severe disease (high ZAP-70, unmutated Ig genes), and who are therefore candidates for aggressive therapy.

[00392] CD38 is a signal transduction molecule as well as an ectoenzyme catalyzing the synthesis and degradation of cyclic ADP ribose (cADPR). CD38 expression is present at high levels in bone marrow precursor B cells, is down-regulated in resting normal B cells, and then is re-expressed in terminally differentiated plasma cells (Campana et al. (2000) *Chem. Immunol.* 75:169-188). CD38 is a reliable prognostic indicator in B-CLL, with the expression of CD38 generally indicating a less favorable outcome (D'Arena et al. (2001) *Leuk. Lymphoma* 42:109; Del Poeta et al. (2001) *Blood* 98:2633; Durig et al. (2002) *Leukemia* 16:30; Ibrahim et al. (2001) *Blood* 98:181; Deaglio et al. (2003) *Blood* 102:2146-2155). The unfavorable clinical indications that CD38 expression has been associated with include an advanced stage of disease, poor responsiveness to chemotherapy, a shorter time before initial treatment is required, and a shorter survival time (Deaglio et al. (2003) *Blood* 102:2146-2155). Initially, a strong correlation between CD38 expression and IgV gene mutation was observed, with patients having unmutated V genes displaying higher percentages of CD38.sup.+ B-CLL cells than those with mutated V genes (Damle et al. (1999) *Blood* 94:1840-1847). However, subsequent studies have indicated that CD38 expression does not always correlate with the rearrangement of the IgV genes (Hamblin et al. (2002) Blood 99:1023; Thunberg et al. (2001) Blood 97:1892).

[00393] p53 is a nuclear phosphoprotein that acts as a tumor suppressor. Wild-type p53 is involved in regulating cell growth and division. p53 binds to DNA, stimulating the production of a protein (p21) that interacts with a cell division-stimulating protein (cdk2). When p21 is bound to cdk2, the cell is blocked from entering the next stage of cell division. Mutant p53 is incapable of binding DNA effectively, thus preventing p21 from acting as the stop signal for cell division, resulting in uncontrolled cell division, and tumor formation. p53 also regulates the induction of programmed cell death (apoptosis) in response to DNA damage, cell stress or the aberrant expression of some oncogenes. Expression of wild type p53 in some cancer cell lines has been shown to restore growth suppression control (Casey et al. (1991) *Oncogene* 6:1791-1797; Takahashi et al. (1992) *Cancer Res.* 52:734-736). Mutations in p53 are found in most tumor types, including tumors of the colon, breast, lung, ovary, bladder, and many other organs. p53 mutations have been found to be associated with Burkitt's lymphoma, L3-type B-cell acute lymphoblastic leukemia, B-cell chronic lymphocytic leukemia (Gaidano et al. (1991) *Proc. Natl. Acad. Sci. U.S.A.* 88:5413-5417). p53 abnormalities have also been found associated with B-cell prolymphocytic leukemia (Lens et al. (1997) *Blood* 89:2015-2023). The gene for p53 is located on the short arm of chromosome 17 at 17p13.105-p12.

[00394] B-2-microglobulin is an extracellular protein that is noncovalently associated with the.alpha. chain of the class I major histocompatibility complex (MHC). It is detectable in the serum, and is an adverse prognostic indicator in CLL (Keating et al. (1998) *Blood* 86:606a) and Hodgkin's lymphoma (Chronowski et al. (2002) *Cancer* 95:2534-2538). It is clinically used for lymphoproliferative diseases including leukemia, lymphoma, and multiple myeloma, where serum β-2-microglobulin levels are related to tumor cell load, prognosis, and disease activity (Bataille et al. (1983) *Br. J. Haematol.* 55:439-447; Aviles et al. (1992) *Rev. Invest. Clin.* 44:215-220). P2 microglobulin is also useful in staging myeloma patients (Pasqualetti et al. (1991) *Eur. J. Cancer* 27:1123-1126).

[00395] Cytogenetic aberrations may also be used as markers to create a predictive profile of a hematological malignancy. For example, chromosome abnormalities are found in a large percentage of CLL patients and are helpful in predicting the course of CLL. For example, a 17p deletion is indicative of aggressive disease progression. In addition, CLL patients with a chromosome 17p deletion or mutation in p53, or both, are known to respond poorly to chemotherapeutics and rituximab. Allelic loss on chromosome 17p may be also be a useful prognostic marker in colorectal cancer, where patients with a 17p deletion are associated with an increased tendency of disease dissemination in colorectal cancer (Khine et al. (1994) *Cancer* 73:28-35).

[00396] Deletions of the long arm of chromosome 11 (11q) are one of the most frequent structural chromosome aberrations in various types of lymphoproliferative disorders. CLL patients with chromosome 11q deletion and possibly ATM mutations have a poor survival compared to patients without either this defect or the 17p deletion. Furthermore, an 11q deletion is often accompanied by extensive lymph node involvement (Dohner et al. (1997) Blood 89:2516-2522). This deletion also identifies patients who are at high risk for disease persistence after high-dose therapy and autologous transplantation.

[00397] The ataxia telangiectasia mutated (ATA4) gene is a tumor suppressor gene that is involved in cell cycle arrest, apoptosis, and repair of DNA double-strand breaks. It is found on chromosome 11. ATM mutations are associated with increased risk for breast cancer among women with a family history of breast cancer (Chenevix-Trench et al. (2002) *J. Natl. Cancer Inst.* 94:205-215; Thorstenson et al. (2003) *Cancer Res.* 63:3325-3333) and/or early-onset breast cancers (Izatt et al. (1999) *Genes Chromosomes Ca*ncer 26:286-294; Teraoka et al. (2001) *Cancer* 92:479-487). There is also a high frequency of association of rhabdomyosarcoma with ATM gene mutation/deletion (Zhang et al. (2003) *Cancer Biol. Ther.* 1:87-91).

[00398] Methods for detecting chromosomal abnormalities in a patient are well known in the art (see, for example, Cuneo et al. (1999) *Blood* 93:1372-1380; Dohner et al. (1997) *Blood* 89:2516-2522). Methods to measure mutated proteins, such as ATM, are well known in the art (see, for example, Butch et al. (2004) *Clin. Chem.* 50: 2302-2308).

[00399] Thus, the biomarkers that are evaluated in the methods described herein include the cell survival and apoptotic proteins described *supra*, and proteins involved in hematological malignancy-related signaling pathways. Determining the expression or presence can be at the protein or nucleic acid level. Thus, the biomarkers include these proteins and the genes encoding these proteins. Where detection is at the protein level, the biomarker protein comprises the full-length polypeptide or any detectable fragment thereof, and can include variants of these protein sequences. Similarly, where detection is at the nucleotide level, the biomarker nucleic acid includes DNA comprising the full-length coding sequence, a fragment of the full-length coding sequence, variants of these sequences, for example naturally occurring variants or splice-variants, or the complement of such a sequence. Biomarker nucleic acids also include RNA, for example, mRNA, comprising the full-length sequence encoding the biomarker protein of interest, a fragment of the full-length RNA sequence of interest, or variants of these sequences. Biomarker proteins and biomarker nucleic acids also include variants of these sequences. By "fragment" is intended a portion of the polynucleotide or a portion of the amino acid sequence and hence protein encoded thereby. Polynucleotides that are fragments of a biomarker nucleotide sequence generally comprise at least 10, 15, 20, 50, 75, 100, 150, 200, 250, 300, 350,

400, 450, 500, 550, 600, 650, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, or 1,400 contiguous nucleotides, or up to the number of nucleotides present in a full-length biomarker polynucleotide disclosed herein. A fragment of a biomarker polynucleotide will generally encode at least 15, 25, 30, 50, 100, 150, 200, or 250 contiguous amino acids, or up to the total number of amino acids present in a full-length biomarker protein of the invention. "Variant" is intended to mean substantially similar sequences. Generally, variants of a particular biomarker of the invention will have at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to that biomarker as determined by sequence alignment programs known in the art.

[00400] As provided above, any method known in the art can be used in the methods for determining the expression or presence of biomarker described herein. Circulating levels of biomarkers in a blood sample obtained from a candidate subject, can be measured, for example, by ELISA, radioimmunoassay (RIA), electrochemiluminescence (ECL), Western blot, multiplexing technologies, or other similar methods. Cell surface expression of biomarkers can be measured, for example, by flow cytometry, immunohistochemistry, Western Blot, immunoprecipitation, magnetic bead selection, and quantification of cells expressing either of these cell surface markers. Biomarker RNA expression levels could be measured by RT-PCR, Qt-PCR, microarray, Northern blot, or other similar technologies.

[00401] As previously noted, determining the expression or presence of the biomarker of interest at the protein or nucleotide level can be accomplished using any detection method known to those of skill in the art. By "detecting expression" or "detecting the level of" is intended determining the expression level or presence of a biomarker protein or gene in the biological sample. Thus, "detecting expression" encompasses instances where a biomarker is determined not to be expressed, not to be detectably expressed, expressed at a low level, expressed at a normal level, or overexpressed.

[00402] In certain aspects of the method provided herein, the one or more subpopulation of lymphocytes are isolated, detected or measured. In certain embodiments, the one or more subpopulation of lymphocytes are isolated, detected or measured using immunophenotyping techniques. In other embodiments, the one or more subpopulation of lymphocytes are isolated, detected or measured using fluorescence activated cell sorting (FACS) techniques.

[00403] In certain embodiments of the methods provided herein, the one or more biomarkers comprises ZAP-70, CD5, t(14;18), CD38, β-2 microglobulin, p53 mutational status, ATM mutational status, chromosome 17p deletion, chromosome 11q deletion, surface or cytoplasmic immunoglobulin, CD138, CD25, 6q deletion, CD19, CD20, CD22, CD11c, CD 103, chromosome 7q deletion, VH mutational status, or a combination thereof.

[00404] In certain aspects, the methods described herein, the determining step requires determining the expression or presence of a combination of biomarkers. In certain embodiment, the combination of biomarkers is CD19 and CD5 or CD20 and CD5.

[00405] In certain aspects, the expression or presence of these various biomarkers and any clinically useful prognostic markers in a biological sample can be detected at the protein or nucleic acid level, using, for example, immunohistochemistry techniques or nucleic acid-based techniques such as in situ hybridization and RT-PCR. In one embodiments, the expression or presence of one or more biomarkers is carried out by a means for nucleic acid amplification, a means for nucleic acid sequencing, a means utilizing a nucleic acid microarray (DNA and RNA), or a means for in situ hybridization using specifically labeled probes.

[00406] In other embodiments, the determining the expression or presence of one or more biomarkers is carried out through gel electrophoresis. In one embodiment, the determination is carried out through transfer to a membrane and hybridization with a specific probe.

[00407] In other embodiments, the determining the expression or presence of one or more biomarkers carried out by a diagnostic imaging technique.

[00408] In still other embodiments, the determining the expression or presence of one or more biomarkers carried out by a detectable solid substrate. In one embodiment, the detectable solid substrate is paramagnetic nanoparticles functionalized with antibodies.

[00409] In another aspect, provided herein are methods for detecting or measuring residual lymphoma following a course of treatment in order to guide continuing or discontinuing treatment or changing from one therapeutic to another comprising determining the expression or presence of one or more biomarkers from one or more subpopulation of lymphocytes in a subject wherein the course of treatment is treatment with a Btk inhibitor.

[00410] Methods for detecting expression of the biomarkers described herein, and optionally cytokine markers, within the test and control biological samples comprise any methods that determine the quantity or the presence of these markers either at the nucleic acid or protein level. Such methods are well known in the art and include but are not limited to western blots, northern blots, ELISA, immunoprecipitation, immunofluorescence, flow cytometry, immunohistochemistry, nucleic acid hybridization techniques, nucleic acid reverse transcription methods, and nucleic acid amplification methods. In particular embodiments, expression of a biomarker is detected on a protein level using, for example, antibodies that are directed against specific biomarker proteins. These antibodies can be used in various methods such as Western blot, ELISA, multiplexing technologies, immunoprecipitation, or immunohistochemistry techniques. In some embodiments, detection of cytokine markers is accomplished by electrochemiluminescence (ECL).

[00411] Any means for specifically identifying and quantifying a biomarker (for example, biomarker, a biomarker of cell survival or proliferation, a biomarker of apoptosis, a biomarker of a Btk-mediated signaling pathway) in the biological sample of a candidate subject is contemplated. Thus, in some embodiments, expression level of a biomarker protein of interest in a biological sample is detected by means of a binding protein capable of interacting specifically with that biomarker protein or a biologically active variant thereof. Preferably, labeled antibodies, binding portions thereof, or other binding partners may be used. The word "label" when used herein refers to a detectable compound or composition that is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition that is detectable.

[00412] The antibodies for detection of a biomarker protein may be monoclonal or polyclonal in origin, or may be synthetically or recombinantly produced. The amount of complexed protein, for example, the amount of biomarker protein associated with the binding protein, for example, an antibody that specifically binds to the biomarker protein, is determined using standard protein detection methodologies known to those of skill in the art. A detailed review of immunological assay design, theory and protocols can be found in numerous texts in the art (see, for example, Ausubel et al., eds. (1995) Current Protocols in Molecular Biology) (Greene Publishing and Wiley-Interscience, NY)); Coligan et al., eds. (1994) Current Protocols in Immunology (John Wiley & Sons, Inc., New York, N.Y.).

[00413] The choice of marker used to label the antibodies will vary depending upon the application. However, the choice of the marker is readily determinable to one skilled in the art. These labeled antibodies may be used in immunoassays as well as in histological applications to detect the presence of any biomarker or protein of interest. The labeled antibodies may be polyclonal or monoclonal. Further, the antibodies for use in detecting a protein of interest may be labeled with a radioactive atom, an enzyme, a chromophoric or fluorescent moiety, or a colorimetric tag as described elsewhere herein. The choice of tagging label also will depend on the detection limitations desired. Enzyme assays (ELISAs) typically allow detection of a colored product formed by interaction of the enzyme-tagged complex with an enzyme substrate. Radionuclides that can serve as detectable labels include, for example, I-131, I-123, I-125, Y-90, Re-188, Re-186, At-211, Cu-67, Bi-212, and Pd-109. Examples of enzymes that can serve as detectable labels include, but are not limited to, horseradish peroxidase, alkaline phosphatase, beta-galactosidase, and glucose-6-phosphate dehydrogenase. Chromophoric moieties include, but are not limited to, fluorescein and rhodamine. The antibodies may be conjugated to these labels by methods known in the art. For example, enzymes and chromophoric molecules may be

conjugated to the antibodies by means of coupling agents, such as dialdehydes, carbodiimides, dimaleimides, and the like. Alternatively, conjugation may occur through a ligand-receptor pair. Examples of suitable ligand-receptor pairs are biotin-avidin or biotin-streptavidin, and antibody-antigen.

[00414] In certain embodiments, expression or presence of one or more biomarkers or other proteins of interest within a biological sample, for example, a sample of bodily fluid, is determined by radioimmunoassays or enzyme-linked immunoassays (ELISAs), competitive binding enzyme-linked immunoassays, dot blot (see, for example, Promega Protocols and Applications Guide (2nd ed.; Promega Corporation (1991), Western blot (see, for example, Sambrook et al. (1989) Molecular Cloning, A Laboratory Manual, Vol. 3, Chapter 18 (Cold Spring Harbor Laboratory Press, Plainview, N.Y.), chromatography, preferably high performance liquid chromatography (HPLC), or other assays known in the art. Thus, the detection assays can involve steps such as, but not limited to, immunoblotting, immunodiffusion, immunoelectrophoresis, or immunoprecipitation.

[00415] In certain other embodiments, the methods of the invention are useful for identifying and treating hematological malignancies, including those listed above, that are refractory to (i.e., resistant to, or have become resistant to) first-line oncotherapeutic treatments.

[00416] The expression or presence of one or more of the biomarkers described herein may also be determined at the nucleic acid level. Nucleic acid-based techniques for assessing expression are well known in the art and include, for example, determining the level of biomarker mRNA in a biological sample. Many expression detection methods use isolated RNA. Any RNA isolation technique that does not select against the isolation of mRNA can be utilized for the purification of RNA (see, e.g., Ausubel et al., ed. (1987-1999) Current Protocols in Molecular Biology (John Wiley & Sons, New York). Additionally, large numbers of tissue samples can readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process disclosed in U.S. Pat. No. 4,843,155.

[00417] Thus, in some embodiments, the detection of a biomarker or other protein of interest is assayed at the nucleic acid level using nucleic acid probes. The term "nucleic acid probe" refers to any molecule that is capable of selectively binding to a specifically intended target nucleic acid molecule, for example, a nucleotide transcript. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled, for example, with a radioactive label, a fluorescent label, an enzyme, a chemiluminescent tag, a colorimetric tag, or other labels or tags that are discussed above or that are known in the art. Examples of molecules that can be utilized as probes include, but are not limited to, RNA and DNA.

[00418] For example, isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. One method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe can be, for example, a full-length cDNA, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to an mRNA or genomic DNA encoding a biomarker, biomarker described herein above. Hybridization of an mRNA with the probe indicates that the biomarker or other target protein of interest is being expressed.

[00419] In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in a gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in detecting the level of mRNA encoding the biomarkers or other proteins of interest.

[00420] An alternative method for determining the level of a mRNA of interest in a sample involves the process of nucleic acid amplification, e.g., by RT-PCR (see, for example, U.S. Pat. No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self-sustained sequence replication (Guatelli et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi et al. (1988) *Bio/Technology* 6:1197), rolling circle replication (U.S. Pat. No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the invention, biomarker expression is assessed by quantitative fluorogenic RT-PCR (i.e., the TaqMan® System).

[00421] Expression levels of an RNA of interest may be monitored using a membrane blot (such as used in hybridization analysis such as Northern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See U.S. Pat. Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The detection of expression may also comprise using nucleic acid probes in solution.

[00422] In one embodiment of the invention, microarrays are used to determine expression or presence of one or more biomarkers. Microarrays are particularly well suited for this purpose because of the reproducibility between different experiments. DNA microarrays provide one method for the simultaneous measurement of the expression levels of large numbers of genes. Each array consists of a reproducible pattern of capture probes attached to a solid support. Labeled RNA or DNA is hybridized to complementary probes on the array and then detected by laser scanning. Hybridization intensities for each probe on the array are determined and converted to a quantitative value representing relative gene expression levels. See, U.S. Pat. Nos. 6,040,138, 5,800,992 and 6,020,135, 6,033,860, and 6,344,316, which are incorporated herein by reference. High-density oligonucleotide arrays are particularly useful for determining the gene expression profile for a large number of RNA's in a sample.

[00423] Techniques for the synthesis of these arrays using mechanical synthesis methods are described in, e.g., U.S. Pat. No. 5,384,261, incorporated herein by reference in its entirety. Although a planar array surface is preferred, the array may be fabricated on a surface of virtually any shape or even a multiplicity of surfaces. Arrays may be peptides or nucleic acids on beads, gels, polymeric surfaces, fibers such as fiber optics, glass or any other appropriate substrate, see U.S. Pat. Nos. 5,770,358, 5,789,162, 5,708,153, 6,040,193 and 5,800,992, each of which is hereby incorporated in its entirety for all purposes. Arrays may be packaged in such a manner as to allow for diagnostics or other manipulation of an all-inclusive device. See, for example, U.S. Pat. Nos. 5,856,174 and 5,922,591, herein incorporated by reference.

Pharmaceutical Compositions/Formulations

[00424] Pharmaceutical compositions may be formulated in a conventional manner using one or more physiologically acceptable carriers including excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. Any of the well-known techniques, carriers, and excipients may be used as suitable and as understood in the art. A summary of pharmaceutical compositions described herein may be found, for example, in *Remington: The Science and Practice of Pharmacy*, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., *Pharmaceutical Dosage Forms*, Marcel Decker, New York, N.Y., 1980; and *Pharmaceutical Dosage Forms and Drug Delivery* Systems, Seventh Ed. (Lippincott Williams & Wilkins1999), herein incorporated by reference in their entirety.

[00425] A pharmaceutical composition, as used herein, refers to a mixture of a compound described herein, such as, for example, compounds of Formula D or the second agent, with other chemical components, such as carriers, stabilizers, diluents, dispersing agents, suspending agents, thickening agents, and/or excipients. The pharmaceutical composition facilitates administration of the compound to an organism. In practicing the methods of treatment or use provided herein, therapeutically effective amounts of compounds described herein are administered in a pharmaceutical composition to a mammal having a disease, disorder, or condition to be treated. Preferably, the mammal is a human. A therapeutically effective amount can vary widely depending on the severity of the disease, the age and relative health of the subject, the potency of the compound used and other factors. The compounds can be used singly or in combination with one or more therapeutic agents as components of mixtures.

[00426] In certain embodiments, compositions may also include one or more pH adjusting agents or buffering agents, including acids such as acetic, boric, citric, lactic, phosphoric and hydrochloric acids; bases such as sodium hydroxide, sodium phosphate, sodium borate, sodium citrate, sodium acetate, sodium lactate and tris-hydroxymethylaminomethane; and buffers such as citrate/dextrose, sodium bicarbonate and ammonium chloride. Such acids, bases and buffers are included in an amount required to maintain pH of the composition in an acceptable range.

[00427] In other embodiments, compositions may also include one or more salts in an amount required to bring osmolality of the composition into an acceptable range. Such salts include those having sodium, potassium or ammonium cations and chloride, citrate, ascorbate, borate, phosphate, bicarbonate, sulfate, thiosulfate or bisulfite anions; suitable salts include sodium chloride, potassium chloride, sodium thiosulfate, sodium bisulfite and ammonium sulfate.

[00428] The term "pharmaceutical combination" as used herein, means a product that results from the mixing or combining of more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients. The term "fixed combination" means that the active ingredients, e.g. a compound described herein and a co-agent, are both administered to a patient simultaneously in the form of a single entity or dosage. The term "non-fixed combination" means that the active ingredients, e.g. a compound described herein and a co-agent, are administered to a patient as separate entities either simultaneously, concurrently or sequentially with no specific intervening time limits, wherein such administration provides effective levels of the two compounds in the body of the patient. The latter also applies to cocktail therapy, e.g. the administration of three or more active ingredients.

[00429] The pharmaceutical formulations described herein can be administered to a subject by multiple administration routes, including but not limited to, oral, parenteral (e.g.,

intravenous, subcutaneous, intramuscular), intranasal, buccal, topical, rectal, or transdermal administration routes. The pharmaceutical formulations described herein include, but are not limited to, aqueous liquid dispersions, self-emulsifying dispersions, solid solutions, liposomal dispersions, aerosols, solid dosage forms, powders, immediate release formulations, controlled release formulations, fast melt formulations, tablets, capsules, pills, delayed release formulations, extended release formulations, pulsatile release formulations, multiparticulate formulations, and mixed immediate and controlled release formulations.

[00430] Pharmaceutical compositions including a compound described herein may be manufactured in a conventional manner, such as, by way of example only, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or compression processes.

[00431] "Antifoaming agents" reduce foaming during processing which can result in coagulation of aqueous dispersions, bubbles in the finished film, or generally impair processing. Exemplary anti-foaming agents include silicon emulsions or sorbitan sesquoleate.

[00432] "Antioxidants" include, for example, butylated hydroxytoluene (BHT), sodium ascorbate, ascorbic acid, sodium metabisulfite and tocopherol. In certain embodiments, antioxidants enhance chemical stability where required.

[00433] In certain embodiments, compositions provided herein may also include one or more preservatives to inhibit microbial activity. Suitable preservatives include mercury-containing substances such as merfen and thiomersal; stabilized chlorine dioxide; and quaternary ammonium compounds such as benzalkonium chloride, cetyltrimethylammonium bromide and cetylpyridinium chloride.

[00434] Formulations described herein may benefit from antioxidants, metal chelating agents, thiol containing compounds and other general stabilizing agents. Examples of such stabilizing agents, include, but are not limited to: (a) about 0.5% to about 2% w/v glycerol, (b) about 0.1% to about 1% w/v methionine, (c) about 0.1% to about 2% w/v monothioglycerol, (d) about 1 mM to about 10 mM EDTA, (e) about 0.01% to about 2% w/v ascorbic acid, (f) 0.003% to about 0.02% w/v polysorbate 80, (g) 0.001% to about 0.05% w/v. polysorbate 20, (h) arginine, (i) heparin, (j) dextran sulfate, (k) cyclodextrins, (l) pentosan polysulfate and other heparinoids, (m) divalent cations such as magnesium and zinc; or (n) combinations thereof.

[00435] "Binders" impart cohesive qualities and include, e.g., alginic acid and salts thereof; cellulose derivatives such as carboxymethylcellulose, methylcellulose (e.g., Methocel®), hydroxypropylmethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose (e.g., Klucel®), ethylcellulose (e.g., Ethocel®), and microcrystalline cellulose (e.g., Avicel®); microcrystalline dextrose; amylose; magnesium aluminum silicate; polysaccharide acids; bentonites; gelatin;

polyvinylpyrrolidone/vinyl acetate copolymer; crospovidone; povidone; starch; pregelatinized starch; tragacanth, dextrin, a sugar, such as sucrose (e.g., Dipac®), glucose, dextrose, molasses, mannitol, sorbitol, xylitol (e.g., Xylitab®), and lactose; a natural or synthetic gum such as acacia, tragacanth, ghatti gum, mucilage of isapol husks, polyvinylpyrrolidone (e.g., Polyvidone® CL, Kollidon® CL, Polyplasdone® XL-10), larch arabogalactan, Veegum®, polyethylene glycol, waxes, sodium alginate, and the like.

[00436] A "carrier" or "carrier materials" include any commonly used excipients in pharmaceutics and should be selected on the basis of compatibility with compounds disclosed herein, such as, compounds of any of Formula D and the second agent, and the release profile properties of the desired dosage form. Exemplary carrier materials include, e.g., binders, suspending agents, disintegration agents, filling agents, surfactants, solubilizers, stabilizers, lubricants, wetting agents, diluents, and the like. "Pharmaceutically compatible carrier materials" may include, but are not limited to, acacia, gelatin, colloidal silicon dioxide, calcium glycerophosphate, calcium lactate, maltodextrin, glycerine, magnesium silicate, polyvinylpyrrollidone (PVP), cholesterol, cholesterol esters, sodium caseinate, soy lecithin, taurocholic acid, phosphotidylcholine, sodium chloride, tricalcium phosphate, dipotassium phosphate, cellulose and cellulose conjugates, sugars sodium stearoyl lactylate, carrageenan, monoglyceride, diglyceride, pregelatinized starch, and the like. See, e.g., *Remington: The Science and Practice of Pharmacy*, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., *Pharmaceutical Dosage Forms*, Marcel Decker, New York, N.Y., 1980; and *Pharmaceutical Dosage Forms and* Drug Delivery Systems, Seventh Ed. (Lippincott Williams & Wilkins1999).

[00437] "Dispersing agents," and/or "viscosity modulating agents" include materials that control the diffusion and homogeneity of a drug through liquid media or a granulation method or blend method. In some embodiments, these agents also facilitate the effectiveness of a coating or eroding matrix. Exemplary diffusion facilitators/dispersing agents include, e.g., hydrophilic polymers, electrolytes, Tween ® 60 or 80, PEG, polyvinylpyrrolidone (PVP; commercially known as Plasdone®), and the carbohydrate-based dispersing agents such as, for example, hydroxypropyl celluloses (e.g., HPC, HPC-SL, and HPC-L), hydroxypropyl methylcelluloses (e.g., HPMC K100, HPMC K4M, HPMC K15M, and HPMC K100M), carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose phthalate, hydroxypropylmethylcellulose acetate stearate (HPMCAS), noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol (PVA), vinyl pyrrolidone/vinyl acetate copolymer (S630), 4-(1,1,3,3-tetramethylbutyl)-

phenol polymer with ethylene oxide and formaldehyde (also known as tyloxapol), poloxamers (e.g., Pluronics F68®, F88®, and F108®, which are block copolymers of ethylene oxide and propylene oxide); and poloxamines (e.g., Tetronic 908®, also known as Poloxamine 908®, which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine (BASF Corporation, Parsippany, N.J.)), polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, polyvinylpyrrolidone K25, or polyvinylpyrrolidone K30, polyvinylpyrrolidone/vinyl acetate copolymer (S-630), polyethylene glycol, e.g., the polyethylene glycol can have a molecular weight of about 300 to about 6000, or about 3350 to about 4000, or about 7000 to about 5400, sodium carboxymethylcellulose, methylcellulose, polysorbate-80, sodium alginate, gums, such as, e.g., gum tragacanth and gum acacia, guar gum, xanthans, including xanthan gum, sugars, cellulosics, such as, e.g., sodium carboxymethylcellulose, methylcellulose, sodium carboxymethylcellulose, polysorbate-80, sodium alginate, polyethoxylated sorbitan monolaurate, polyethoxylated sorbitan monolaurate, povidone, carbomers, polyvinyl alcohol (PVA), alginates, chitosans and combinations thereof. Plasticizers such as cellulose or triethyl cellulose can also be used as dispersing agents. Dispersing agents particularly useful in liposomal dispersions and self-emulsifying dispersions are dimyristoyl phosphatidyl choline, natural phosphatidyl choline from eggs, natural phosphatidyl glycerol from eggs, cholesterol and isopropyl myristate.

[00438] Combinations of one or more erosion facilitator with one or more diffusion facilitator can also be used in the present compositions.

[00439] The term "diluent" refers to chemical compounds that are used to dilute the compound of interest prior to delivery. Diluents can also be used to stabilize compounds because they can provide a more stable environment. Salts dissolved in buffered solutions (which also can provide pH control or maintenance) are utilized as diluents in the art, including, but not limited to a phosphate buffered saline solution. In certain embodiments, diluents increase bulk of the composition to facilitate compression or create sufficient bulk for homogenous blend for capsule filling. Such compounds include e.g., lactose, starch, mannitol, sorbitol, dextrose, microcrystalline cellulose such as Avicel®; dibasic calcium phosphate, dicalcium phosphate dihydrate; tricalcium phosphate, calcium phosphate; anhydrous lactose, spray-dried lactose; pregelatinized starch, compressible sugar, such as Di-Pac® (Amstar); mannitol, hydroxypropylmethylcellulose, hydroxypropylmethylcellulose acetate stearate, sucrose-based diluents, confectioner's sugar; monobasic calcium sulfate monohydrate, calcium sulfate dihydrate; calcium lactate trihydrate, dextrates; hydrolyzed cereal solids, amylose; powdered cellulose, calcium carbonate; glycine, kaolin; mannitol, sodium chloride; inositol, bentonite, and the like.

[00440] The term "disintegrate" includes both the dissolution and dispersion of the dosage form when contacted with gastrointestinal fluid. "Disintegration agents or disintegrants" facilitate the breakup or disintegration of a substance. Examples of disintegration agents include a starch, e.g., a natural starch such as corn starch or potato starch, a pregelatinized starch such as National 1551 or Amijel®, or sodium starch glycolate such as Promogel® or Explotab®, a cellulose such as a wood product, methylcrystalline cellulose, e.g., Avicel®, Avicel® PH101, Avicel® PH102, Avicel® PH105, Elcema® P100, Emcocel®, Vivacel®, Ming Tia®, and Solka-Floc®, methylcellulose, croscarmellose, or a cross-linked cellulose, such as cross-linked sodium carboxymethylcellulose (Ac-Di-Sol®), cross-linked carboxymethylcellulose, or cross-linked croscarmellose, a cross-linked starch such as sodium starch glycolate, a cross-linked polymer such as crospovidone, a cross-linked polyvinylpyrrolidone, alginate such as alginic acid or a salt of alginic acid such as sodium alginate, a clay such as Veegum® HV (magnesium aluminum silicate), a gum such as agar, guar, locust bean, Karaya, pectin, or tragacanth, sodium starch glycolate, bentonite, a natural sponge, a surfactant, a resin such as a cation-exchange resin, citrus pulp, sodium lauryl sulfate, sodium lauryl sulfate in combination starch, and the like.

[00441] "Drug absorption" or "absorption" typically refers to the process of movement of drug from site of administration of a drug across a barrier into a blood vessel or the site of action, e.g., a drug moving from the gastrointestinal tract into the portal vein or lymphatic system.

[00442] An "enteric coating" is a substance that remains substantially intact in the stomach but dissolves and releases the drug in the small intestine or colon. Generally, the enteric coating comprises a polymeric material that prevents release in the low pH environment of the stomach but that ionizes at a higher pH, typically a pH of 6 to 7, and thus dissolves sufficiently in the small intestine or colon to release the active agent therein.

[00443] "Erosion facilitators" include materials that control the erosion of a particular material in gastrointestinal fluid. Erosion facilitators are generally known to those of ordinary skill in the art. Exemplary erosion facilitators include, e.g., hydrophilic polymers, electrolytes, proteins, peptides, and amino acids.

[00444] "Filling agents" include compounds such as lactose, calcium carbonate, calcium phosphate, dibasic calcium phosphate, calcium sulfate, microcrystalline cellulose, cellulose powder, dextrose, dextrates, dextran, starches, pregelatinized starch, sucrose, xylitol, lactitol, mannitol, sorbitol, sodium chloride, polyethylene glycol, and the like.

[00445] "Flavoring agents" and/or "sweeteners" useful in the formulations described herein, include, e.g., acacia syrup, acesulfame K, alitame, anise, apple, aspartame, banana, Bavarian cream, berry, black currant, butterscotch, calcium citrate, camphor, caramel, cherry,

cherry cream, chocolate, cinnamon, bubble gum, citrus, citrus punch, citrus cream, cotton candy, cocoa, cola, cool cherry, cool citrus, cyclamate, cylamate, dextrose, eucalyptus, eugenol, fructose, fruit punch, ginger, glycyrrhetinate, glycyrrhiza (licorice) syrup, grape, grapefruit, honey, isomalt, lemon, lime, lemon cream, monoammonium glyrrhizinate (MagnaSweet®), maltol, mannitol, maple, marshmallow, menthol, mint cream, mixed berry, neohesperidine DC, neotame, orange, pear, peach, peppermint, peppermint cream, Prosweet® Powder, raspberry, root beer, rum, saccharin, safrole, sorbitol, spearmint, spearmint cream, strawberry, strawberry cream, stevia, sucralose, sucrose, sodium saccharin, saccharin, aspartame, acesulfame potassium, mannitol, talin, sylitol, sucralose, sorbitol, Swiss cream, tagatose, tangerine, thaumatin, tutti fruitti, vanilla, walnut, watermelon, wild cherry, wintergreen, xylitol, or any combination of these flavoring ingredients, e.g., anise-menthol, cherry-anise, cinnamon-orange, cherry-cinnamon, chocolate-mint, honey-lemon, lemon-lime, lemon-mint, menthol-eucalyptus, orange-cream, vanilla-mint, and mixtures thereof.

[00446] "Lubricants" and "glidants" are compounds that prevent, reduce or inhibit adhesion or friction of materials. Exemplary lubricants include, e.g., stearic acid, calcium hydroxide, talc, sodium stearyl fumerate, a hydrocarbon such as mineral oil, or hydrogenated vegetable oil such as hydrogenated soybean oil (Sterotex®), higher fatty acids and their alkali-metal and alkaline earth metal salts, such as aluminum, calcium, magnesium, zinc, stearic acid, sodium stearates, glycerol, talc, waxes, Stearowet®, boric acid, sodium benzoate, sodium acetate, sodium chloride, leucine, a polyethylene glycol (e.g., PEG-4000) or a methoxypolyethylene glycol such as Carbowax™, sodium oleate, sodium benzoate, glyceryl behenate, polyethylene glycol, magnesium or sodium lauryl sulfate, colloidal silica such as Syloid™, Cab-O-Sil®, a starch such as corn starch, silicone oil, a surfactant, and the like.

[00447] A "measurable serum concentration" or "measurable plasma concentration" describes the blood serum or blood plasma concentration, typically measured in mg, □g, or ng of therapeutic agent per ml, dl, or l of blood serum, absorbed into the bloodstream after administration. As used herein, measurable plasma concentrations are typically measured in ng/ml or □g/ml.

[00448] "Pharmacodynamics" refers to the factors which determine the biologic response observed relative to the concentration of drug at a site of action.

[00449] "Pharmacokinetics" refers to the factors which determine the attainment and maintenance of the appropriate concentration of drug at a site of action.

[00450] "Plasticizers" are compounds used to soften the microencapsulation material or film coatings to make them less brittle. Suitable plasticizers include, e.g., polyethylene glycols such as PEG 300, PEG 400, PEG 600, PEG 1450, PEG 3350, and PEG 800, stearic acid,

propylene glycol, oleic acid, triethyl cellulose and triacetin. In some embodiments, plasticizers can also function as dispersing agents or wetting agents.

[00451] "Solubilizers" include compounds such as triacetin, triethylcitrate, ethyl oleate, ethyl caprylate, sodium lauryl sulfate, sodium doccusate, vitamin E TPGS, dimethylacetamide, N-methylpyrrolidone, N-hydroxyethylpyrrolidone, polyvinylpyrrolidone, hydroxypropylmethyl cellulose, hydroxypropyl cyclodextrins, ethanol, n-butanol, isopropyl alcohol, cholesterol, bile salts, polyethylene glycol 200-600, glycofurol, transcutol, propylene glycol, and dimethyl isosorbide and the like.

[00452] "Stabilizers" include compounds such as any antioxidation agents, buffers, acids, preservatives and the like.

[00453] "Steady state," as used herein, is when the amount of drug administered is equal to the amount of drug eliminated within one dosing interval resulting in a plateau or constant plasma drug exposure.

[00454] "Suspending agents" include compounds such as polyvinylpyrrolidone, e.g., polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, polyvinylpyrrolidone K25, or polyvinylpyrrolidone K30, vinyl pyrrolidone/vinyl acetate copolymer (S630), polyethylene glycol, e.g., the polyethylene glycol can have a molecular weight of about 300 to about 6000, or about 3350 to about 4000, or about 7000 to about 5400, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, hydroxymethylcellulose acetate stearate, polysorbate-80, hydroxyethylcellulose, sodium alginate, gums, such as, e.g., gum tragacanth and gum acacia, guar gum, xanthans, including xanthan gum, sugars, cellulosics, such as, e.g., sodium carboxymethylcellulose, methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, hydroxyethylcellulose, polysorbate-80, sodium alginate, polyethoxylated sorbitan monolaurate, polyethoxylated sorbitan monolaurate, povidone and the like.

[00455] "Surfactants" include compounds such as sodium lauryl sulfate, sodium docusate, Tween 60 or 80, triacetin, vitamin E TPGS, sorbitan monooleate, polyoxyethylene sorbitan monooleate, polysorbates, polaxomers, bile salts, glyceryl monostearate, copolymers of ethylene oxide and propylene oxide, e.g., Pluronic® (BASF), and the like. Some other surfactants include polyoxyethylene fatty acid glycerides and vegetable oils, *e.g.*, polyoxyethylene (60) hydrogenated castor oil; and polyoxyethylene alkylethers and alkylphenyl ethers, *e.g.*, octoxynol 10, octoxynol 40. In some embodiments, surfactants may be included to enhance physical stability or for other purposes.

[00456] "Viscosity enhancing agents" include, e.g., methyl cellulose, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose,

hydroxypropylmethyl cellulose acetate stearate, hydroxypropylmethyl cellulose phthalate, carbomer, polyvinyl alcohol, alginates, acacia, chitosans and combinations thereof.

[00457] "Wetting agents" include compounds such as oleic acid, glyceryl monostearate, sorbitan monooleate, sorbitan monolaurate, triethanolamine oleate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monolaurate, sodium docusate, sodium oleate, sodium lauryl sulfate, sodium doccusate, triacetin, Tween 80, vitamin E TPGS, ammonium salts and the like.

Dosage Forms

[00458] The compositions described herein can be formulated for administration to a subject via any conventional means including, but not limited to, oral, parenteral (e.g., intravenous, subcutaneous, or intramuscular), buccal, intranasal, rectal or transdermal administration routes. As used herein, the term "subject" is used to mean an animal, preferably a mammal, including a human or non-human. The terms patient and subject may be used interchangeably.

[00459] Moreover, the pharmaceutical compositions described herein, which include a compound of any of Formula D or the second agent can be formulated into any suitable dosage form, including but not limited to, aqueous oral dispersions, liquids, gels, syrups, elixirs, slurries, suspensions and the like, for oral ingestion by a patient to be treated, solid oral dosage forms, aerosols, controlled release formulations, fast melt formulations, effervescent formulations, lyophilized formulations, tablets, powders, pills, dragees, capsules, delayed release formulations, extended release formulations, pulsatile release formulations, multiparticulate formulations, and mixed immediate release and controlled release formulations.

[00460] Pharmaceutical preparations for oral use can be obtained by mixing one or more solid excipient with one or more of the compounds described herein, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include, for example, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methylcellulose, microcrystalline cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose; or others such as: polyvinylpyrrolidone (PVP or povidone) or calcium phosphate. If desired, disintegrating agents may be added, such as the cross-linked croscarmellose sodium, polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[00461] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc,

polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

[00462] Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

[00463] In some embodiments, the solid dosage forms disclosed herein may be in the form of a tablet, (including a suspension tablet, a fast-melt tablet, a bite-disintegration tablet, a rapid-disintegration tablet, an effervescent tablet, or a caplet), a pill, a powder (including a sterile packaged powder, a dispensable powder, or an effervescent powder) a capsule (including both soft or hard capsules, e.g., capsules made from animal-derived gelatin or plant-derived HPMC, or "sprinkle capsules"), solid dispersion, solid solution, bioerodible dosage form, controlled release formulations, pulsatile release dosage forms, multiparticulate dosage forms, pellets, granules, or an aerosol. In other embodiments, the pharmaceutical formulation is in the form of a powder. In still other embodiments, the pharmaceutical formulation is in the form of a tablet, including but not limited to, a fast-melt tablet. Additionally, pharmaceutical formulations described herein may be administered as a single capsule or in multiple capsule dosage form. In some embodiments, the pharmaceutical formulation is administered in two, or three, or four, capsules or tablets.

[00464] In some embodiments, solid dosage forms, e.g., tablets, effervescent tablets, and capsules, are prepared by mixing particles of a compound of any of Formula (A1-A6), Formula (B1-B6), Formula (C1-C6), or Formula (D1-D6), with one or more pharmaceutical excipients to form a bulk blend composition. When referring to these bulk blend compositions as homogeneous, it is meant that the particles of the compound of any of Formula (A1-A6), Formula (B1-B6), Formula (C1-C6), or Formula (D1-D6), are dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms, such as tablets, pills, and capsules. The individual unit dosages may also include film coatings, which disintegrate upon oral ingestion or upon contact with diluent. These formulations can be manufactured by conventional pharmacological techniques.

[00465] Conventional pharmacological techniques include, e.g., one or a combination of methods: (1) dry mixing, (2) direct compression, (3) milling, (4) dry or non-aqueous granulation, (5) wet granulation, or (6) fusion. See, e.g., Lachman et al., *The Theory and Practice of Industrial Pharmacy* (1986). Other methods include, e.g., spray drying, pan coating, melt granulation, granulation, fluidized bed spray drying or coating (e.g., wurster coating), tangential coating, top spraying, tableting, extruding and the like.

[00466] The pharmaceutical solid dosage forms described herein can include a compound described herein and one or more pharmaceutically acceptable additives such as a compatible carrier, binder, filling agent, suspending agent, flavoring agent, sweetening agent, disintegrating agent, dispersing agent, surfactant, lubricant, colorant, diluent, solubilizer, moistening agent, plasticizer, stabilizer, penetration enhancer, wetting agent, anti-foaming agent, antioxidant, preservative, or one or more combination thereof. In still other aspects, using standard coating procedures, such as those described in *Remington's Pharmaceutical Sciences*, 20th Edition (2000), a film coating is provided around the formulation of the compound of any of Formula (A1-A6), Formula (B1-B6), Formula (C1-C6), or Formula (D1-D6). In one embodiment, some or all of the particles of the compound of any of Formula (A1-A6), Formula (B1-B6), Formula (C1-C6), or Formula (D1-D6), are coated. In another embodiment, some or all of the particles of the compound of any of Formula (A1-A6), Formula (B1-B6), Formula (C1-C6), or Formula (D1-D6), are microencapsulated. In still another embodiment, the particles of the compound of any of Formula (A1-A6), Formula (B1-B6), Formula (C1-C6), or Formula (D1-D6), are not microencapsulated and are uncoated.

[00467] Suitable carriers for use in the solid dosage forms described herein include, but are not limited to, acacia, gelatin, colloidal silicon dioxide, calcium glycerophosphate, calcium lactate, maltodextrin, glycerine, magnesium silicate, sodium caseinate, soy lecithin, sodium chloride, tricalcium phosphate, dipotassium phosphate, sodium stearoyl lactylate, carrageenan, monoglyceride, diglyceride, pregelatinized starch, hydroxypropylmethylcellulose, hydroxypropylmethylcellulose acetate stearate, sucrose, microcrystalline cellulose, lactose, mannitol and the like.

[00468] Suitable filling agents for use in the solid dosage forms described herein include, but are not limited to, lactose, calcium carbonate, calcium phosphate, dibasic calcium phosphate, calcium sulfate, microcrystalline cellulose, cellulose powder, dextrose, dextrates, dextran, starches, pregelatinized starch, hydroxypropylmethycellulose (HPMC), hydroxypropylmethycellulose phthalate, hydroxypropylmethylcellulose acetate stearate (HPMCAS), sucrose, xylitol, lactitol, mannitol, sorbitol, sodium chloride, polyethylene glycol, and the like.

[00469] In order to release the compound of any of Formula (A1-A6), Formula (B1-B6), Formula (C1-C6), or Formula (D1-D6), from a solid dosage form matrix as efficiently as possible, disintegrants are often used in the formulation, especially when the dosage forms are compressed with binder. Disintegrants help rupturing the dosage form matrix by swelling or capillary action when moisture is absorbed into the dosage form. Suitable disintegrants for use in the solid dosage forms described herein include, but are not limited to, natural starch such as corn starch or potato starch, a pregelatinized starch such as National 1551 or Amijel®, or sodium starch glycolate such as Promogel® or Explotab®, a cellulose such as a wood product, methylcrystalline cellulose, e.g., Avicel®, Avicel® PH101, Avicel® PH102, Avicel® PH105, Elcema® P100, Emcocel®, Vivacel®, Ming Tia®, and Solka-Floc®, methylcellulose, croscarmellose, or a cross-linked cellulose, such as cross-linked sodium carboxymethylcellulose (Ac-Di-Sol®), cross-linked carboxymethylcellulose, or cross-linked croscarmellose, a cross-linked starch such as sodium starch glycolate, a cross-linked polymer such as crospovidone, a cross-linked polyvinylpyrrolidone, alginate such as alginic acid or a salt of alginic acid such as sodium alginate, a clay such as Veegum® HV (magnesium aluminum silicate), a gum such as agar, guar, locust bean, Karaya, pectin, or tragacanth, sodium starch glycolate, bentonite, a natural sponge, a surfactant, a resin such as a cation-exchange resin, citrus pulp, sodium lauryl sulfate, sodium lauryl sulfate in combination starch, and the like.

[00470] Binders impart cohesiveness to solid oral dosage form formulations: for powder filled capsule formulation, they aid in plug formation that can be filled into soft or hard shell capsules and for tablet formulation, they ensure the tablet remaining intact after compression and help assure blend uniformity prior to a compression or fill step. Materials suitable for use as binders in the solid dosage forms described herein include, but are not limited to, carboxymethylcellulose, methylcellulose (e.g., Methocel®), hydroxypropylmethylcellulose (e.g. Hypromellose USP Pharmacoat-603, hydroxypropylmethylcellulose acetate stearate (Aqoate HS-LF and HS), hydroxyethylcellulose, hydroxypropylcellulose (e.g., Klucel®), ethylcellulose (e.g., Ethocel®), and microcrystalline cellulose (e.g., Avicel®), microcrystalline dextrose, amylose, magnesium aluminum silicate, polysaccharide acids, bentonites, gelatin, polyvinylpyrrolidone/vinyl acetate copolymer, crospovidone, povidone, starch, pregelatinized starch, tragacanth, dextrin, a sugar, such as sucrose (e.g., Dipac®), glucose, dextrose, molasses, mannitol, sorbitol, xylitol (e.g., Xylitab®), lactose, a natural or synthetic gum such as acacia, tragacanth, ghatti gum, mucilage of isapol husks, starch, polyvinylpyrrolidone (e.g., Povidone® CL, Kollidon® CL, Polyplasdone® XL-10, and Povidone® K-12), larch arabogalactan, Veegum®, polyethylene glycol, waxes, sodium alginate, and the like.

[00471] In general, binder levels of 20-70% are used in powder-filled gelatin capsule formulations. Binder usage level in tablet formulations varies whether direct compression, wet granulation, roller compaction, or usage of other excipients such as fillers which itself can act as moderate binder. Formulators skilled in art can determine the binder level for the formulations, but binder usage level of up to 70% in tablet formulations is common.

[00472] Suitable lubricants or glidants for use in the solid dosage forms described herein include, but are not limited to, stearic acid, calcium hydroxide, talc, corn starch, sodium stearyl fumerate, alkali-metal and alkaline earth metal salts, such as aluminum, calcium, magnesium, zinc, stearic acid, sodium stearates, magnesium stearate, zinc stearate, waxes, Stearowet®, boric acid, sodium benzoate, sodium acetate, sodium chloride, leucine, a polyethylene glycol or a methoxypolyethylene glycol such as Carbowax™, PEG 4000, PEG 5000, PEG 6000, propylene glycol, sodium oleate, glyceryl behenate, glyceryl palmitostearate, glyceryl benzoate, magnesium or sodium lauryl sulfate, and the like.

[00473] Suitable diluents for use in the solid dosage forms described herein include, but are not limited to, sugars (including lactose, sucrose, and dextrose), polysaccharides (including dextrates and maltodextrin), polyols (including mannitol, xylitol, and sorbitol), cyclodextrins and the like.

[00474] The term "non water-soluble diluent" represents compounds typically used in the formulation of pharmaceuticals, such as calcium phosphate, calcium sulfate, starches, modified starches and microcrystalline cellulose, and microcellulose (e.g., having a density of about 0.45 g/cm^3, e.g. Avicel, powdered cellulose), and talc.

[00475] Suitable wetting agents for use in the solid dosage forms described herein include, for example, oleic acid, glyceryl monostearate, sorbitan monooleate, sorbitan monolaurate, triethanolamine oleate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monolaurate, quaternary ammonium compounds (e.g., Polyquat 10®), sodium oleate, sodium lauryl sulfate, magnesium stearate, sodium docusate, triacetin, vitamin E TPGS and the like.

[00476] Suitable surfactants for use in the solid dosage forms described herein include, for example, sodium lauryl sulfate, sorbitan monooleate, polyoxyethylene sorbitan monooleate, polysorbates, polaxomers, bile salts, glyceryl monostearate, copolymers of ethylene oxide and propylene oxide, e.g., Pluronic® (BASF), and the like.

[00477] Suitable suspending agents for use in the solid dosage forms described here include, but are not limited to, polyvinylpyrrolidone, e.g., polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, polyvinylpyrrolidone K25, or polyvinylpyrrolidone K30, polyethylene glycol, e.g., the polyethylene glycol can have a molecular weight of about 300 to

about 6000, or about 3350 to about 4000, or about 7000 to about 5400, vinyl pyrrolidone/vinyl acetate copolymer (S630), sodium carboxymethylcellulose, methylcellulose, hydroxy-propylmethylcellulose, polysorbate-80, hydroxyethylcellulose, sodium alginate, gums, such as, e.g., gum tragacanth and gum acacia, guar gum, xanthans, including xanthan gum, sugars, cellulosics, such as, e.g., sodium carboxymethylcellulose, methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, hydroxyethylcellulose, polysorbate-80, sodium alginate, polyethoxylated sorbitan monolaurate, polyethoxylated sorbitan monolaurate, povidone and the like.

[00478] Suitable antioxidants for use in the solid dosage forms described herein include, for example, e.g., butylated hydroxytoluene (BHT), sodium ascorbate, and tocopherol.

[00479] It should be appreciated that there is considerable overlap between additives used in the solid dosage forms described herein. Thus, the above-listed additives should be taken as merely exemplary, and not limiting, of the types of additives that can be included in solid dosage forms described herein. The amounts of such additives can be readily determined by one skilled in the art, according to the particular properties desired.

[00480] In other embodiments, one or more layers of the pharmaceutical formulation are plasticized. Illustratively, a plasticizer is generally a high boiling point solid or liquid. Suitable plasticizers can be added from about 0.01% to about 50% by weight (w/w) of the coating composition. Plasticizers include, but are not limited to, diethyl phthalate, citrate esters, polyethylene glycol, glycerol, acetylated glycerides, triacetin, polypropylene glycol, polyethylene glycol, triethyl citrate, dibutyl sebacate, stearic acid, stearol, stearate, and castor oil.

[00481] Compressed tablets are solid dosage forms prepared by compacting the bulk blend of the formulations described above. In various embodiments, compressed tablets which are designed to dissolve in the mouth will include one or more flavoring agents. In other embodiments, the compressed tablets will include a film surrounding the final compressed tablet. In some embodiments, the film coating can provide a delayed release of the compound of any of Formula D or the second agent, from the formulation. In other embodiments, the film coating aids in patient compliance (e.g., Opadry® coatings or sugar coating). Film coatings including Opadry® typically range from about 1% to about 3% of the tablet weight. In other embodiments, the compressed tablets include one or more excipients.

[00482] A capsule may be prepared, for example, by placing the bulk blend of the formulation of the compound of any of Formula D or the second agent, described above, inside of a capsule. In some embodiments, the formulations (non-aqueous suspensions and solutions) are placed in a soft gelatin capsule. In other embodiments, the formulations are placed in

standard gelatin capsules or non-gelatin capsules such as capsules comprising HPMC. In other embodiments, the formulation is placed in a sprinkle capsule, wherein the capsule may be swallowed whole or the capsule may be opened and the contents sprinkled on food prior to eating. In some embodiments, the therapeutic dose is split into multiple (e.g., two, three, or four) capsules. In some embodiments, the entire dose of the formulation is delivered in a capsule form.

[00483] In various embodiments, the particles of the compound of any of Formula D or the second agent, and one or more excipients are dry blended and compressed into a mass, such as a tablet, having a hardness sufficient to provide a pharmaceutical composition that substantially disintegrates within less than about 30 minutes, less than about 35 minutes, less than about 40 minutes, less than about 45 minutes, less than about 50 minutes, less than about 55 minutes, or less than about 60 minutes, after oral administration, thereby releasing the formulation into the gastrointestinal fluid.

[00484] In another aspect, dosage forms may include microencapsulated formulations. In some embodiments, one or more other compatible materials are present in the microencapsulation material. Exemplary materials include, but are not limited to, pH modifiers, erosion facilitators, anti-foaming agents, antioxidants, flavoring agents, and carrier materials such as binders, suspending agents, disintegration agents, filling agents, surfactants, solubilizers, stabilizers, lubricants, wetting agents, and diluents.

[00485] Materials useful for the microencapsulation described herein include materials compatible with compounds of any of Formula D or the second agent, which sufficiently isolate the compound of any of Formula D or the second agent, from other non-compatible excipients. Materials compatible with compounds of any of Formula D or the second agent, are those that delay the release of the compounds of any of Formula D or the second agent, *in vivo*.

[00486] Exemplary microencapsulation materials useful for delaying the release of the formulations including compounds described herein, include, but are not limited to, hydroxypropyl cellulose ethers (HPC) such as Klucel® or Nisso HPC, low-substituted hydroxypropyl cellulose ethers (L-HPC), hydroxypropyl methyl cellulose ethers (HPMC) such as Seppifilm-LC, Pharmacoat®, Metolose SR, Methocel®-E, Opadry YS, PrimaFlo, Benecel MP824, and Benecel MP843, methylcellulose polymers such as Methocel®-A, hydroxypropylmethylcellulose acetate stearate Aqoat (HF-LS, HF-LG,HF-MS) and Metolose®, Ethylcelluloses (EC) and mixtures thereof such as E461, Ethocel®, Aqualon®-EC, Surelease®, Polyvinyl alcohol (PVA) such as Opadry AMB, hydroxyethylcelluloses such as Natrosol®, carboxymethylcelluloses and salts of carboxymethylcelluloses (CMC) such as Aqualon®-CMC, polyvinyl alcohol and polyethylene glycol co-polymers such as Kollicoat IR®, monoglycerides

(Myverol), triglycerides (KLX), polyethylene glycols, modified food starch, acrylic polymers and mixtures of acrylic polymers with cellulose ethers such as Eudragit® EPO, Eudragit® L30D-55, Eudragit® FS 30D Eudragit® L100-55, Eudragit® L100, Eudragit® S100, Eudragit® RD100, Eudragit® E100, Eudragit® L12.5, Eudragit® S12.5, Eudragit® NE30D, and Eudragit® NE 40D, cellulose acetate phthalate, sepifilms such as mixtures of HPMC and stearic acid, cyclodextrins, and mixtures of these materials.

[00487] In still other embodiments, plasticizers such as polyethylene glycols, e.g., PEG 300, PEG 400, PEG 600, PEG 1450, PEG 3350, and PEG 800, stearic acid, propylene glycol, oleic acid, and triacetin are incorporated into the microencapsulation material. In other embodiments, the microencapsulating material useful for delaying the release of the pharmaceutical compositions is from the USP or the National Formulary (NF). In yet other embodiments, the microencapsulation material is Klucel. In still other embodiments, the microencapsulation material is methocel.

[00488] Microencapsulated compounds of any of Formula D or the second agent, may be formulated by methods known by one of ordinary skill in the art. Such known methods include, e.g., spray drying processes, spinning disk-solvent processes, hot melt processes, spray chilling methods, fluidized bed, electrostatic deposition, centrifugal extrusion, rotational suspension separation, polymerization at liquid-gas or solid-gas interface, pressure extrusion, or spraying solvent extraction bath. In addition to these, several chemical techniques, e.g., complex coacervation, solvent evaporation, polymer-polymer incompatibility, interfacial polymerization in liquid media, in situ polymerization, in-liquid drying, and desolvation in liquid media could also be used. Furthermore, other methods such as roller compaction, extrusion/spheronization, coacervation, or nanoparticle coating may also be used.

[00489] In one embodiment, the particles of compounds of any of Formula D or the second agent, are microencapsulated prior to being formulated into one of the above forms. In still another embodiment, some or most of the particles are coated prior to being further formulated by using standard coating procedures, such as those described in *Remington's Pharmaceutical Sciences*, 20th Edition (2000).

[00490] In other embodiments, the solid dosage formulations of the compounds of any of Formula D or the second agent, are plasticized (coated) with one or more layers. Illustratively, a plasticizer is generally a high boiling point solid or liquid. Suitable plasticizers can be added from about 0.01% to about 50% by weight (w/w) of the coating composition. Plasticizers include, but are not limited to, diethyl phthalate, citrate esters, polyethylene glycol, glycerol, acetylated glycerides, triacetin, polypropylene glycol, polyethylene glycol, triethyl citrate, dibutyl sebacate, stearic acid, stearol, stearate, and castor oil.

[00491] In other embodiments, a powder including the formulations with a compound of any of Formula D or the second agent, described herein, may be formulated to include one or more pharmaceutical excipients and flavors. Such a powder may be prepared, for example, by mixing the formulation and optional pharmaceutical excipients to form a bulk blend composition. Additional embodiments also include a suspending agent and/or a wetting agent. This bulk blend is uniformly subdivided into unit dosage packaging or multi-dosage packaging units.

[00492] In still other embodiments, effervescent powders are also prepared in accordance with the present disclosure. Effervescent salts have been used to disperse medicines in water for oral administration. Effervescent salts are granules or coarse powders containing a medicinal agent in a dry mixture, usually composed of sodium bicarbonate, citric acid and/or tartaric acid. When salts of the compositions described herein are added to water, the acids and the base react to liberate carbon dioxide gas, thereby causing "effervescence." Examples of effervescent salts include, e.g., the following ingredients: sodium bicarbonate or a mixture of sodium bicarbonate and sodium carbonate, citric acid and/or tartaric acid. Any acid-base combination that results in the liberation of carbon dioxide can be used in place of the combination of sodium bicarbonate and citric and tartaric acids, as long as the ingredients were suitable for pharmaceutical use and result in a pH of about 6.0 or higher.

[00493] In some embodiments, the solid dosage forms described herein can be formulated as enteric coated delayed release oral dosage forms, i.e., as an oral dosage form of a pharmaceutical composition as described herein which utilizes an enteric coating to affect release in the small intestine of the gastrointestinal tract. The enteric coated dosage form may be a compressed or molded or extruded tablet/mold (coated or uncoated) containing granules, powder, pellets, beads or particles of the active ingredient and/or other composition components, which are themselves coated or uncoated. The enteric coated oral dosage form may also be a capsule (coated or uncoated) containing pellets, beads or granules of the solid carrier or the composition, which are themselves coated or uncoated.

[00494] The term "delayed release" as used herein refers to the delivery so that the release can be accomplished at some generally predictable location in the intestinal tract more distal to that which would have been accomplished if there had been no delayed release alterations. In some embodiments the method for delay of release is coating. Any coatings should be applied to a sufficient thickness such that the entire coating does not dissolve in the gastrointestinal fluids at pH below about 5, but does dissolve at pH about 5 and above. It is expected that any anionic polymer exhibiting a pH-dependent solubility profile can be used as an enteric coating in the methods and compositions described herein to achieve delivery to the lower gastrointestinal

tract. In some embodiments the polymers described herein are anionic carboxylic polymers. In other embodiments, the polymers and compatible mixtures thereof, and some of their properties, include, but are not limited to:

[00495] Shellac, also called purified lac, a refined product obtained from the resinous secretion of an insect. This coating dissolves in media of pH >7;

[00496] Acrylic polymers. The performance of acrylic polymers (primarily their solubility in biological fluids) can vary based on the degree and type of substitution. Examples of suitable acrylic polymers include methacrylic acid copolymers and ammonium methacrylate copolymers. The Eudragit series E, L, S, RL, RS and NE (Rohm Pharma) are available as solubilized in organic solvent, aqueous dispersion, or dry powders. The Eudragit series RL, NE, and RS are insoluble in the gastrointestinal tract but are permeable and are used primarily for colonic targeting. The Eudragit series E dissolve in the stomach. The Eudragit series L, L-30D and S are insoluble in stomach and dissolve in the intestine;

[00497] Cellulose Derivatives. Examples of suitable cellulose derivatives are: ethyl cellulose; reaction mixtures of partial acetate esters of cellulose with phthalic anhydride. The performance can vary based on the degree and type of substitution. Cellulose acetate phthalate (CAP) dissolves in pH >6. Aquateric (FMC) is an aqueous based system and is a spray dried CAP psuedolatex with particles <1 μm. Other components in Aquateric can include pluronics, Tweens, and acetylated monoglycerides. Other suitable cellulose derivatives include: cellulose acetate trimellitate (Eastman); methylcellulose (Pharmacoat, Methocel); hydroxypropylmethyl cellulose phthalate (HPMCP); hydroxypropylmethyl cellulose succinate (HPMCS); and hydroxypropylmethylcellulose acetate succinate (e.g., AQOAT (Shin Etsu)). The performance can vary based on the degree and type of substitution. For example, HPMCP such as, HP-50, HP-55, HP-55S, HP-55F grades are suitable. The performance can vary based on the degree and type of substitution. For example, suitable grades of hydroxypropylmethylcellulose acetate succinate include, but are not limited to, AS-LG (LF), which dissolves at pH 5, AS-MG (MF), which dissolves at pH 5.5, and AS-HG (HF), which dissolves at higher pH. These polymers are offered as granules, or as fine powders for aqueous dispersions; Poly Vinyl Acetate Phthalate (PVAP). PVAP dissolves in pH >5, and it is much less permeable to water vapor and gastric fluids.

[00498] In some embodiments, the coating can, and usually does, contain a plasticizer and possibly other coating excipients such as colorants, talc, and/or magnesium stearate, which are well known in the art. Suitable plasticizers include triethyl citrate (Citroflex 2), triacetin (glyceryl triacetate), acetyl triethyl citrate (Citroflec A2), Carbowax 400 (polyethylene glycol 400), diethyl phthalate, tributyl citrate, acetylated monoglycerides, glycerol, fatty acid esters,

propylene glycol, and dibutyl phthalate. In particular, anionic carboxylic acrylic polymers usually will contain 10-25% by weight of a plasticizer, especially dibutyl phthalate, polyethylene glycol, triethyl citrate and triacetin. Conventional coating techniques such as spray or pan coating are employed to apply coatings. The coating thickness must be sufficient to ensure that the oral dosage form remains intact until the desired site of topical delivery in the intestinal tract is reached.

[00499] Colorants, detackifiers, surfactants, antifoaming agents, lubricants (e.g., carnuba wax or PEG) may be added to the coatings besides plasticizers to solubilize or disperse the coating material, and to improve coating performance and the coated product.

[00500] In other embodiments, the formulations described herein, which include compounds of Formula D or the second agent, are delivered using a pulsatile dosage form. A pulsatile dosage form is capable of providing one or more immediate release pulses at predetermined time points after a controlled lag time or at specific sites. Many other types of controlled release systems known to those of ordinary skill in the art and are suitable for use with the formulations described herein. Examples of such delivery systems include, e.g., polymer-based systems, such as polylactic and polyglycolic acid, plyanhydrides and polycaprolactone; porous matrices, nonpolymer-based systems that are lipids, including sterols, such as cholesterol, cholesterol esters and fatty acids, or neutral fats, such as mono-, di- and triglycerides; hydrogel release systems; silastic systems; peptide-based systems; wax coatings, bioerodible dosage forms, compressed tablets using conventional binders and the like. See, e.g., Liberman et al., *Pharmaceutical Dosage Forms*, 2 Ed., Vol. 1, pp. 209-214 (1990); Singh et al., *Encyclopedia of Pharmaceutical Technology*, 2nd Ed., pp. 751-753 (2002); U.S. Pat. Nos. 4,327,725, 4,624,848, 4,968,509, 5,461,140, 5,456,923, 5,516,527, 5,622,721, 5,686,105, 5,700,410, 5,977,175, 6,465,014 and 6,932,983, each of which is specifically incorporated by reference.

[00501] In some embodiments, pharmaceutical formulations are provided that include particles of the compounds of any of Formula D or the second agent, described herein and at least one dispersing agent or suspending agent for oral administration to a subject. The formulations may be a powder and/or granules for suspension, and upon admixture with water, a substantially uniform suspension is obtained.

[00502] Liquid formulation dosage forms for oral administration can be aqueous suspensions selected from the group including, but not limited to, pharmaceutically acceptable aqueous oral dispersions, emulsions, solutions, elixirs, gels, and syrups. See, e.g., Singh et al., *Encyclopedia of Pharmaceutical Technology*, 2nd Ed., pp. 754-757 (2002). In addition to the particles of compounds of Formula (A1-A6), the liquid dosage forms may include additives,

such as: (a) disintegrating agents; (b) dispersing agents; (c) wetting agents; (d) at least one preservative, (e) viscosity enhancing agents, (f) at least one sweetening agent, and (g) at least one flavoring agent. In some embodiments, the aqueous dispersions can further include a crystalline inhibitor.

[00503] The aqueous suspensions and dispersions described herein can remain in a homogenous state, as defined in The USP Pharmacists' Pharmacopeia (2005 edition, chapter 905), for at least 4 hours. The homogeneity should be determined by a sampling method consistent with regard to determining homogeneity of the entire composition. In one embodiment, an aqueous suspension can be re-suspended into a homogenous suspension by physical agitation lasting less than 1 minute. In another embodiment, an aqueous suspension can be re-suspended into a homogenous suspension by physical agitation lasting less than 45 seconds. In yet another embodiment, an aqueous suspension can be re-suspended into a homogenous suspension by physical agitation lasting less than 30 seconds. In still another embodiment, no agitation is necessary to maintain a homogeneous aqueous dispersion.

[00504] Examples of disintegrating agents for use in the aqueous suspensions and dispersions include, but are not limited to, a starch, e.g., a natural starch such as corn starch or potato starch, a pregelatinized starch such as National 1551 or Amijel®, or sodium starch glycolate such as Promogel® or Explotab®; a cellulose such as a wood product, methylcrystalline cellulose, e.g., Avicel®, Avicel® PH101, Avicel® PH102, Avicel® PH105, Elcema® P100, Emcocel®, Vivacel®, Ming Tia®, and Solka-Floc®, methylcellulose, croscarmellose, or a cross-linked cellulose, such as cross-linked sodium carboxymethylcellulose (Ac-Di-Sol®), cross-linked carboxymethylcellulose, or cross-linked croscarmellose; a cross-linked starch such as sodium starch glycolate; a cross-linked polymer such as crospovidone; a cross-linked polyvinylpyrrolidone; alginate such as alginic acid or a salt of alginic acid such as sodium alginate; a clay such as Veegum® HV (magnesium aluminum silicate); a gum such as agar, guar, locust bean, Karaya, pectin, or tragacanth; sodium starch glycolate; bentonite; a natural sponge; a surfactant; a resin such as a cation-exchange resin; citrus pulp; sodium lauryl sulfate; sodium lauryl sulfate in combination starch; and the like.

[00505] In some embodiments, the dispersing agents suitable for the aqueous suspensions and dispersions described herein are known in the art and include, for example, hydrophilic polymers, electrolytes, Tween ® 60 or 80, PEG, polyvinylpyrrolidone (PVP; commercially known as Plasdone®), and the carbohydrate-based dispersing agents such as, for example, hydroxypropylcellulose and hydroxypropyl cellulose ethers (e.g., HPC, HPC-SL, and HPC-L), hydroxypropyl methylcellulose and hydroxypropyl methylcellulose ethers (e.g. HPMC K100, HPMC K4M, HPMC K15M, and HPMC K100M), carboxymethylcellulose sodium,

methylcellulose, hydroxyethylcellulose, hydroxypropylmethyl-cellulose phthalate, hydroxypropylmethyl-cellulose acetate stearate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol (PVA), polyvinylpyrrolidone/vinyl acetate copolymer (Plasdone®, e.g., S-630), 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde (also known as tyloxapol), poloxamers (e.g., Pluronics F68®, F88®, and F108®, which are block copolymers of ethylene oxide and propylene oxide); and poloxamines (e.g., Tetronic 908®, also known as Poloxamine 908®, which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine (BASF Corporation, Parsippany, N.J.)). In other embodiments, the dispersing agent is selected from a group not comprising one of the following agents: hydrophilic polymers; electrolytes; Tween ® 60 or 80; PEG; polyvinylpyrrolidone (PVP); hydroxypropylcellulose and hydroxypropyl cellulose ethers (e.g., HPC, HPC-SL, and HPC-L); hydroxypropyl methylcellulose and hydroxypropyl methylcellulose ethers (e.g. HPMC K100, HPMC K4M, HPMC K15M, HPMC K100M, and Pharmacoat® USP 2910 (Shin-Etsu)); carboxymethylcellulose sodium; methylcellulose; hydroxyethylcellulose; hydroxypropylmethyl-cellulose phthalate; hydroxypropylmethyl-cellulose acetate stearate; non-crystalline cellulose; magnesium aluminum silicate; triethanolamine; polyvinyl alcohol (PVA); 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde; poloxamers (e.g., Pluronics F68®, F88®, and F108®, which are block copolymers of ethylene oxide and propylene oxide); or poloxamines (e.g., Tetronic 908®, also known as Poloxamine 908®).

[00506] Wetting agents suitable for the aqueous suspensions and dispersions described herein are known in the art and include, but are not limited to, cetyl alcohol, glycerol monostearate, polyoxyethylene sorbitan fatty acid esters (e.g., the commercially available Tweens® such as e.g., Tween 20® and Tween 80® (ICI Specialty Chemicals)), and polyethylene glycols (e.g., Carbowaxs 3350® and 1450®, and Carbopol 934® (Union Carbide)), oleic acid, glyceryl monostearate, sorbitan monooleate, sorbitan monolaurate, triethanolamine oleate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monolaurate, sodium oleate, sodium lauryl sulfate, sodium docusate, triacetin, vitamin E TPGS, sodium taurocholate, simethicone, phosphotidylcholine and the like

[00507] Suitable preservatives for the aqueous suspensions or dispersions described herein include, for example, potassium sorbate, parabens (e.g., methylparaben and propylparaben), benzoic acid and its salts, other esters of parahydroxybenzoic acid such as butylparaben, alcohols such as ethyl alcohol or benzyl alcohol, phenolic compounds such as phenol, or quaternary compounds such as benzalkonium chloride. Preservatives, as used herein, are incorporated into the dosage form at a concentration sufficient to inhibit microbial growth.

[00508] Suitable viscosity enhancing agents for the aqueous suspensions or dispersions described herein include, but are not limited to, methyl cellulose, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, Plasdon® S-630, carbomer, polyvinyl alcohol, alginates, acacia, chitosans and combinations thereof. The concentration of the viscosity enhancing agent will depend upon the agent selected and the viscosity desired.

[00509] Examples of sweetening agents suitable for the aqueous suspensions or dispersions described herein include, for example, acacia syrup, acesulfame K, alitame, anise, apple, aspartame, banana, Bavarian cream, berry, black currant, butterscotch, calcium citrate, camphor, caramel, cherry, cherry cream, chocolate, cinnamon, bubble gum, citrus, citrus punch, citrus cream, cotton candy, cocoa, cola, cool cherry, cool citrus, cyclamate, cylamate, dextrose, eucalyptus, eugenol, fructose, fruit punch, ginger, glycyrrhetinate, glycyrrhiza (licorice) syrup, grape, grapefruit, honey, isomalt, lemon, lime, lemon cream, monoammonium glyrrhizinate (MagnaSweet®), maltol, mannitol, maple, marshmallow, menthol, mint cream, mixed berry, neohesperidine DC, neotame, orange, pear, peach, peppermint, peppermint cream, Prosweet® Powder, raspberry, root beer, rum, saccharin, safrole, sorbitol, spearmint, spearmint cream, strawberry, strawberry cream, stevia, sucralose, sucrose, sodium saccharin, saccharin, aspartame, acesulfame potassium, mannitol, talin, sucralose, sorbitol, swiss cream, tagatose, tangerine, thaumatin, tutti fruitti, vanilla, walnut, watermelon, wild cherry, wintergreen, xylitol, or any combination of these flavoring ingredients, e.g., anise-menthol, cherry-anise, cinnamon-orange, cherry-cinnamon, chocolate-mint, honey-lemon, lemon-lime, lemon-mint, menthol-eucalyptus, orange-cream, vanilla-mint, and mixtures thereof. In one embodiment, the aqueous liquid dispersion can comprise a sweetening agent or flavoring agent in a concentration ranging from about 0.001% to about 1.0% the volume of the aqueous dispersion. In another embodiment, the aqueous liquid dispersion can comprise a sweetening agent or flavoring agent in a concentration ranging from about 0.005% to about 0.5% the volume of the aqueous dispersion. In yet another embodiment, the aqueous liquid dispersion can comprise a sweetening agent or flavoring agent in a concentration ranging from about 0.01% to about 1.0% the volume of the aqueous dispersion.

[00510] In addition to the additives listed above, the liquid formulations can also include inert diluents commonly used in the art, such as water or other solvents, solubilizing agents, and emulsifiers. Exemplary emulsifiers are ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propyleneglycol, 1,3-butyleneglycol, dimethylformamide, sodium lauryl sulfate, sodium doccusate, cholesterol, cholesterol esters, taurocholic acid, phosphotidylcholine, oils, such as cottonseed oil, groundnut oil, corn germ oil,

olive oil, castor oil, and sesame oil, glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols, fatty acid esters of sorbitan, or mixtures of these substances, and the like.

[00511] In some embodiments, the pharmaceutical formulations described herein can be self-emulsifying drug delivery systems (SEDDS). Emulsions are dispersions of one immiscible phase in another, usually in the form of droplets. Generally, emulsions are created by vigorous mechanical dispersion. SEDDS, as opposed to emulsions or microemulsions, spontaneously form emulsions when added to an excess of water without any external mechanical dispersion or agitation. An advantage of SEDDS is that only gentle mixing is required to distribute the droplets throughout the solution. Additionally, water or the aqueous phase can be added just prior to administration, which ensures stability of an unstable or hydrophobic active ingredient. Thus, the SEDDS provides an effective delivery system for oral and parenteral delivery of hydrophobic active ingredients. SEDDS may provide improvements in the bioavailability of hydrophobic active ingredients. Methods of producing self-emulsifying dosage forms are known in the art and include, but are not limited to, for example, U.S. Pat. Nos. 5,858,401, 6,667,048, and 6,960,563, each of which is specifically incorporated by reference.

[00512] It is to be appreciated that there is overlap between the above-listed additives used in the aqueous dispersions or suspensions described herein, since a given additive is often classified differently by different practitioners in the field, or is commonly used for any of several different functions. Thus, the above-listed additives should be taken as merely exemplary, and not limiting, of the types of additives that can be included in formulations described herein. The amounts of such additives can be readily determined by one skilled in the art, according to the particular properties desired.

Intranasal Formulations

[00513] Intranasal formulations are known in the art and are described in, for example, U.S. Pat. Nos. 4,476,116, 5,116,817 and 6,391,452, each of which is specifically incorporated by reference. Formulations that include a compound of any of Formula (A1-A6), Formula (B1-B6), Formula (C1-C6), or Formula (D1-D6), which are prepared according to these and other techniques well-known in the art are prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, fluorocarbons, and/or other solubilizing or dispersing agents known in the art. See, for example, Ansel, H. C. et al., Pharmaceutical Dosage Forms and Drug Delivery Systems, Sixth Ed. (1995). Preferably these compositions and formulations are prepared with suitable nontoxic pharmaceutically acceptable ingredients. These ingredients are known to those skilled in the preparation of nasal dosage forms and some of these can be found in REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY, 21st edition, 2005, a standard reference in the field. The choice of suitable carriers is highly dependent upon the exact

nature of the nasal dosage form desired, e.g., solutions, suspensions, ointments, or gels. Nasal dosage forms generally contain large amounts of water in addition to the active ingredient. Minor amounts of other ingredients such as pH adjusters, emulsifiers or dispersing agents, preservatives, surfactants, gelling agents, or buffering and other stabilizing and solubilizing agents may also be present. The nasal dosage form should be isotonic with nasal secretions.

[00514] For administration by inhalation, the compounds of any of Formula D or the second agent, described herein may be in a form as an aerosol, a mist or a powder. Pharmaceutical compositions described herein are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, such as, by way of example only, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound described herein and a suitable powder base such as lactose or starch.

Buccal Formulations

[00515] Buccal formulations that include compounds of any of Formula D or the second agent may be administered using a variety of formulations known in the art. For example, such formulations include, but are not limited to, U.S. Pat. Nos. 4,229,447, 4,596,795, 4,755,386, and 5,739,136, each of which is specifically incorporated by reference. In addition, the buccal dosage forms described herein can further include a bioerodible (hydrolysable) polymeric carrier that also serves to adhere the dosage form to the buccal mucosa. The buccal dosage form is fabricated so as to erode gradually over a predetermined time period, wherein the delivery of the compound of any of Formula D or the second agent, is provided essentially throughout. Buccal drug delivery, as will be appreciated by those skilled in the art, avoids the disadvantages encountered with oral drug administration, e.g., slow absorption, degradation of the active agent by fluids present in the gastrointestinal tract and/or first-pass inactivation in the liver. With regard to the bioerodible (hydrolysable) polymeric carrier, it will be appreciated that virtually any such carrier can be used, so long as the desired drug release profile is not compromised, and the carrier is compatible with the compound of any of Formula D or the second agent, and any other components that may be present in the buccal dosage unit. Generally, the polymeric carrier comprises hydrophilic (water-soluble and water-swellable) polymers that adhere to the wet surface of the buccal mucosa. Examples of polymeric carriers useful herein include acrylic acid polymers and co, e.g., those known as "carbomers" (Carbopol®, which may be obtained from B.F. Goodrich, is one such polymer). Other components may also be incorporated into the

buccal dosage forms described herein include, but are not limited to, disintegrants, diluents, binders, lubricants, flavoring, colorants, preservatives, and the like. For buccal or sublingual administration, the compositions may take the form of tablets, lozenges, or gels formulated in a conventional manner.

Transdermal Formulations

[00516] Transdermal formulations described herein may be administered using a variety of devices which have been described in the art. For example, such devices include, but are not limited to, U.S. Pat. Nos. 3,598,122, 3,598,123, 3,710,795, 3,731,683, 3,742,951, 3,814,097, 3,921,636, 3,972,995, 3,993,072, 3,993,073, 3,996,934, 4,031,894, 4,060,084, 4,069,307, 4,077,407, 4,201,211, 4,230,105, 4,292,299, 4,292,303, 5,336,168, 5,665,378, 5,837,280, 5,869,090, 6,923,983, 6,929,801 and 6,946,144, each of which is specifically incorporated by reference in its entirety.

[00517] The transdermal dosage forms described herein may incorporate certain pharmaceutically acceptable excipients which are conventional in the art. In one embodiments, the transdermal formulations described herein include at least three components: (1) a formulation of a compound of any of Formula D or the second agent; (2) a penetration enhancer; and (3) an aqueous adjuvant. In addition, transdermal formulations can include additional components such as, but not limited to, gelling agents, creams and ointment bases, and the like. In some embodiments, the transdermal formulation can further include a woven or non-woven backing material to enhance absorption and prevent the removal of the transdermal formulation from the skin. In other embodiments, the transdermal formulations described herein can maintain a saturated or supersaturated state to promote diffusion into the skin.

[00518] Formulations suitable for transdermal administration of compounds described herein may employ transdermal delivery devices and transdermal delivery patches and can be lipophilic emulsions or buffered, aqueous solutions, dissolved and/or dispersed in a polymer or an adhesive. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents. Still further, transdermal delivery of the compounds described herein can be accomplished by means of iontophoretic patches and the like. Additionally, transdermal patches can provide controlled delivery of the compounds of any of Formula D or the second agent. The rate of absorption can be slowed by using rate-controlling membranes or by trapping the compound within a polymer matrix or gel. Conversely, absorption enhancers can be used to increase absorption. An absorption enhancer or carrier can include absorbable pharmaceutically acceptable solvents to assist passage through the skin. For example, transdermal devices are in the form of a bandage comprising a backing member, a reservoir containing the compound optionally with carriers, optionally a rate controlling barrier to deliver the compound to the skin

of the host at a controlled and predetermined rate over a prolonged period of time, and means to secure the device to the skin.

Injectable Formulations

[00519] Formulations that include a compound of any of Formula D or the second agent, suitable for intramuscular, subcutaneous, or intravenous injection may include physiologically acceptable sterile aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and non-aqueous carriers, diluents, solvents, or vehicles including water, ethanol, polyols (propyleneglycol, polyethylene-glycol, glycerol, cremophor and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. Formulations suitable for subcutaneous injection may also contain additives such as preserving, wetting, emulsifying, and dispensing agents. Prevention of the growth of microorganisms can be ensured by various antibacterial and antifungal agents, such as parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, such as aluminum monostearate and gelatin.

[00520] For intravenous injections, compounds described herein may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. For other parenteral injections, appropriate formulations may include aqueous or nonaqueous solutions, preferably with physiologically compatible buffers or excipients. Such excipients are generally known in the art.

[00521] Parenteral injections may involve bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The pharmaceutical composition described herein may be in a form suitable for parenteral injection as a sterile suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty

acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

Other Formulations

[00522] In certain embodiments, delivery systems for pharmaceutical compounds may be employed, such as, for example, liposomes and emulsions. In certain embodiments, compositions provided herein can also include an mucoadhesive polymer, selected from among, for example, carboxymethylcellulose, carbomer (acrylic acid polymer), poly(methylmethacrylate), polyacrylamide, polycarbophil, acrylic acid/butyl acrylate copolymer, sodium alginate and dextran.

[00523] In some embodiments, the compounds described herein may be administered topically and can be formulated into a variety of topically administrable compositions, such as solutions, suspensions, lotions, gels, pastes, medicated sticks, balms, creams or ointments. Such pharmaceutical compounds can contain solubilizers, stabilizers, tonicity enhancing agents, buffers and preservatives.

[00524] The compounds described herein may also be formulated in rectal compositions such as enemas, rectal gels, rectal foams, rectal aerosols, suppositories, jelly suppositories, or retention enemas, containing conventional suppository bases such as cocoa butter or other glycerides, as well as synthetic polymers such as polyvinylpyrrolidone, PEG, and the like. In suppository forms of the compositions, a low-melting wax such as, but not limited to, a mixture of fatty acid glycerides, optionally in combination with cocoa butter is first melted.

Dosing and Treatments

[00525] 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; and (b) analyzing the mobilized plurality of cells. 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 amount of the irreversible Btk inhibitor is from 300 mg/day up to, and including, 1000 mg/day. In some embodiments, the amount of the irreversible Btk inhibitor is from 420 mg/day up to, and including, 840 mg/day. In some embodiments, the amount of the irreversible Btk inhibitor is about 420 mg/day, about 560 mg/day, or about 840 mg/day. In some embodiments, the amount

of the irreversible Btk inhibitor is about 420 mg/day. In some embodiments, the AUC_{0-24} of the Btk inhibitor is between about 150 and about 3500 ng*h/mL. In some embodiments, the AUC_{0-24} of the Btk inhibitor is between about 500 and about 1100 ng*h/mL. In some embodiments, the Btk inhibitor is administered orally. In some embodiments, the Btk inhibitor is administered once per day, twice per day, or three times per day. In some embodiments, the Btk inhibitor is administered until disease progression, unacceptable toxicity, or individual choice. In some embodiments, the Btk inhibitor is administered daily until disease progression, unacceptable toxicity, or individual choice. In some embodiments, the Btk inhibitor is administered every other day until disease progression, unacceptable toxicity, or individual choice. In some embodiments, the Btk inhibitor is a maintenance therapy.

[00526] The compounds described herein can be used in the preparation of medicaments for the inhibition of Btk or a homolog thereof, or for the treatment of diseases or conditions that would benefit, at least in part, from inhibition of Btk or a homolog thereof, including a patient and/or subject diagnosed with a hematological malignancy. In addition, a method for treating any of the diseases or conditions described herein in a subject in need of such treatment, involves administration of pharmaceutical compositions containing at least one compound of any of Formula (A), Formula (B), Formula (C), or Formula (D), described herein, or a pharmaceutically acceptable salt, pharmaceutically acceptable N-oxide, pharmaceutically active metabolite, pharmaceutically acceptable prodrug, or pharmaceutically acceptable solvate thereof, in therapeutically effective amounts to said subject.

[00527] The compositions containing the compound(s) described herein can be administered for prophylactic, therapeutic, or maintenance treatment. In some embodiments, compositions containing the compounds described herein are administered for therapeutic applications (e.g., administered to a patient diagnosed with a hematological malignancy). In some embodiments, compositions containing the compounds described herein are administered for therapeutic applications (e.g., administered to a patient susceptible to or otherwise at risk of developing a hematological malignancy). In some embodiments, compositions containing the compounds described herein are administered to a patient who is in remission as a maintenance therapy.

[00528] Amounts of a compound disclosed herein will depend on the use (e.g., therapeutic, prophylactic, or maintenance). Amounts of a compound disclosed herein will depend on severity and course of the disease or condition, previous therapy, the patient's health status, weight, and response to the drugs, and the judgment of the treating physician. It is considered well within the skill of the art for one to determine such therapeutically effective amounts by routine experimentation (including, but not limited to, a dose escalation clinical

trial). In some embodiments, the amount of the irreversible Btk inhibitor is from 300 mg/day up to, and including, 1000 mg/day. In some embodiments, the amount of the irreversible Btk inhibitor is from 420 mg/day up to, and including, 840 mg/day. In some embodiments, the amount of the Btk inhibitor is from 400 mg/day up to, and including, 860 mg/day. In some embodiments, the amount of the Btk inhibitor is about 360 mg/day. In some embodiments, the amount of the Btk inhibitor is about 420 mg/day. In some embodiments, the amount of the Btk inhibitor is about 560 mg/day. In some embodiments, the amount of the Btk inhibitor is about 840 mg/day. In some embodiments, the amount of the Btk inhibitor is from 2 mg/kg/day up to, and including, 13 mg/kg/day. In some embodiments, the amount of the Btk inhibitor is from 2.5 mg/kg/day up to, and including, 8 mg/kg/day. In some embodiments, the amount of the Btk inhibitor is from 2.5 mg/kg/day up to, and including, 6 mg/kg/day. In some embodiments, the amount of the Btk inhibitor is from 2.5 mg/kg/day up to, and including, 4 mg/kg/day. In some embodiments, the amount of the Btk inhibitor is about 2.5 mg/kg/day. In some embodiments, the amount of the Btk inhibitor is about 8 mg/kg/day.

[00529] In some embodiments, a Btk inhibitor disclosed herein is administered daily. In some embodiments, a Btk inhibitor disclosed herein is administered every other day.

[00530] In some embodiments, a Btk inhibitor disclosed herein is administered once per day. In some embodiments, a Btk inhibitor disclosed herein is administered twice per day. In some embodiments, a Btk inhibitor disclosed herein is administered here times per day. In some embodiments, a Btk inhibitor disclosed herein is administered times per day.

[00531] In some embodiments, the Btk inhibitor is administered until disease progression, unacceptable toxicity, or individual choice. In some embodiments, the Btk inhibitor is administered daily until disease progression, unacceptable toxicity, or individual choice. In some embodiments, the Btk inhibitor is administered every other day until disease progression, unacceptable toxicity, or individual choice.

[00532] In the case wherein the patient's status does improve, upon the doctor's discretion the administration of the compounds may be given continuously; alternatively, the dose of drug being administered may be temporarily reduced or temporarily suspended for a certain length of time (i.e., a "drug holiday"). The length of the drug holiday can vary between 2 days and 1 year, including by way of example only, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 28 days, 35 days, 50 days, 70 days, 100 days, 120 days, 150 days, 180 days, 200 days, 250 days, 280 days, 300 days, 320 days, 350 days, or 365 days. The dose reduction during a drug holiday may be from 10%-100%, including, by way of example only, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

[00533] Once improvement of the patient's conditions has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, can be reduced, as a function of the symptoms, to a level at which the improved disease, disorder or condition is retained. Patients can, however, require intermittent treatment on a long-term basis upon any recurrence of symptoms.

[00534] The amount of a given agent that will correspond to such an amount will vary depending upon factors such as the particular compound, the severity of the disease, the identity (e.g., weight) of the subject or host in need of treatment, but can nevertheless be routinely determined in a manner known in the art according to the particular circumstances surrounding the case, including, e.g., the specific agent being administered, the route of administration, and the subject or host being treated. In general, however, doses employed for adult human treatment will typically be in the range of 0.02-5000 mg per day, or from about 1-1500 mg per day. The desired dose may conveniently be presented in a single dose or as divided doses administered simultaneously (or over a short period of time) or at appropriate intervals, for example as two, three, four or more sub-doses per day.

[00535] The pharmaceutical composition described herein may be in unit dosage forms suitable for single administration of precise dosages. In unit dosage form, the formulation is divided into unit doses containing appropriate quantities of one or more compound. The unit dosage may be in the form of a package containing discrete quantities of the formulation. Non-limiting examples are packaged tablets or capsules, and powders in vials or ampoules. Aqueous suspension compositions can be packaged in single-dose non-reclosable containers. Alternatively, multiple-dose reclosable containers can be used, in which case it is typical to include a preservative in the composition. By way of example only, formulations for parenteral injection may be presented in unit dosage form, which include, but are not limited to ampoules, or in multi-dose containers, with an added preservative. In some embodiments, each unit dosage form comprises 210 mg of a compound disclosed herein. In some embodiments, an individual is administered 1 unit dosage form per day. In some embodiments, an individual is administered 2 unit dosage forms per day. In some embodiments, an individual is administered 3 unit dosage forms per day. In some embodiments, an individual is administered 4 unit dosage forms per day.

[00536] The foregoing ranges are merely suggestive, as the number of variables in regard to an individual treatment regime is large, and considerable excursions from these recommended values are not uncommon. Such dosages may be altered depending on a number of variables, not limited to the activity of the compound used, the disease or condition to be treated, the mode of administration, the requirements of the individual subject, the severity of the disease or condition being treated, and the judgment of the practitioner.

[00537] Toxicity and therapeutic efficacy of such therapeutic s can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, including, but not limited to, the determination of the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between the toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD_{50} and ED_{50}. Compounds exhibiting high therapeutic indices are preferred. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with minimal toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

Kits/Articles of Manufacture

[00538] The present invention also encompasses kits for carrying out the methods of the present invention. For example, the kit can comprise a labeled compound or agent capable of detecting a biomarker described herein, e.g., a biomarker of apoptosis, cellular proliferation or survival, or a Btk-mediated signaling pathway, either at the protein or nucleic acid level, in a biological sample and means for determining the amount of the biomarker in the sample (for example, an antibody or an oligonucleotide probe that binds to RNA encoding a biomarker of interest) following incubation of the sample with a BCLD therapeutic agent of interest. Kits can be packaged to allow for detection of multiple biomarkers of interest by including individual labeled compounds or agents capable of detecting each individual biomarker of interest and means for determining the amount of each biomarker in the sample.

[00539] The particular choice of the second agent used will depend upon the diagnosis of the attending physicians and their judgment of the condition of the patient and the appropriate treatment protocol of the Btk inhibitors.

EXAMPLES

[00540] The following specific and non-limiting examples are to be construed as merely illustrative, and do not limit the present disclosure in any way whatsoever. Without further elaboration, it is believed that one skilled in the art can, based on the description herein, utilize the present disclosure to its fullest extent. All publications cited herein are hereby incorporated by reference in their entirety. Where reference is made to a URL or other such identifier or address, it is understood that such identifiers can change and particular information on the internet can come and go, but equivalent information can be found by searching the internet. Reference thereto evidences the availability and public dissemination of such information.

[00541] The clinical studies provided hereinbelow are exemplified with the irreversible Btk inhibitor (R)-1-(3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one (i.e. PCI-32765/ibrutinib). In some embodiments, such studies are performed using a Btk inhibitor of any of Formulas (A), (A1), (B), (B1), (C), (C1), (D), (D1), (E) or (F). In some embodiments, such studies are performed using a Btk inhibitor of Formula D.

Example 1: Clinical Trial to Determine Safety and Efficacy of the Btk Inhibitor PCI-32765

[00542] The primary endpoints of the first-in-human dose-escalation study of PCI-32765 were to determine the adverse event profile; to determine Btk active site occupancy; to find the Maximum Tolerated Dose (MTD) (if the MTD was not reached, the maximum dose would be 3 dose levels above the dose that achieved full Btk occupancy); and to determine the pharmacokinetics (PK) of PCI-32765. The secondary endpoint was to assess tumor response to PCI-32765 monotherapy.

[00543] The dose-escalation portion of this open-label trial enrolled subjects with B-cell malignancies (histologies listed in Table 2, below) and is now completed.

[00544] Five dose levels (total n=56) were tested using a 28 day on and 7 day off treatment schedule; the dose levels were 1.25 (n=7), 2.5 (n=9), 5.0 (n=6), 8.3 (n=8) and 12.5 (n=7) mg/kg/day. Two additional groups of patients received continuous daily dosing in 35 day schedules: one group received 8.3 mg/kg/day (n=10) while the other group received 560 mg/day ("fixed" cohort; n=9). Patients with ABC DLBCL were treated at 560 mg/day; enrollment of patients with other histologies has been completed and data from these patients have been reported (Advani *et al.*, 2010).

[00545] Seven complete responses (CRs) and 23 partial response (PRs) were documented in 56 total patients; an additional 10 subjects had stable disease (SD). Responses have been observed at all dose levels and in all histologies. Response data is summarized in Table 2 (below).

Table 2. Clinical Responses to PCI-32765

	Patients	CR	PR	SD	PD	not eval	ORR (evaluable pts)	ORR (ITT[a])
CLL/SLL	16	1	10	3	0	2	79%	69%
FL	16	3	3	3	4	3	46%	38%
MCL	9	3	4	1	1	0	78%	78%
DLBCL	7	0	2	1	4	0	29%	29%
MZL/MALT	4	0	1	1	1	1	33%	25%
WM	4	0	3	1	0	0	75%	75%
Overall	**56**	**7**	**23**	**10**	**10**	**6**	**60%**	**54%**

[a] Intent-to-Treat

[00546] The MTD was not reached. Two dose limiting toxicities (DLTs) were documented: one subject had a prolonged neutropenia at the 2.5 mg/kg/day dose level and another subject had an allergic reaction at the 8.3 mg/kg/day dose level. No DLTs were observed at the final dose level (12.5 mg/kg/day). Full Btk occupancy was observed in all patients at the 2.5 mg/kg dose level.

[00547] Day 1 and Day 8 (steady-state) pharmacokinetic results are presented in Tables 3 and 4, below. Total plasma concentrations of the compound of formula D (total = bound fraction + unbound fraction) generally increased with increasing body-weight normalized doses of 1.25, 2.5, 5.0, 8.3, and 12.5 mg/kg on Day 1.

Table 3. Mean (Coefficient of Variation) Pharmacokinetic Parameters of Compound of Formula D on Day 1

Cohort	N	Dose (mg/kg)	C_{max}[a] (ng/mL)	T_{max} (h)	$AUC_{0-\infty}$ (ng•h/mL)	$t_{1/2}$[b] (h)
1	5[c]	1.25	48.7 (54%)	1.0 (0%)	181 (50%)	1.7 (27%)
2	9	2.5	90.4 (92%)	2.1 (60%)	494 (83%)	1.5 (33%)
3	6	5	86.1 (136%)	2.3 (59%)	419 (95%)	2.5 (51%)
4	8	8.3	135 (71%)	1.8 (59%)	923 (84%)	2.1 (28%)
CD	10	8.3	155 (81%)	2.2 (49%)	1027 (77%)	2.1 (35%)
5	7	12.5	383 (72%)	1.7 (28%)	1550 (57%)	1.5 (27%)
Fixed	9	560 mg fixed dose	156 (91%)	1.8 (55%)	749 (69%)	2.65 (69%)

AUC = area under the curve; CD = continuous dosing; C_{max} = maximum observed drug concentration; $t_{1/2}$ = half-life; T_{max} = time to maximum concentration

a lower limit of quantitation was 0.050 ng/mL for PCI-32765

b half-life from T_{max} to 6 hours postdose

c 2 out of 7 subjects were considered outliers

Table 4. Mean (Coefficient of Variation) Pharmacokinetic Parameters of PCI-32765 at Steady State (Day 8) (Trial PCYC-04753)

Cohort	N	Dose (mg/kg)	C_{max}[a] (ng/mL)	C_{max}/dose (g/L)	AUC_{0-24h} (ng•h/mL)	AUC_{0-24h}/dose (g•h/L)
1	5	1.25	27.0 (56%)b	22.3 (49%)	301 (61%)	250 (53%)
2	9	2.5	114 (93%)	37.2 (108%)	1840 (122%)	408 (114%)
3	6	5	112 (96%)	22.4 (96%)	1580 (89%)	291 (94%)
4	8	8.3	183 (124%)	22.0 (124%)	2330 (121%)	281 (121%)
CD	10	8.3	130 (84%)	15.6 (84%)	1545 (89%)	186 (89%)
5	7	12.5	236 (67%)	18.9 (67%)	2936 (67%)	235 (68%)
Fixed	9	560 mg fixed dose	122 (29%)	---	1553 (25%)	---

AUC = area under the curve; CD = continuous dosing; C_{max} = maximum observed drug concentration
a lower limit of quantitation was 0.050 ng/mL for PCI-32765

[00548] Day 1 area-under-the-curve values were estimated from 0 to infinity ($AUC_{0-\infty}$) and at steady-state from 0 to 24 hours post-dose (AUC_{0-24h}). C_{max} and AUC values increased with increasing dose from 1.25 to 12.5 mg/kg on Day 1 and at steady-state. Dose-normalized C_{max} and AUC values at steady-state generally showed dose proportional increases, however greater than dose proportional increases were observed at the 2.5-mg/kg dose level on Day 1 and at steady-state. The time to maximum plasma concentration (T_{max}) ranged from 1.0 to 2.3 hours. The mean half-life of the compound of formula D post-T_{max} ranged from 1.5 to 2.5 hours. In patients who received body-weight normalized doses (mg/kg/day), high intersubject variability was seen across all dose levels with regard to Day 1 $AUC_{0-\infty}$ and mean steady-state (Day 8) AUC_{0-24h}. Administration of a 560 mg/day fixed dose resulted in a mean systemic exposure to compound of formula D, measured as $AUC_{0-\infty}$, that was intermediate to mean exposures measured at the 5 and 8.3 mg/kg dose levels. At steady state (Day 8), systemic exposures in subjects that received a 560-mg fixed dose had less intersubject variability (measured as coefficient of variation for AUC_{0-24}) when compared with exposures in subjects who received body-weight normalized doses.

[00549] Analysis of PK and pharmacodynamic profiles on Day 1 showed that Btk active-site occupancy was saturated 4 and 24 hours postdose at AUC values of ≥200 ng•h/mL. At steady-state, all subjects who received dosages ≥2.5 mg/kg/day had AUC values ≥245 ng•h/mL. This indicates that despite the brief plasma half-life of compound of PCI-32765, it is an effective irreversible inhibitor for at least 24 hours, and therefore once-daily dosing is sufficient to maintain complete occupancy of the Btk active site.

[00550] In CLL, PCI-32765 inhibits chemokine-secretion and chemokine-mediated malignant cell migration and adhesion. As a correlative study within clinical trial, patients' primary tumor samples were co-cultured with nurse-like cells and incubated for 24 hours with 1 nM PCI-32765. After treatment, secreted levels of CCL3 dropped from 393 ± 172 pg/μL to 54 ± 46 pg/μL ($p<0.05$) and levels of CCL4 dropped from 2550 ± 678 pg/μL to 394 ± 188 pg/mL ($p<0.05$). Furthermore, in primary CLL cultures derived from patient samples from the same

trial, 1 μM PCI-32765 reduced CXCL12-mediated chemotaxis (57 ± 9% of control, n=10) and CXCL13-mediated chemotaxis (46 ± 5% of control, n=10). Plasma samples from CLL patients on this trial revealed high pre-treatment CCL3/4 levels, and these levels were significantly decreased after treatment: 24 hours following the first dose of PCI-32765, CCL3 levels decreased from 60 ± 29 pg/mL to 16 ± 13 pg/mL, and CCL4 pre-treatment levels decreased from 106 ± 55 pg/mL to 23 ± 12 pg/mL (n=6)

Example 2: Clinical Trial with PCI-32765 in Patients with CLL

[00551] A phase Ib/II clinical trial was performed to study the effects of PCI-32765 on individuals with Relapsed or Refractory (R/R) Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL).

[00552] Study Type: Interventional

[00553] Allocation: Non-Randomized

[00554] Endpoint Classification: Safety Study

[00555] Intervention Model: Parallel Assignment

[00556] Masking: Open Label

[00557] Primary Purpose: Treatment

[00558] Group I (elderly, naïve, individuals) received 420 mg/day of PCI-32765. Group II (elderly, naïve, individuals) received 840 mg/day of PCI-32765. Group III (R/R individuals, who had twice been treated with fludara) received 420 mg/day of PCI-32765. Group IV (R/R individuals, who had twice been treated with fludara) received 840 mg/day of PCI-32765. Patient characteristics are summarized in Tables 5 and 6.

Table 5: Patient Characteristics

	Group I - Naïve 420 mg/d (N=26)	Group II - Naïve 840 mg/d (N=5)	Total (N=31)
Age, years			
Median:	71 (66 – 84)	71 (65 – 77)	71 (65 – 84)
=70 years, # (%)	20 (77)	3 (60)	23 (74)
ECOG Performance Status, # (%)			
0	18/25 (72)	2 (40)	20/30 (67)
1	7/25 (28)	3 (60)	10/30 (33)
β2 Microglobulin > 3mg/L	7 (27)	0	7 (23)
Cytopenia at baseline, # (%)			
HGB < 11g/dL	8 (31)	2 (40)	10 (32)
Platelets < 100,000/μL	11 (42)	1 (20)	12 (39)
HGB < 11g/dL or PLT < 100,000 μL	16 (62)	3 (60)	19 (61)
Prognostic Markers, # (%)			
IgVH unmutated:	11 (42)	2/4 (50)	13/30 (43)
Del(17p):	2 (8)	0	2 (6)

	Group III - R/R CLL 420 mg/d (N=27)	Group IV - R/R CLL 840 mg/d (N=34)	Total (N=61)
Age, years			
Median:	64	64	64
Range:	40 - 81	44 - 80	40 – 81
=70 years, # (%)	9 (33)	10 (29)	19 (31)
Diagnosis, # (%)			
CLL:	26 (96)	33 (97)	59 (97)
SLL :	1 (4)	1 (3)	2 (3)
ECOG Performance Status, # (%)			
0	11 (41)	13 (38)	24 (39)
1/2	16 (59)	21 (62)	37 (61)
Prior Rx, #			
Median:	3	5	4
Range:	2 - 10	1 - 12	1 – 12
Prior therapy, # (%)			
Nucleoside analog	27 (100)	34 (100)	61 (100)
Rituximab	25 (93)	33 (97)	58 (95)
Alkylator	24 (89)	28 (82)	52 (85)
Alemtuzumab	5 (19)	3 (9)	8 (13)
Bendamustine	8 (30)	13 (38)	21 (34)
Ofatumumab	8 (30)	10 (29)	18 (30)
Bulky Disease, # (%)	13 (48)	20 (59)	33 (54)
≥ 5 cm	1 (4)	9 (26)	10 (16)
≥ 10 cm			
Cytopenia at baseline, # (%)			
ANC < 1500/μL	7 (26)	17 (50)	24 (39)
HGB < 11g/dL	4 (15)	18 (53)	22 (36)
Platelets < 100,000/μL	8 (30)	24 (71)	32 (52)
HGB < 11g/dL or PLT < 100,000 μL	9 (33)	27 (79)	36 (59)
Purine Analog Refractory, # (%) (< 12 month treatment free interval following purine analog regimen)	10 (37)	18 (53)	28 (46)
Prognostic Markers, # (%)			
IgVH unmutated:	19/25 (76)	23/28 (82)	42/53 (79)
Del(17p):	9/24 (38)	11/32 (34)	20/56 (36)
Del(11q):	8/24 (33)	14/32 (44)	22/56 (39)
β2 Microglobulin > 3mg/L	9/25 (36)	20/32 (63)	29/57 (51)

[00559] Tumor assessment was performed every 2 treatment cycles.

[00560] Study Objectives:

[00561] 1. Describe the characteristics of the antitumor effect of PCI-32765 in individuals with CLL/SLL, e.g., reduction in lymphadenopathy/splenomegaly, and kinetics of change in absolute lymphocyte count (ACL).

[00562] 2. Summarize the safety profile of PCI-32765.

[00563] Inclusion Criteria:

- FOR TREATMENT-NAIVE GROUP ONLY: Men and women ≥ 65 years of age with confirmed diagnosis of CLL/SLL, who require treatment per NCI or International Working Group guidelines11-14
- FOR RELAPSED/REFRACTORY GROUP ONLY: Men and women ≥ 18 years of age with a confirmed diagnosis of relapsed/refractory CLL/SLL unresponsive to therapy (ie, failed ≥ 2 previous treatments for CLL/SLL and at least 1 regimen had to have had a purine analog [e.g., fludarabine] for subjects with CLL)
- Body weight ≥ 40 kg
- ECOG performance status of ≤ 2
- Agreement to use contraception during the study and for 30 days after the last dose of study drug if sexually active and able to bear children
- Willing and able to participate in all required evaluations and procedures in this study protocol including swallowing capsules without difficulty
- Ability to understand the purpose and risks of the study and provide signed and dated informed consent and authorization to use protected health information (in accordance with national and local subject privacy regulations)

[00564] Exclusion Criteria:

- A life-threatening illness, medical condition or organ system dysfunction which, in the investigator's opinion, could compromise the subject's safety, interfere with the absorption or metabolism of PCI-32765 PO, or put the study outcomes at undue risk
- Any immunotherapy, chemotherapy, radiotherapy, or experimental therapy within 4 weeks before first dose of study drug (corticosteroids for disease-related symptoms allowed but require 1-week washout before study drug administration)
- Central nervous system (CNS) involvement by lymphoma
- Major surgery within 4 weeks before first dose of study drug

- Creatinine > 1.5 x institutional upper limit of normal (ULN); total bilirubin > 1.5 x ULN (unless due to Gilbert's disease); and aspartate aminotransferase (AST) or alanine aminotransferase (ALT) > 2.5 x ULN unless disease related
- Concomitant use of medicines known to cause QT prolongation or torsades de pointes
- Significant screening electrocardiogram (ECG) abnormalities including left bundle branch block, 2nd degree AV block type II, 3rd degree block, bradycardia, and QTc > 470 msec
- Lactating or pregnant

[00565] Response Criteria:

[00566] NHL IWG criteria1 were applied to SLL cases without modification.

[00567] The 2008 CLL IWG criteria were applied to CLL cases with the following modifications:

[00568] 1) An isolated lymphocytosis, in the absence of other parameters meeting the criteria for PD, was not considered PD

[00569] 2) Patients experiencing a lymphocytosis, but obtaining a PR by other measurable parameters, were classified as "nodal" response until there was a 50% reduction in ALC from baseline in which case they were categorized as PR.

[00570] 3) Patients with a normal ALC (<5K) at baseline with treatment-related lymphocytosis required normalization to <5K to be categorized as PR.

[00571] Results:

[00572] Results of the study are presented in Tables 6-11.

Table 6: Subject Disposition - Treatment Naive

	Group I - Naïve 420 mg/d (N=26)	**Group II - Naïve 840 mg/d (N=5)**
Subjects Discontinued, # (%)	4 (15)	1 (20)
Primary Reasons for Discontinuation, # (%)		
Disease Progression	1 (4)[a]	0 (0)
Adverse event	2 (8)[b,c]	1 (20)[e]
Unrelated: viral syndrome		
Unrelated: Worsening GI hemorrhage		
Possibly related: fatigue		
Subject withdrew consent "desired faster response"	1 (4)[d]	
Death on study, # (%)	0	0

days on ibrutinib: a) 280 days; b) 41 days; c) 115 days; d) 41 days; e) 9 days

Table 7: Subject Disposition - R/R CLL

	Group III - R/R CLL 420 mg/d (N=27)	Group IV - R/R CLL 840 mg/d (N=34)
Subjects Discontinued, # (%)	7 (26)	8 (24)
Primary Reasons for Discontinuation, # (%)		
Disease Progression	2 (7)	1 (3)
Adverse event	1 (4)	1 (3)
Other [2]	1 (4)[d]	
Death on study[1]	1 (4)	2 (6)

[1]Cause of death: 1 pneumonia, 1 ARDS/cryptococcal pneumonia, 1 histiocytic sarcoma
[2]Other: 5 transplant, 1 NSCLC diagnosed day 5, 1 off study drug > 2 weeks

Table 8: Best Response- Treatment Naïve

	Group I - Naïve 420 mg/d (N=26) Median f/u = 14.4 mos #(%)	Group II - Naïve 840 mg/d (N=5) Median f/u = 7.4 mos #(%)	Total (N=31) Median f/u = 12.8 mos #(%)
CR	3 (12)	0	3 (10)
PR	18 (69)	2 (40)	20 (65)
ORR (per IWCLL 2008 criteria)	81%	40%	74%
Nodal	3 (12)	1 (20)	4 (13)
SD	1 (4)	1 (20)	2 (6)
PD	0	0	0
NE	1 (4)	1 (20)	2 (6)

Table 9: Best Response - R/R CLL

	Group III - R/R CLL 420 mg/d (N=27) Median f/u = 14.4 mos #(%)	Group IV - R/R CLL 840 mg/d (N=34) Median f/u = 7.4 mos #(%)	Total (N=31) Median f/u = 12.8 mos #(%)
CR	1 (4)	0	1 (2)
PR	17 (63)	23 (68)	40 (66)
ORR (per IWCLL 2008 criteria)	67%	6 %	67%
Nodal	6 (22)	8 (24)	12 (23)
SD	1 (4)	1 (3)	2 (3)
PD	1 (4)	0	1 (2)
NE	1 (4)	2 (6)	3 (5)

Table 10: Best Response by Risk Features - Treatment Naïve

	N	ORR % (n)	CR % (n)
All Patients	31	74 (23)	10 (3)
≥ 70 years age	23	70 (16)	13 (3)
Hgb < 11 g/dL or PLT < 100K/μL at screening	19	79 (15)	11 (2)
IgVH unmutated	13	92 (12)	15 (2)
Del17p present	2	100 (2)	0 (0)
β2 Microglobulin > 3mg/L	7	86 (6)	29 (2)

Table 11: Best Response by Risk Features - R/R CLL

	n/N	ORR %
All Patients	41/61	67
≥ 70 years age	13/19	68
Bulky disease ≥ 5 cm	24/33	73
Bulky disease ≥ 10 cm	7/10	70
Hgb < 11 g/dL or PLT < 100K/μL at screening	22/36	61
IgVH unmutated	31/42	74
Del17p present	13/20	65
Del11lq present	16/22	73
β2 Microglobulin > 3mg/L	19/29	66
Purine Analog Refractory (>12 mos from any purine analog to next therapy)	17/28	61

[00573] Results from this study are further summarized in Figs. 2-7. Figure 2 depicts the LN response in patient suffering from CLL prior to and following treatment with PCI-32765. Figure 3 shows the decrease in tumor burden over the course treatment with PCI-32765 in R/R CLL patients administered 420 mg/day or 840 mg/day PCI-32765. Figure 4 presents the absolute lymphocyte count (ALC) and the sum of the product of the diameters (SPD) of the lymph nodes (LN) during the course of treatment with PCI-32765 in treatment naïve or R/R CLL patients administered 420 mg/day PCI-32765. Figure 5 presents the cumulative best response in treatment naïve patients administered 420 mg/day PCI-32765 over successive cycles (cycles 2, 5, 8, 11 and best response) of treatment. Figure 6 presents the cumulative best response in R/R CLL patients administered 420 mg/day PCI-32765 over successive cycles (cycles 2, 5, 8, 11 and best response) of treatment. Figure 7 presents a comparison between the cumulative best response in R/R CLL patients (RR) versus treatment naïve (TN) patients administered 420 mg/day PCI-32765 over successive cycles of treatment.

[00574] Conclusions:

[00575] The interim Phase II data confirm that PCI-32765 is highly active in both treatment-naïve and relapsed/ refractory CLL/ SLL patients. Class-specific rapid lymph node reduction with concurrent lymphocytosis seen in the majority of patients. 2008 CLL IWG

objective responses (PR + CR) and nodal responses appear to be durable and independent of high risk genomic features. A high proportion (86%) of relapsed or refractory patients are free-of-progression at 12 months (420mg cohort).

Example 3: Long Term Follow-Up Trial for Individuals Taking PCI-32765

[00576] The purpose of this study is to determine the long-term safety of a fixed-dose, daily regimen of PCI-32765 in subjects with B cell lymphoma or chronic lymphocytic leukemia/small lymphocytic leukemia (CLL/SLL).

[00577] Study Type: Interventional

[00578] Allocation: Non-Randomized

[00579] Endpoint Classification: Safety Study

[00580] Intervention Model: Single Group Assignment

[00581] Masking: Open Label

[00582] Primary Purpose: Treatment

[00583] Intervention: 420 mg/day of PCI-32765

[00584] Applicable conditions: B-cell Chronic Lymphocytic Leukemia; Small Lymphocytic Lymphoma; Diffuse Well-Differentiated Lymphocytic Lymphoma; B Cell Lymphoma; Follicular Lymphoma; Mantle Cell Lymphoma; Non-Hodgkin's Lymphoma; Waldenström Macroglobulinemia; Burkitt's Lymphoma; B-Cell Diffuse Lymphoma

[00585] Primary Outcome Measures:

[00586] Adverse Events/ Safety Tolerability [Time Frame: 30 days after last dose of study drug] - frequency, severity, and relatedness of adverse events

[00587] Secondary Outcome Measures:

[00588] 1. Tumor Response [Time Frame: frequency of tumor assessments done per standard of care] - tumor response will be assessed per established response criteria. This study will capture time to disease progression and duration of response.

[00589] 2. Tumor Response [Time Frame: Time to disease progression] - Duration of response as measured by established response criteria for B cell lymphoma and chronic lymphocytic leukemia

[00590] Inclusion Criteria:

- Men and women with B cell lymphoma or CLL/small lymphocytic lymphoma (SLL) who had stable disease or response to PCI-32765 PO for at least 6 months on a prior PCI-32765 study and want to continue study drug or who had disease progression on PCYC-04753 study and want to try a higher dose
- Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 2

- Agreement to use contraception during the study and for 30 days after the last dose of study drug if sexually active and able to bear children
- Willing and able to participate in all required evaluations and procedures in this study protocol including swallowing capsules without difficulty
- Ability to understand the purpose and risks of the study and provide signed and dated informed consent and authorization to use protected health information (in accordance with national and local subject privacy regulations)

[00591] Exclusion Criteria:

- A life-threatening illness, medical condition or organ system dysfunction which, in the investigator's opinion, could compromise the subject's safety, interfere with the absorption or metabolism of PCI-32765 PO, or put the study outcomes at undue risk
- Concomitant immunotherapy, chemotherapy, radiotherapy, corticosteroids (at dosages equivalent to prednisone > 20 mg/day), or experimental therapy
- Concomitant use of medicines known to cause QT prolongation or torsades de pointes
- Central nervous system (CNS) involvement by lymphoma
- Creatinine > 1.5 x institutional upper limit of normal (ULN); total bilirubin > 1.5 x ULN (unless due to Gilbert's disease); and aspartate aminotransferase (AST) or alanine aminotransferase (ALT) > 2.5 x ULN unless disease related
- Lactating or pregnant

Example 4: Phase II Study of PCI-32765 in Relapsed/Refractory MCL

[00592] The purpose of this study is to: Evaluate the efficacy of PCI-32765 in relapsed/refractory subjects with MCL who have not had prior bortezomib, and who have had prior bortezomib. The secondary objective is to evaluate the safety of a fixed daily dosing regimen of PCI-32765 capsules in this population.

[00593] Study Type: Interventional

[00594] Allocation: Non-Randomized

[00595] Endpoint Classification: Safety/Efficacy Study

[00596] Intervention Model: Parallel Assignment

[00597] Masking: Open Label

[00598] Primary Purpose: Treatment

[00599] Intervention: 560 mg/day of PCI-32765

[00600] **Primary Outcome Measures:**

[00601] To Measure the Number of Participants with a Response to Study Drug [Time Frame: Participants will be followed until progression of disease or start of another anti-cancer treatment.]

[00602] **Secondary Outcome Measures**

[00603] 1. To Measure the Number of Participants with Adverse Events as a Measure of Safety and Tolerability [Time Frame: Participants will be followed until progression of disease or start of another anti-cancer treatment.]

[00604] 2. To Measure the Number of Participants Pharmacokinetics to Assist in Determining How the Body Responds to the Study Drug [Time Frame: Procedure to be Performed During the First Month of Receiving Study Drug.]

[00605] 3. Patient Reported Outcomes [Time Frame: Participants will be followed until progression of disease or start of another anti-cancer treatment.]

[00606] 4. To measure the number of participants reported outcomes in determining the health related quality of life.

[00607] **Inclusion Criteria:**

- Men and women $\geq$ 18 years of age
- ECOG performance status of $\leq$ 2
- Pathologically confirmed MCL, with documentation of either overexpression of cyclin D1 or t(11;14), and measurable disease on cross sectional imaging that is $\geq$ 2 cm in the longest diameter and measurable in 2 perpendicular dimensions
- Documented failure to achieve at least partial response (PR) with, or documented disease progression disease after, the most recent treatment regimen
- At least 1, but no more than 5, prior treatment regimens for MCL (Note: Subjects having received $\geq$2 cycles of prior treatment with bortezomib, either as a single agent or as part of a combination therapy regimen, will be considered to be bortezomib-exposed.)
- Willing and able to participate in all required evaluations and procedures in this study protocol including swallowing capsules without difficulty
- Ability to understand the purpose and risks of the study and provide signed and dated informed consent and authorization to use protected health information (in accordance with national and local subject privacy regulations)

[00608] **Major exclusion criteria:**

- Prior chemotherapy within 3 weeks, nitrosoureas within 6 weeks, therapeutic anticancer antibodies within 4 weeks, radio- or toxin-immunoconjugates within 10

weeks, radiation therapy within 3 weeks, or major surgery within 2 weeks of first dose of study drug

- Any life-threatening illness, medical condition or organ system dysfunction which, in the investigator's opinion, could compromise the subject's safety, interfere with the absorption or metabolism of PCI-32765 capsules, or put the study outcomes at undue risk
- Clinically significant cardiovascular disease such as uncontrolled or symptomatic arrhythmias, congestive heart failure, or myocardial infarction within 6 months of screening, or any Class 3 or 4 cardiac disease as defined by the New York Heart Association Functional Classification
- Malabsorption syndrome, disease significantly affecting gastrointestinal function, or resection of the stomach or small bowel or ulcerative colitis, symptomatic inflammatory bowel disease, or partial or complete bowel obstruction
- Any of the following laboratory abnormalities: Absolute neutrophil count (ANC) < 750 cells/mm^3 (0.75 x 10^9/L) unless there is documented bone marrow involvement; Platelet count < 50,000 cells/mm^3 (50 x 10^9/L) independent of transfusion support unless there is documented bone marrow involvement; Serum aspartate transaminase (AST/SGOT) or alanine transaminase (ALT/SGPT) ≥ 3.0 x upper limit of normal (ULN); Creatinine > 2.0 x ULN.

[00609] Characteristics of the patients enrolled in the study are presented in Tables 12 and 13 below.

Table 12

	Bortezomib-Naive (N=41)	Bortezomib-Exposed (N=27)	Total (N=68)
Age: Median:	66	69	67
Range:	47 – 83	54 – 83	47 – 83
Gender: Male	31 (76)	23 (85)	54 (79)
Time from Initial Diagnosis, # (%)			
< 3 yrs to 1st dose	20 (49)	6 (22)	26 (38)
≥ 3 yrs to 1st dose	21 (51)	21 (78)	42 (62)
ECOG Status: 0	24 (59)	13 (48)	37 (54)
1	12 (29)	12 (44)	24 (35)
2	5 (12)	2 (7)	7 (10)
Prior regimens, # (%)			
Median	2	3	2
Range	1 – 5	1 – 5	1 – 5
< 3 regimens	28 (68)	11 (41)	39 (57)
≥ 3 regimens	13 (32)	16 (59)	29 (43)

Table 13

	Bortezomib-Naive (N=41)	Bortezomib-Exposed (N=27)	Total (N=68)
Prior high intensity therapy, # (%)			
HyperCVAD	17 (41)	11 (41)	28 (41)
Stem cell transplant	5 (12)	2 (7)	7 (10)
Platinum-salvage therapy	2 (5)	0 (0)	2 (3)
MIPI Score, # (%)			
Low risk	7 (17)	3 (11)	10 (15)
Intermediate risk	16 (39)	10 (37)	26 (38)
High risk	17 (41)	11 (41)	28 (41)
Bulky disease (mass ≥10 cm LD), # (%)	5 (12)	4 (15)	9 (13)
Stage IV Disease, # (%)	32 (78)	23 (85)	55 (81)
Refractory Disease*, # (%)	14 (34)	13 (48)	27 (40)

MIPI=MCL International Prognostic Index; LD=Longest Diameter
* Refractory disease = failure to achieve at least PR to the last therapy prior to study entry

[00610] Patient disposition for the study is presented in Table 14.

Table 14

	Bortezomib-Naive (N=41)	Bortezomib-Exposed (N=27)	Total (N=68)
Time on Study (mos), #			
N	36	25	61
Median	3.7	3.7	3.7
Range	0.9 – 7.0	0.7 – 7.5	0.7 – 7.5
Patients Still on Study Treatment, # (%)	29 (71)	19 (70)	48 (71)
Patients Discontinued Treatment, # (%)	12 (29)	8 (30)	20 (29)
Primary Reasons for Discontinuation, # (%)			
Disease Progression	7 (17)	6 (22)	13 (19)
Adverse event	2 (5)	1 (4)	3 (4)
Sponsor decision	1 (2)	0 (0)	1 (1)
Investigator decision	2 (5)	1 (4)	3 (4)
Death on study[1], # (%)	0 (0)	1 (4)	1 (2)

[1]Cause of death: pneumonia

[00611] Results:

[00612] Results for the best response for the bortezomib-naïve and bortezomib-exposed patients with relapsed or refractory MCL are presented in Figure 19 and Table 15 below.

Table 15

	n/N	ORR %
All Patients	**35/51**	**69**
Bulky Disease	4/7	57
Refractory		
Yes	14/21	67
No	21/30	70
Prior cancer treatments		
< 3 regimens	23/30	77
≥ 3 regimens	12/21	57
Prior high intensity therapy		
Yes	22/31	71
No	13/20	65
MIPI Score: Low Risk	6/8	75
Intermediate Risk	13/20	65
High Risk	15/20	75

[00613] PCI-32765 induced a high response rate for relapsed or refractory MCL and was associated with a favorable safety profile. No significant myelosuppression in the patients was observed during the study.

Example 5: Phase II Study of PCI-32765 + ofatumumab in Relapsed/Refractory CLL

[00614] The purpose of this study was to determine the efficacy and safety of a fixed-dose, daily regimen of orally administered PCI-32765 combined with ofatumumab in subjects with relapsed/refractory CLL/SLL and related diseases.

[00615] Study Type: Interventional

[00616] Allocation: Non-Randomized

[00617] Endpoint Classification: Safety Study

[00618] Intervention Model: Single Group Assignment

[00619] Masking: Open Label

[00620] Primary Purpose: Treatment

[00621] Intervention: 420 mg/day of PCI-32765, standard dose of ofatumumab

[00622] Applicable conditions: B-cell Chronic Lymphocytic Leukemia; Small Lymphocytic Lymphoma; Diffuse Well-Differentiated Lymphocytic Lymphoma; Prolymphocyctic Leukemia; Richter's Transformation

[00623] Primary Outcome Measures:

[00624] Response and safety of PCI-32765 [Time Frame: At the end of cycles 1 and 3]

[00625] Response rate as defined by recent guidelines in Chronic Lymphocytic Leukemia

[00626] Secondary Outcome Measures:

[00627] 1. Pharmacokinetic/Pharmacodynamic assessments [Time Frame: during 1-2 cycles]

[00628] 2. Pharmacodynamics of PCI-32765 (ie, drug occupancy of Btk and effect on biological market 1/2) of PCI-32765.

[00629] 3. Tumor Response [Time Frame: at the end of Cycles 2,4 and 6 (28 days for each cycle)]

[00630] 4. Overall response rate as defined by recent guidelines on CLL

[00631] Inclusion Criteria:

[00632] Subjects with histologically confirmed chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), prolymphocytic leukemia (PLL) as defined by WHO classification of hematopoietic neoplasms, or Richter's transformation arising out of CLL/SLL and satisfying ≥ 1 of the following conditions:

- Progressive splenomegaly and/or lymphadenopathy identified by physical examination or radiographic studies; Anemia (<11 g/dL) or thrombocytopenia (<100,000/μL) due to bone marrow involvement;
- Presence of unintentional weight loss > 10% over the preceding 6 months;
- NCI CTCAE Grade 2 or 3 fatigue;
- Fevers > 100.5 degree or night sweats for > 2 weeks without evidence of infection
- Progressive lymphocytosis with an increase of > 50% over a 2 month period or an anticipated doubling time of < 6 months
- Need for cytoreduction prior to stem cell transplant
- Subjects must have failed ≥ 2 prior therapies for CLL including a nucleoside analog or ≥ 2 prior therapies not including nucleoside analog if there is a contraindication to such therapy
- > 10% expression of CD20 on tumor cells
- ECOG performance status ≤ 2
- Life expectancy ≥ 12 weeks
- Subjects must have organ and marrow function as defined below:
- Absolute neutrophil count (ANC) ≥ 1000/μL in the absence of bone marrow involvement Platelets ≥ 30,000/μL Total bilirubin ≤ 1.5 x institutional upper limit of normal unless due to Gilbert's disease AST(SGOT) ≤ 2.5 x institutional upper limit of normal unless due to infiltration of the liver Creatinine ≤ 2.0 mg/dL OR creatinine clearance ≥ 50 mL/min
- No history of prior anaphylactic reaction to rituximab

- No history of prior exposure to ofatumumab
- Age ≥ 18 years
- Body weight ≥ 40 kg
- Able to swallow capsules without difficulty and no history of malabsorption syndrome, disease significantly affecting gastrointestinal function, or resection of the stomach or small bowel or ulcerative colitis, symptomatic inflammatory bowel disease, or partial or complete bowel obstruction

[00633] Exclusion Criteria:

[00634] A life-threatening illness, medical condition or organ system dysfunction which, in the investigator's opinion, could compromise the subject's safety, interfere with the absorption or metabolism of PCI-32765 PO, or put the study outcomes at undue risk

[00635] Any anticancer immunotherapy, chemotherapy, radiotherapy, or experimental therapy within 4 weeks before first dose of study drug. Corticosteroids for disease-related symptoms are allowed provided 1 week washout occurs.

[00636] Active central nervous system (CNS) involvement by lymphoma

[00637] Major surgery within 4 weeks before first dose of study drug

[00638] Lactating or pregnant

[00639] History of prior malignancy, except for adequately treated basal cell or squamous cell skin cancer, in situ cervical cancer, or other cancer from which the subject has been disease free for at least 2 years or which will not limit survival to < 2 years

[00640] History of Grade ≥ 2 toxicity (other than alopecia) continuing from prior anticancer therapy.

[00641] Characteristics of the patients enrolled in the study are presented in Tables 16 and 17 below.

Table 16

		(N=27)
Age, years	Median: Range: ≥70 years, # (%)	66 51 – 85 12 (44)
Diagnosis, #(%)	CLL SLL PLL Richter's	22 (81) 1 (4) 1 (4) 3 (11)
ECOG PS, #(%)	0 1	10 (37) 17 (63)
Prior Rx	Median: Range: ≥ 3 regimens:	3 2 –10 16 (59)
mRai Staging, #(%)	Low Risk Intermediate Risk High Risk	1 (4) 13 (48) 13 (48)

Table 17

	(N=27) (%)
Purine Analog Refractory, # (%)	11 (41)
Cytopenia at baseline, # (%)	
ANC < 1500/μL	4 (15)
HGB < 11g/dL	9 (33)
Platelets < 100,000/μL	9 (33)
HGB < 11g/dL or PLT < 100,000 μL	13 (48)
Prognostic Markers, # (%)	
IgVH unmutated:	20/22 (91)
Del(17p):	10 (37)
Del(11q):	9 (33)

[00642] Patient disposition data is presented in Table 18.

Table 18

	(N=27)
Follow-Up Median (months)	9.8
Range	5.2 – 12.9
Patients Still on Study, # (%)	24 (89)
Patients Discontinued, # (%)	3 (11)
Primary Reasons for Discontinuation, # (%)	
Went to Transplant	1 (4)
Disease Progression	1 (4)
Death	1 (4)

[00643] Results:

[00644] Results of the best response rate are shown in Table 19. Figure 20 shows the mobilization of lymphocytes and decrease in lymph node size following treatment with PCI-32756 or combination therapy with ofatumumab. Combination therapy decreased the total number of lymphocytes in circulation. Figure 21 shows histology of bone marrow response in a patient.

Table 18

	CLL/SLL/PLL (N=24)	Richter's (N=3)
	# (%)	# (%)
CR	1 (4%)	0
PR	23 (96%)	2 (67%)
ORR	**100 %**	**67%**
SD	0	1 (33%)
PD	0	0
NE	0	0

[00645] PCI-32756 in combination with ofatumumab is well-tolerated and highly active in patient with R/R CLL/SLL/PLL. 6 Patients have been evaluated for dose limiting toxicity (DLT) through end of cycle 2. 0 DLTs occurred in these patients. 4 patients have had end of cycle 3 scans and blood counts. 3 of 4 are responders per IWG criteria. Among the patients with CLL/SSL/PLL, the combination results in a 100% ORR irrespective of genomics.

Example 6: Phase II Study of PCI-32765 in combination with Rituximab in Relapsed/Refractory CLL

[00646] CLL patients with high-risk disease features have shorter remissions and a poor outcome with conventional chemo-immunotherapy, particularly in the relapsed disease setting. Bruton's tyrosine kinase (BTK) inhibitor, ibrutinib (PCI-32765), thwarts B cell receptor (BCR)

signaling and is a promising new targeted therapy for patients with mature B cell malignancies, particularly patients with CLL. Data from the Phase 1/2 trials demonstrated that high-risk CLL patients responded equally as well as low-risk patients to ibrutinib. Single-agent ibrutinib-treated CLL patients characteristically have delayed responses or stable disease due to persistent lymphocytosis, caused by re-distribution of tissue-resident CLL cells into the peripheral blood. To accelerate and improve responses and to expand upon the ibrutinib experience in high-risk CLL patients, a Phase 2 single-center clinical trial of ibrutinib plus rituximab was conducted.

[00647] Patients were treated with ibrutinib 420 mg PO daily, in combination with weekly rituximab (375 mg/m^2) for weeks 1-4 (cycle 1), then daily ibrutinib plus monthly rituximab until cycle 6, followed by daily single-agent ibrutinib. Study inclusion required high-risk disease (del17p or TP53 mutation [treated or untreated], patients with PFS < 36 months after frontline chemo-immunotherapy, or relapsed CLL with del11q.

[00648] Patient characteristics included a median age of 65 (range 35–82); median of 2 prior therapies, 14 female and 26 male patients. Median Rai stage was 4 (range 1–4), β2 microglobulin 4.2 mg/L (2.2 –12.3), 31 patients had unmutated IGHV, only one patient mutated IGHV, the remaining patients had inconclusive IGHV results. 19 patients had del17p or TP53 mutation (4 without prior therapy), and 13 patients had del11q. At a median follow up of 4 months, 38 of 40 patients continue on therapy without disease progression. 1 patient died from an unrelated infectious complication, and one patient withdrew consent before starting therapy. Out of 20 patients evaluable for early response assessment at 3 months, 17 patients achieved a partial remission (PR) for an ORR of 85%, and three achieved a PR with persistent lymphocytosis. Interestingly, on this combination trial, the re-distribution lymphocytosis peaked earlier and the duration was shorter (see Figure) than with single-agent ibrutinib, presumably due to the addition of rituximab.

[00649] Treatment was well tolerated, with only 13 cases of grade 3 (n=11) or grade 4 (n=2) toxicities, which were largely unrelated and transient, such as neutropenia, fatigue, pneumonia (n=1), insomnia, and bone aches. Questionnaires revealed an improved overall health and quality of life after 3 cycles of treatment in the evaluable patients (n=21). Conclusion: ibrutinib in combination with rituximab is a safe, well tolerated regimen for high-risk CLL patients, which induces very high early response rates.

Example 7: Phase I Study of PCI-32765 in combination with Bendamustine and Rituximab in Patients with Relapsed/Refractory Non-Hodgkin's Lymphoma

[00650] This phase I study was designed to determine the maximum tolerated dose, dose limiting toxicity (DLT), toxicities, and preliminary efficacy of R-bendamustine in combination with ibrutinib in patients with relapsed/refractory NHL.

[00651] Eligibility included patients with relapsed/refractory FL, MZL, MCL, transformed NHL, and DLBCL, and patients with previously untreated MCL not candidates for autologous stem cell transplantation (ASCT). ANC > 1000/mm^3 , platelets > 50,000/mm^3 , and creatinine < 2.0 mg/dL were required at study entry. Prior ASCT, rituximab, bendamustine, and ibrutinib were permitted. Treatment consisted of R 375 mg/m^2 day 1, bendamustine 90 mg/m^2 days 1 and 2, and escalating doses of ibrutinib (280 mg or 560 mg) days 1-28 every 28 days for 6 cycles. Six patients were enrolled at each dose level. Responding patients could continue ibrutinib alone after cycle 6 until disease progression or unacceptable toxicity. Pegfilgrastim was permitted for patients with grade 4 neutropenia during cycles 1-6. Response was assessed after cycles 3 and 6 by International Harmonization Criteria (Cheson, JCO 2007).

[00652] Eleven patients (9 males) with a median age of 72 (range 45-84) previously treated with a median of 3 prior therapies (range 0-10) were enrolled. Six patients were refractory to their most recent therapy, 4 patients had prior ASCT, 2 patients had received prior bendamustine, and 0 patients had prior ibrutinib. Other characteristics included stage III-IV disease in 82%, elevated IPI > 3 in 64%, extranodal involvement in 64%, bulky adenopathy > 5 cm in 45%, B-symptoms in 45%, and elevated LDH in 36%. Histologies included MCL (n=3), DLBCL (n=3), transformed NHL (n=2), FL (n=2), MZL (n=1). Nine patients completed two or more cycles of therapy (median 3, range 1-6) with 280 mg of ibrutinib (n=6) and 560 mg of ibrutinib (n=3), and 2 patients discontinued therapy prior to completing cycle 1 for progressive disease (PD) at 280 mg and 560 mg of ibrutinib, respectively, were replaced. Six patients continue to receive protocol treatment. The 5 patients off study included the 2 patients with DLBCL and transformed NHL who were replaced for PD prior to completing cycle 1, 2 patients with DLBCL and PD after cycles 3 and 4, and 1 patient with MCL receiving 280 mg ibrutinib with bendamustine (90 mg/m^2) who discontinued due to grade 3 neutropenia lasting > 14 days after cycle 4. No DLTs have been observed. Grade 3-4 events included lymphopenia (64%), neutropenia (27%), thrombocytopenia (18%), pancreatitis (9%), vomiting (9%), shingles (9%), and rash (9%). Dose reductions from 280 mg ibrutinib to 140 mg were required in 3 patients for grade 3 thrombocytopenia, pancreatitis, and rash. Bendamustine dose reductions to 60 mg/m^2 were required in 1 patient for grade 3 thrombocytopenia. Dose delays occurred in 4 patients for thrombocytopenia (n=1), neutropenia (n=1), pancreatitis (n=1), and rash (n=1). ORR was 38% in 8 evaluable patients, with 3 patients currently receiving protocol treatment who have not yet undergone restaging scans. Responses included 2 complete responses and 1 partial response in the 3 patients with MCL. Conclusions: Combined ibrutinib with R-bendamustine is well tolerated without unexpected toxicity and with significant activity in patients with previously untreated and relapsed MCL. Three additional patients will be accrued to the 560 mg dose level

and expansion cohorts examining this combination specifically in patients with FL, DLBCL, and MCL are planned.

Example 8: Phase II Study of PCI-32765 in combination with Bendamustine and Rituximab or FCR in Relapsed/Refractory CLL

[00653] The purpose of this study is to establish the safety of orally administered PCI-32765 in combination with fludarabine/cyclophosphamide/rituximab (FCR) and bendamustine/rituximab (BR) in patients with chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma(SLL).

[00654] Study Type: Interventional

[00655] Allocation: Non-Randomized

[00656] Endpoint Classification: Safety Study

[00657] Intervention Model: Single Group Assignment

[00658] Masking: Open Label

[00659] Primary Purpose: Treatment

[00660] Intervention: 420 mg/day of PCI-32765, standard FCR or BR regimen

[00661] Applicable conditions: B-cell Chronic Lymphocytic Leukemia; Small Lymphocytic Lymphoma; Diffuse Well-differentiated Lymphocytic Lymphoma

[00662] Primary Outcome Measures:

[00663] To measure the number of participants with prolonged hematologic toxicity [Time Frame: 8 weeks from first dose]

[00664] Secondary Outcome Measures:

[00665] 1. To measure the number of participants with adverse events as a measure of safety and tolerability [Time Frame: For 30 days after the last dose of PCI-32765]

[00666] 2. To measure the number of patients who respond to treatment by measuring the increase or decrease of disease in the lymph nodes and/or blood test results [Time Frame: Patients may remain on study until the last subject enrolled completes a maximum of 12 cycles of PCI-32765. Any subjects still receiving PCI-32765at that time may enroll in a long-term follow-up study to continue to receive PCI-32765capsules]

[00667] Inclusion Criteria:

[00668] Histologically confirmed CLL or SLL and satisfying at least 1 of the following criteria for requiring treatment:

- Progressive splenomegaly and/or lymphadenopathy identified by physical examination or radiographic studies
- Anemia (<11 g/dL) or thrombocytopenia (<100,000/μL) due to bone marrow involvement

- Presence of unintentional weight loss > 10% over the preceding 6 months
- NCI CTCAE Grade 2 or 3 fatigue
- Fevers > 100.5°C or night sweats for > 2 weeks without evidence of infection
- Progressive lymphocytosis with an increase of > 50% over a 2 month period or an anticipated doubling time of < 6 months
- 1 to 3 prior treatment regimens for CLL/SLL
- ECOG performance status of ≤ 1
- ≥ 18 years of age
- Willing and able to participate in all required evaluations and procedures in this study protocol including swallowing capsules without difficulty
- Ability to understand the purpose and risks of the study and provide signed and dated informed consent and authorization to use protected health information (in accordance with national and local subject privacy regulations)

[00669] Exclusion Criteria:

- Any chemotherapy, therapeutic antineoplastic antibodies (not including radio- or toxin immunoconjugates), radiation therapy, or experimental antineoplastic therapy within 4 weeks of first dose of study drug Radio- or toxin-conjugated antibody therapy within 10 weeks of first dose of study drug
- Concomitant use of medicines known to cause QT prolongation or torsades de pointes
- Transformed lymphoma or Richter's transformation
- Any life-threatening illness, medical condition or organ system dysfunction which, in the investigator's opinion, could compromise the subject's safety, interfere with the absorption or metabolism of PCI-32765 PO, or put the study outcomes at undue risk
- Any of the following laboratory abnormalities: Absolute neutrophil count (ANC) < 1000 cells/mm^3 (1.0 x 109/L); Platelet count < 50,000/mm^3 (50 x 109/L); Serum aspartate transaminase (AST/SGOT) or alanine transaminase (ALT/SGPT) ≥ 3.0 x upper limit of normal (ULN); Creatinine > 2.0 x ULN or creatinine clearance < 40 mL/min

[00670] Characteristics for the patients enrolled in the study are shown in Tables 19 and 20.

Table 19

		Ibrutinib + BR (N=30)
Age, years	Median:	61.5
	Range:	41 – 82
	≥70 years, # (%)	7 (23)
Diagnosis, # (%)	CLL	29 (97)
	SLL	1 (3)
ECOG Performance Status, # (%)	0	15 (50)
	1	15 (50)
Prior Rx, # (%)	Median:	2
	Range:	1 – 3
	3 prior regimens:	12 (40)
Bulky Disease, # (%)	≥ 5 cm	16 (53)
Hgb < 11 g/dL or PLT < 100K/μL at screening		14 (47)

Table 20

	Ibrutinib + BR (N=30)
Purine Analog Refractory, # (%) (≤12 month treatment free interval following purine analog regimen)	11 (37)
Bendamustine Refractory, # (%) (≤ 12 month treatment free interval following bendamustine containing regimen)	4 (13)
Prognostic Markers, # (%)	
Del(17p):	7 (23)
Del(11q):	13 (43)
β2 Microglobulin > 3mg/L	16 (59)

[00671] Patient disposition is presented in Table 21.

Table 21

		Ibrutinib + BR (N=30)
Follow-Up	Median (months)	8.1
	Range	2.7 – 11.3
Patients Still on Study, # (%)		23 (77)
Patients Discontinued, # (%)		7 (23)
Primary Reasons for Discontinuation, # (%)		
Proceeded to SCT		5 (17)
Disease Progression		2 (7)

[00672] A summary of the treatment regimen is presented in Table 22.

Table 22

	Ibrutinib + BR (N=30)
Number of Cycles of Bendamustine Received	
Median	6
Range	2 – 6
Number of Cycles of Rituximab Received	
Median	6
Range	2 – 6
Ibrutinib % Total Planned Dose Received	
Median	97
Range	73 – 100
Subjects with Dose Modifications, # (%)	
Bendamustine	7 (23)
Rituximab	0
Ibrutinib	1 (3)

[00673] Results:

[00674] Results of the best response rate are shown in Tables 23 and 24. Figure 22 shows the mobilization of lymphocytes and decrease in lymph node size following treatment with PCI-32756 or combination therapy with bendamustine and rituximab. Combination therapy decreased the total number of lymphocytes in circulation.

Table 23

	Ibrutinib + BR (N=30) *8.1 mos median f/u*
	# (%)
CR	4 (13)
PR	24 (80)
ORR	93%
Nodal	1 (3)
SD	0 (0)
PD	1 (3)
NE	0 (0)

Table 24

	N	ORR %	CR %
All Patients	30	93	13
≥ 70 years age	7	86	29
Prior Rx, = 3 regimens	12	83	0
Hgb < 11 g/dL or PLT < 100K/μL	14	93	7
Del 11q present	13	100	15
β2 Microglobulin > 3mg/L	16	94	0
Purine analog refractory	11	91	0
Bendamustine refractory	4	75	0
Del 17p present	7	71	14

[00675] Administration of PCI-32765 in combination with bendamustine and rituximab resulted in 93% if patients achieving a IWCLL response with 13% complete responses (CRs). No added toxicity was observed when adding PCI-32765 to bendamustine and rituximab. In previous studies, bendamustine and rituximab combination therapy only achieved 59% response including 9% CRs. Thus, PCI-32765 significantly enhances treatment when administered in combination with bendamustine and rituximab.

[00676] Studies with PCI-32765 in combination with FCR in patients with CLL/SLL are in progress. 3 patients were treated in the initial study for 6 cycles. Treatment was well-tolerate in all 3 patients with an overall response of 100% (3/3) with 2 confirmed MRD-negative CRs (MRD negative at 10^{-4}). All 3 patients remains progression free on PCI-32765 with a median follow-up of 8.5 months.

Example 9: Phase II Study of PCI-32765 in Relapsed/Refractory DLBCL

[00677] The purpose of this study is to evaluate the efficacy of PCI-32765 in relapsed/refractory de novo activated B-cell (ABC) and germinal-cell B-Cell (GCB) Diffuse Large B-cell Lymphoma (DLBCL).

[00678] Study Type: Interventional

[00679] Allocation: Non-Randomized

[00680] Endpoint Classification: Safety Study

[00681] Intervention Model: Single Group Assignment

[00682] Masking: Open Label

[00683] Primary Purpose: Treatment

[00684] Intervention: 560 mg/day PCI-32765

[00685] Primary Outcome Measures:

[00686] To measure the number of patients with a response to study drug [Time Frame: 24 weeks from first dose]. Participants will be followed until progression of disease or start of another anti-cancer treatment.

[00687] Secondary Outcome Measures:

[00688] 1. To measure the number of patients with adverse events as a measure of safety and tolerability. [Time Frame: For 30 days after the last dose of PCI-32765] Participants will be followed until progression of the disease or start of another anticancer treatment.

[00689] 2. To measure the number of participants pharmacokinetics to assist in determining how the body responses to the study drug. [Time Frame: Procedure will be performed during the first month of receiving study drug.]

[00690] Inclusion Criteria:

- Men and women ≥ 18 years of age.
- Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 2.
- Pathologically confirmed de novo DLBCL; subjects must have available archival tissue for central review to be eligible.
- Relapsed or refractory disease, defined as either: 1) recurrence of disease after a complete remission (CR), or 2) partial response (PR), stable disease (SD), or progressive disease (PD) at completion of the treatment regimen preceding entry to the study (residual disease):Subjects must have previously received an appropriate first-line treatment regimen. Subjects with suspected residual disease after the treatment regimen directly preceding study enrollment must have biopsy demonstration of residual DLBCL.
- Subjects who have not received high dose chemotherapy/autologous stem cell transplant (HDT/ASCT) must be ineligible for HDT/ASCT as defined by meeting any of the following criteria:
  - Age ≥ 70 years
  - Diffuse lung capacity for carbon monoxide (DLCO) < 50% by pulmonary function test (PFT)
  - Left ventricular ejection fraction (LVEF) < 50% by multiple gated acquisition(MUGA)/echocardiograph (ECHO)
  - Other organ dysfunction or comorbidities precluding the use of HDT/ASCT on the basis of unacceptable risk of treatment-related morbidity
  - Subject refusal of HDT/ASCT
- Subjects must have ≥ 1 measurable (> 2 cm in longest dimension) disease sites on computed tomography (CT) scan.

[00691] Exclusion Criteria:

- Transformed DLBCL or DLBCL with coexistent histologies (e.g., follicular or mucosa-associated lymphoid tissue [MALT] lymphoma)
- Primary mediastinal (thymic) large B-cell lymphoma (PMBL)

- Known central nervous system (CNS) lymphoma
- Any chemotherapy, external beam radiation therapy, or anticancer antibodies within 3 weeks of the first dose of study drug
- Radio- or toxin-immunoconjugates within 10 weeks of the first dose of study drug
- Major surgery within 2 weeks of first dose of study drug
- Any life-threatening illness, medical condition or organ system dysfunction which, in the investigator's opinion, could compromise the subject's safety, or put the study outcomes at undue risk
- Clinically significant cardiovascular disease such as uncontrolled or symptomatic arrhythmias, congestive heart failure, or myocardial infarction within 6 months of screening, or any Class 3 or 4 cardiac disease as defined by the New York Heart Association Functional Classification
- Unable to swallow capsules or malabsorption syndrome, disease significantly affecting gastrointestinal function, or resection of the stomach or small bowel or ulcerative colitis, symptomatic inflammatory bowel disease, or partial or complete bowel obstruction
- Any of the following laboratory abnormalities:
  - Absolute neutrophil count (ANC) < 750 cells/mm^3 (0.75 x 109/L) unless there is documented bone marrow involvement;
  - Platelet count < 50,000 cells/mm^3 (50 x 109/L) independent of transfusion support unless there is documented bone marrow involvement; S
  - Serum aspartate transaminase (AST/SGOT) or alanine transaminase (ALT/SGPT) ≥ 3.0 upper limit of normal (ULN);
  - Creatinine > 2.0 x ULN

Example 10: Growth Inhibition by PCI-32765 in a subset of B cell lymphoma derived cell lines

[00692] The PCI-32765 inhibited the growth of a subset of B cell lymphoma derived cell lines, with GI_{50} values ranging from 0.1 to 5.5 μM (see Table 5, below).

[00693] In cell lines, PCI-32765 has demonstrated weak synergy with lenalidomide, bortezomib, sorafenib, gemcitabine, dexamethasone, bendamustine, 3-((dimethylamino)methyl)-N-(2-(4-(hydroxycarbamoyl)phenoxy)ethyl)benzofuran-2-carboxamide, and the Syk inhibitor R-406; *and* additivity with taxol, vincristine, doxorubicin, temsirolimus and carboplatin. Combination drug testing in xenografts has recently begun; an initial experiment in a DLBCL xenograft demonstrated greater than additivity for the PCI-32765 and bortezomib.

[00694] Treatment of primary CLL cells with 0.01 - 100 mcM of PCI-32765 resulted in: 1) dose and time-dependent apoptosis, 2) apoptosis that was not affected by genetic changes which are known to predict for poor response to other agents, *i.e.*, del11q, del17p, and IgVH gene mutational status; 3) cytotoxicity accompanied by PARP cleavage and induction of caspase-3 activity; and 4) apoptosis independent of the presence or absence of fibronectin or the Hs5 stromal cell line, suggesting that the activity of PCI-32765 was not diminished by microenvironmental influences.

[00695] PCI-32765 inhibited the growth of a subset of B cell lymphoma derived cell lines, with GI_{50} values ranging from 0.1 to 5.5 μM (see Table 25, below).

Table 25. Growth Inhibition by PCI-32765 in a Subset of Human Lymphoma Cell Lines

B-Lymphoma Cell Line	Origin	Subtype	GI_{50} (μM)
LY10	DLBCL	ABC	0.10
DHL-6	DLBCL	GCB	0.18
DHL-4	DLBCL	GCB	0.53
DHL-10	DLBCL	GCB	3.7
LY3	DLBCL	ABC (CARD11)	> 10
LY19	DLBCL	GCB (CARD11)	> 10
DB	DLBCL	na	> 10
WSU-NHL	FL-transformed	na	0.12
DOHH2	FL-transformed	na	0.12
WSU-DLCL2	FL-transformed	na	0.5
Ramos	Burkitt's	na	5.5
Mino	Mantle cell	na	0.15
Granta-519	Mantle cell	na	> 10
Jeko-1	Mantle cell	na	> 10

na = not applicable

Example 11: *In vitro* Assay of a Btk inhibitor Combinations in DLBCL cells

[00696] Combinations of the Btk inhibitor PCI-32765 and additional anti-cancer agents were assayed using DoHH2 cells. DOHH2 is a DLBCL (diffuse large B-cell lymphoma) cell line, from a transformed follicular lymphoma patient. The cell line is moderately sensitive to PCI-32765. PCI-32765 was incubated with other cancer drugs for 2 days. Assay was an Alamar blue assay.

[00697] The combinations tested were:

[00698] PCI-32765 and Gemicitabine;

[00699] PCI-32765 and Dexamethasone;

[00700] PCI-32765 and Lenalinomide;

[00701] PCI-32765 and R-406;

[00702] PCI-32765 and Temsirolimus;

[00703] PCI-32765 and Carboplatin;

[00704] PCI-32765 and Bortezomib; and

[00705] PCI-32765 and Doxorubicin.

[00706] Results are presented in Figures 23-25.

Example 10: *In vitro* Assay of a Btk inhibitor Combinations in ABC-DLBCL cells

[00707] Combinations of the Btk inhibitor PCI-32765 and additional anti-cancer cancer agents were assayed using TMD8 cells. TMD8 is a NF-kB signaling-dependent ABC-DLBCL cell line. It is sensitive to BTK inhibitors alone at low nanomolar concentrations (GI50 ~1-3 nM). A Btk inhibitor was incubated with other cancer drugs for 2 days. The assay was an Alamar blue assay.

[00708] The combinations tested were:

[00709] PCI-32765 and CAL-101;

[00710] PCI-32765 and Lenalinomide;

[00711] PCI-32765 and R-406;

[00712] PCI-32765 and Bortezomib;

[00713] PCI-32765 and Vincristine;

[00714] PCI-32765 and Taxol;

[00715] PCI-32765 and Fludarabine; and

[00716] PCI-32765 and Doxorubicin.

[00717] Results are presented in figures 26-33.

Example 11: Clinical Trial of Btk Inhibitor in Combination with BR

[00718] A clinical trial is performed to determine the effects of combining a Btk inhibitor (e.g. PCI-32765) with BR (bendamustine and rituximab) in patients with non-Hodgkin's lymphoma. The Btk inhibitor is administered. Following an increase in the concentration of lymphoid cells in the peripheral blood, BR is administered.

Example 12: Clinical Trial of Btk Inhibitor in Combination with Bortezomib

[00719] A clinical trial is initiated to determine the effects of combining a Btk inhibitor (e.g. PCI-32765) with bortezomib in patients with non-Hodgkin's lymphoma. The Btk inhibitor is administered. Following an increase in the concentration of lymphoid cells in the peripheral blood, bortezomib is administered.

Example 13: Clinical Trial of Btk Inhibitor in Combination with BR

[00720] A clinical trial is performed to determine the effects of combining a Btk inhibitor (e.g. PCI-32765) with BR (bendamustine and rituximab) in patients with CLL. The Btk

inhibitor is administered. Following an increase in the concentration of lymphoid cells in the peripheral blood, BR is administered.

Example 14: Clinical Trial of Btk Inhibitor in Combination with FCR

[00721] A clinical trial is performed to determine the effects of combining a Btk inhibitor (e.g. PCI-32765) with FCR (fludarabine, cyclophosphamide, rituximab) in patients with CLL. The Btk inhibitor is administered. Following an increase in the concentration of lymphoid cells in the peripheral blood, BR is administered.

Example 15: Clinical Trial of Btk Inhibitor in Combination with Ofatumumab

[00722] A clinical trial is performed to determine the effects of combining a Btk inhibitor (e.g. PCI-32765) with ofatumumab in patients with CLL. The Btk inhibitor is administered. Following an increase in the concentration of lymphoid cells in the peripheral blood, ofatumumab is administered.

Example 16: Clinical Trial of Btk Inhibitor in Combination with Rituximab

[00723] A clinical trial is performed to determine the effects of combining a Btk inhibitor (e.g. PCI-32765) with rituximab in patients with CLL. The Btk inhibitor is administered. Following an increase in the concentration of lymphoid cells in the peripheral blood, rituximab is administered.

Example 17: Clinical Trial of Btk Inhibitor in Combination with Lenalidomide

[00724] A clinical trial is performed to determine the effects of combining a Btk inhibitor (e.g. PCI-32765) with lenalidomide in patients with relapsed or refractory B-cell malignancies. The Btk inhibitor is administered. Following an increase in the concentration of lymphoid cells in the peripheral blood, lenalidomide is administered.

Example 18: Clinical Trial of Btk Inhibitor in Combination with Lenalidomide

[00725] A clinical trial is performed to determine the effects of combining a Btk inhibitor (e.g. PCI-32765) with lenalidomide in patients with DLBCL, indolent B cell lymphoma, CLL, and multiple myeloma. The Btk inhibitor is administered. Following an increase in the concentration of lymphoid cells in the peripheral blood, lenalidomide is administered.

Example 19: Clinical Trial of Btk Inhibitor in Combination with R-CHOP

[00726] A clinical trial is performed to determine the effects of combining a Btk inhibitor (e.g. PCI-32765) with R-CHOP (Rituximab, Cyclophosphamide, Doxorubicin Hydrochloride, Vincristine Sulfate, Prednisone) in patients with relapsed or refractory B-cell malignancies. The Btk inhibitor is administered. Following an increase in the concentration of lymphoid cells in the peripheral blood, R-CHOP is administered.

Example 20: Clinical Trial of Btk Inhibitor in Combination with R-CHOP

[00727] A clinical trial is performed to determine the effects of combining a Btk inhibitor (e.g. PCI-32765) with R-CHOP in patients with DLBCL, indolent B cell lymphoma and Waldenstrom's Macroglobulinemia. The Btk inhibitor is administered. Following an increase in the concentration of lymphoid cells in the peripheral blood, R-CHOP is administered.

Example 21: Clinical Trial of Btk Inhibitor in Combination with Temsirolimus

[00728] A clinical trial is performed to determine the effects of combining a Btk inhibitor (e.g. PCI-32765) with temsirolimus in patients with relapsed or refractory B-cell malignancies. The Btk inhibitor is administered. Following an increase in the concentration of lymphoid cells in the peripheral blood, temsirolimus is administered.

Example 22: Clinical Trial of Btk Inhibitor in Combination with Temsirolimus

[00729] A clinical trial is performed to determine the effects of combining a Btk inhibitor (e.g. PCI-32765) with temsirolimus in patients with MCL, DLBCL and indolent B cell lymphomas. The Btk inhibitor is administered. Following an increase in the concentration of lymphoid cells in the peripheral blood, temsirolimus is administered.

Example 23: *In Vitro* Assay of a Btk Inhibitor in Combination with Second Treatment

[00730] Combinations of the Btk inhibitor PCI-32765 and a second treatment are assayed using TMD8 cells.

[00731] TMD8 is a NF-κB signaling-dependent ABC-DLBCL cell line. It is sensitive to BTK inhibitors alone at low nanomolar concentrations (GI50 ~1-3 nM). A Btk inhibitor is incubated with other cancer drugs for 2 days. Assay is an Alamar blue assay.

[00732] Combinations are:

[00733] PCI-32765 and lenalidomide and dexamethasone

[00734] PCI-32765 and bortezomib

[00735] PCI-32765 and R-CHOP (cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone, and optionally, rituximab)

[00736] PCI-32765 and R-EPOCH (etoposide, doxorubicin, vinristine, cyclophosphamide, prednisolone, and optionally, rituximab)

[00737] PCI-32765 and R-ICE (ifosfamide, carboplatin, etoposide)

[00738] PCI-32765 and ofatumumab

[00739] PCI-32765 and rituximab

[00740] PCI-32765 and GA101 (Genentech)

[00741] PCI-32765 and BR (Bendamustine/Rituximab)

Example 24: Pharmaceutical Compositions:

[00742] The compositions described below are presented with a compound of Formula (D) for illustrative purposes; any of the compounds of any of Formulas (A), (B), (C), or (D) can be used in such pharmaceutical compositions. In particular examples, the compound 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 (i.e. PCI-32765/ibrutinib).

Example 24a: Parenteral Composition

[00743] To prepare a parenteral pharmaceutical composition suitable for administration by injection, 100 mg of a water-soluble salt of a compound of Formula (D) (e.g. PCI-32765/ibrutinib) is dissolved in DMSO and then mixed with 10 mL of 0.9% sterile saline. The mixture is incorporated into a dosage unit form suitable for administration by injection.

Example 24b: Oral Composition

[00744] To prepare a pharmaceutical composition for oral delivery, 100 mg of a compound of Formula (D) (e.g. PCI-32765/ibrutinib) is mixed with 750 mg of starch. The mixture is incorporated into an oral dosage unit for, such as a hard gelatin capsule, which is suitable for oral administration.

Example 24c: Sublingual (Hard Lozenge) Composition

[00745] To prepare a pharmaceutical composition for buccal delivery, such as a hard lozenge, mix 100 mg of a compound of Formula (D) (e.g. PCI-32765/ibrutinib), with 420 mg of powdered sugar mixed, with 1.6 mL of light corn syrup, 2.4 mL distilled water, and 0.42 mL mint extract. The mixture is gently blended and poured into a mold to form a lozenge suitable for buccal administration.

Example 24d: Inhalation Composition

[00746] To prepare a pharmaceutical composition for inhalation delivery, 20 mg of a compound of Formula (D) (e.g. PCI-32765/ibrutinib) is mixed with 50 mg of anhydrous citric acid and 100 mL of 0.9% sodium chloride solution. The mixture is incorporated into an inhalation delivery unit, such as a nebulizer, which is suitable for inhalation administration.

Example 24e: Rectal Gel Composition

[00747] To prepare a pharmaceutical composition for rectal delivery, 100 mg of a compound of Formula (D) (e.g. PCI-32765/ibrutinib) is mixed with 2.5 g of methylcellulose (1500 mPa), 100 mg of methylparapen, 5 g of glycerin and 100 mL of purified water. The resulting gel mixture is then incorporated into rectal delivery units, such as syringes, which are suitable for rectal administration.

Example 24f: Topical Gel Composition

[00748] To prepare a pharmaceutical topical gel composition, 100 mg of a compound of Formula (D) (e.g. PCI-32765/ibrutinib) is mixed with 1.75 g of hydroxypropyl cellulose, 10 mL of propylene glycol, 10 mL of isopropyl myristate and 100 mL of purified alcohol USP. The resulting gel mixture is then incorporated into containers, such as tubes, which are suitable for topical administration.

Example 24g: Ophthalmic Solution Composition

[00749] To prepare a pharmaceutical ophthalmic solution composition, 100 mg of a compound of Formula (D) (e.g. PCI-32765/ibrutinib) is mixed with 0.9 g of NaCl in 100 mL of purified water and filtered using a 0.2 micron filter. The resulting isotonic solution is then incorporated into ophthalmic delivery units, such as eye drop containers, which are suitable for ophthalmic administration.

Example 25: Effect of PCI-32765 on Lymphocyte Mobilization in Mantle Cell Lymphoma

[00750] Patients with chronic lymphocytic leukemia (CLL) often have marked but transient increases of circulating CLL lymphocytes following treatment with ibrutinib, as seen with other inhibitors of the B cell receptor (BCR) pathway. In the course of the Phase I study of ibrutinib, similar effects were noted among treated patients with other types of non-Hodgkin lymphoma (NHL) including mantle cell lymphoma (MCL). In this example, we characterized the patterns and phenotypes of cells mobilized among patients with MCL, and further investigate the mechanism of this effect. Peripheral blood CD19+CD5+ cells from MCL patients treated with ibrutinib (PCI-32765) after 7 days were found to have significant reduction in the expression of CXCR4, CD38 and Ki67 compared to pre-treatment. In addition, plasma chemokines such as MDC, MIP-1β, and CXCL13 were reduced 40-60% after one week of treatment. Mechanistically, we found ibrutinib inhibited BCR, and chemokine mediated adhesion and chemotaxis of MCL cell lines, and dose-dependently inhibited BCR, stromal cell and CXCL12/CXCL13 stimulations of pBtk, pPLCγ2, pErk or pAkt. Importantly, ibrutinib dose-dependently inhibited the pseudoemperipoleisis of MCL in co-culture. We propose that Btk is essential for the homing of MCL cells into secondary lymphoid organs, and its inhibition results in an egress of malignant cells into peripheral blood.

Materials and Methods

[00751] **Primary Human MCL Specimens from Drug Treated Patients**: Blood was drawn from MCL patients enrolled in PCYC-04753 or PCYC-1104 studies in the US in accordance with GCP guidelines provided by ICH and principles of the Declaration of Helsinki with informed consent and in compliance with the protocols approved by the relevant Institutional Review Board(s). The whole blood samples were drawn into sodium heparin CPT

tubes (BD), mixed for 5 min then spun at 1500 rcf for 20 min at the collection sites. The samples were shipped overnight to Pharmacyclics with 36 hrs. In a laminar flow hood, the PBMCs were removed from the top layer of the tubes, washed with PBS, and frozen in 90% FBS + 10% DMSO (Sigma, St Louis, MO) in liquid nitrogen until use.

[00752] **Cell lines and primary material for *ex vivo* studies**: MCL cell lines HBL2 (kindly provided by Dr. Wolfram Klapper, Department of Pathology, University of Kiel, Germany), JeKo1 (kindly provided by Dr. Lydia Visser, Department of Pathology, University Medical Center Groningen, the Netherlands) and Mino (DSMZ, Germany) were cultured in RPMI 1640 supplemented with 10% heat inactivated fetal bovine serum, 2 mM L-glutamine, 100U/ml penicillin and 100μg/ml streptomycin (Life Technologies, The Netherlands). Mino cells for the co-culture analysis and migration assays and the murine stromal cell line M2-10B4 were obtained from ATCC and maintained in RPMI media supplemented with 15% or 10% fetal bovine serum, respectively. All cell culture reagents were obtained from Life Technologies (Grand Island, NY).

[00753] For *ex vivo* studies, peripheral blood derived from MCL patients were provided by the department of Hematology of the Academic Medical Center (AMC) Amsterdam, PBMC's were isolated with Ficoll and B cells were purified using MACS with negative selection (Miltenyi Biotec). This study was conducted and approved by the AMC Medical Committee on Human Experimentation. Informed consent was obtained in accordance with the Declaration of Helsinki.

[00754] With informed consent in accordance with the Declaration of Helsinki and approval from the NIH institutional review board, peripheral blood (PB) and Lymph node biopsies (LN) were collected from treatment-naive MCL patients enrolled in the National Cancer Institute Study #05-C-0170 (http://clinicaltrials.gov identifier: NCT00114738). Matched PB and LN samples were obtained on the same day, processed, and analyzed in parallel. Mononuclear cells were isolated by density-gradient centrifugation (Ficoll Lymphocyte Separation Media; ICN Biomedicals) and viably frozen in 90% fetal bovine serum (FBS), 10% dimethyl sulfoxide (DSMO) (Sigma) in liquid nitrogen until use.

[00755] **Antibodies:** Antibodies used in flow cytometry were purchased from BD (San Jose, CA) and used according to instructions: CD3-V500, CD19-APCCy7,CD19-APC, CD5-PerCPCy5.5, CD5- FITC, CXCR4-PECy7, CXCR4-PE, CD38-PE, CD62L-PE, CCR7-V450, CXCR3-Alexa488, CXCR5-Alexa647, CD49d-APC, CD29-PE, CD44-V450, CD54-PE, CD11a-APC, CD11c-V450, CD18-FITC, CD40-PECy7, Ki67-Alexa488, Ig κ light chain-APC, Ig □ light chain-FITC. Antibodies used for Western blots: phospho-p44/42 MAP kinase [T202/Y204] against ERK1 and 2, phospho-AKT [Ser473] against PKB/AKT (New England

Biolabs, Ipswich, MA), phospho-BTK [Y551] against BTK (BD Biosciences), phospho-BTK [Y223] against BTK (Epitomics, Burlingame, CA) and phospho-PLCγ2 [Y759] against PLCγ2 (BD Biosciences); anti-ERK2 (C-14; Santa Cruz Biotechnology, Santa Cruz, CA), anti-AKT (H-136; Santa Cruz Biotechnology), anti-BTK (Clone 53; BD Biosciences), goat $F(ab)'_2$ anti-human IgM (LE/AF; Southern Biotech, Birmingham, AL), horseradish peroxidase (HRP)-conjugated rabbit anti-mouse and HRP-conjugated goat anti-rabbit (DAKO, Houston, TX).

[00756] **Compounds and Reagents for *in vitro* experiments**: Ibrutinib was from Pharmacyclics (Sunnyvale, CA), R406 from Axon Medchem (Groningen, Netherlands), Wortmannin and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma-Aldrich (St Louis, MO); recombinant human sVCAM-1, human plasma fibronectin, BSA (fraction V), rhCXCL12 and rhCXCL13 were from R&D Systems (Minneapolis, MN), rhCCL19 and rhCCL21 from MT-diagnostics (Netherlands, BV) and poly-l-Lysine (PLL) from Sigma-Aldrich.

[00757] **MCL Phenotyping:** Frozen PBMCs were thawed in a 37°C water bath, resuspended in RPMI + 10% FBS, and recovered in a 37°C, 5% CO_2 incubator in 5 ml polypropylene tubes (BD-Falcon) for 2 hours before phenotyping analysis. The PBMCs were washed with PBS + 2% FBS, pelleted and resuspended in PBS + 2% FBS containing phenotyping surface antibodies. All staining cocktails were run in duplicate tubes. The cells were stained for 30 minutes, washed with PBS, pelleted at 1300 rpm for 5 min, then fixed in PBS + 1.6% paraformaldehyde (Electron Microscopy Services, Hatfield, PA). Cells to be analyzed for proliferation with Ki67 were permeabilized with 70% ethanol at -20°C overnight, rehydrated with PBS and stained with Ki-67 antibody.

[00758] **Flow Cytometry:** BD FACS Canto II (BD, San Jose, CA) was used for all flow cytometry collection. The instrument was maintained according to manufacturer's recommendations. CS&T beads (BD) are used daily for baseline and reproducibility measurements according to manufacturer's instructions. Phosphoflow assays were stained and performed as described. The above antibodies were used with BD CompBead Plus to establish compensation settings and antibody staining consistency. 10,000 $CD19^+$ cells were collected from each staining sample. The data was analyzed and quantified using FlowJo7.6 (Tree Star, Ashland, OR).

[00759] **Co-culture assays:** Co-cultures of M2-10B4 stromal cells and the B cell line Mino were established according to the method of Burger et al. *Blood.* 1999;94(11):3658-3667. Mino cells were treated with vehicle, pertussis toxin (Sigma), or ibrutinib for one hour at 37°C, washed with media, and then added to plates containing confluent monolayers of stromal cells. The co-cultures were incubated at 37°C for 5 hrs to overnight to allow migration of Mino cells

beneath the stromal cell layer, after which they were washed extensively to remove unmigrated cells. For co-cultures using live-cell tracer dyes, cells were first loaded with Alexa Fluor CellTracker (Life Technologies, Grand Island, NY) according to the manufacturer's instructions. For microscopy, cells were fixed with paraformaldehyde and mounted onto slides with DAPI mounting medium (Vectashield, Vector Laboratories, Burlingame, CA). For quantification of migration of Mino cells in co-cultures by flow cytometry, cells were trypsinized and stained with APC-Cy7-labeled anti-CD19 antibody (BD Laboratories). Cells were counted using CountBright Absolute counting beads (Life Technologies) on a BD CantoII flow cytometer.

[00760] **Actin polymerization in Mino cells:** Mino cells were allowed to adhere to coverslips in serum-free media for 30 min at 37°C and then treated with DMSO, pertussis toxin, or ibrutinib for 1 hr. Cells were fixed with paraformaldehyde, permeabilized with Triton X-100, and stained with Alexa Fluor495-labeled phalloidin (Molecular Probes, Grand Island, NY). Coverslips were mounted on glass slides using Vectashield mounting medium containing DAPI (Vector Laboratories). Microscopy was performed on a Zeiss Axioplan2 microscope using a 63x/1.40 oil-immersion Plan-Apochromat objective, and images were acquired with a Zeiss AxioCam MRm CCD camera, and AxioVision v.4.8 software. For densitometry, at least 30 cells were imaged for each condition.

[00761] **Adhesion assay:** The cell adhesion assays were performed essentially as described previously. In detail, adhesion assays were done in triplicate on EIA/RIA 96-well plates (Costar) coated overnight at 4°C with PBS containing, 10 μg/ml fibronectin or 500ng/ml VCAM-1, 4% BSA, or for 15 min at 37°C with 1mg/mL poly-l-lysine (PLL), and blocked for 2h at 37°C with 4% BSA in RPMI 1640. Cells were pretreated with 100 nM ibrutinib, 100 nM Wortmannin, 1μM R406 or at 37°C for 1h in RPMI with 1% BSA. Subsequently, cells were stimulated with either 100ng/ml goat $(Fab')_2$ anti-human IgM, or 50 ng/mL PMA, and $1.5x10^5$ Namalwa or $3x10^5$ CLL-cells were immediately plated in 100 μl/well and incubated at 37°C for 30 min. After extensive washing of the plate with RPMI containing 1% BSA to remove non-adhered cells, the adherent cells were fixed for 10 min with 10% glutaraldehyde in PBS and subsequently stained for 45 min with 0.5% crystal violet in 20% methanol. After extensive washing with water, the dye was eluted in methanol and absorbance was measured after 40 min at 570 nm on a spectrophotometer (Multiskan RC spectrophotometer, Thermo Fisher Scientific, Philadephia, PA). Background absorbance (no cells added) was subtracted. Absorbance due to nonspecific adhesion, as determined in wells coated with 4% BSA, was always less than 10% of the absorbance of anti-IgM-stimulated cells. Maximal adhesion (100%) was determined by applying the cells to wells coated with PLL, without washing the wells before fixation.

Adhesion of the nonpretreated anti-IgM-stimulated cells was normalized to 100% and the bars represent the means + SEM of independent experiments, each assayed in triplicate.

[00762] Chemokine-mediated adhesion was assayed as described above, except that the chemokines 100 ng/mL CXCL12, 100 ng/mL CXCL13, 100 ng/mL CCL19 or 100 ng/mL CCL21 were coimmobilized with 500 ng/mL VCAM-1. The plates were spun directly after applying the cells to the plate, and the cells were allowed to adhere for 2 min.

[00763] Alternatively, serum starved cells were first stimulated and allowed to adhere as described, and ibrutinib (1μM) was added afterwards for 2h at 37°C and subsequently the plates were washed to remove the unbound cells.

[00764] **Migration assay:** Migration assays were performed essentially as described (de Gorter et al. *Immunity*. 2007;26(1):93-104; de Gorter et al. *Blood*. 2008;111(7):3364-3372). In detail, migration assays in triplicate with transwells (pore size 5μm, Costar) coated with 500 ng/mL VCAM-1. The lower compartment contained 100 ng/mL CXCL12, The cells, pretreated with 100 nM ibrutinib at 37°C for 1h in RPMI with 0.5% BSA, were applied to the upper compartment and allowed to migrate for 2h at 37°C. The amount of viable migrated cells was determined by FACS and expressed as a percentage of the input.

[00765] **Immunoblotting:** Immunoblotting was performed essentially as described. (de Rooij et al. *Blood*. 2012;119(11):2590-2594; de Gorter et al. *Blood*. 2008;111(7):3364-3372). In detail, 10^7 cells/mL RPMI were pretreated with 100nM ibrutinib at 37°C for 1h. After stimulation with 100ng/ml goat anti-human IgM, $(Fab')_2$ or 100ng/mL CXCL12 for 5 min (or as indicated), cells were directly lysed in SDS-PAGE sample buffer. $2x10^5$ cells were applied on a 10% SDS-PAGE gel and blotted with rabbit anti-phospho-ERK1/2 (Cell Signaling, Danvers, MA), rabbit anti-phospho-AKT, mouse anti-phospho-BTK or mouse anti-β-actin followed by HRP-conjugated goat anti-rabbit or rabbit anti-mouse and developed by enhanced chemiluminescence (GE Healthcare, Piscataway, NJ). To confirm equal expression and loading, the blots were stripped and incubated with the antibodies rabbit anti-ERK2, rabbit anti-AKT and mouse anti-BTK.

[00766] **Statistical analysis:** Analyses were performed using GraphPad Prism 4.0 (San Diego, CA). Statistically significant differences were determined using either ANOVA with Bonferroni's post hoc comparison or unpaired two-tailed Student's t- test was used to determine the significance of differences between two means. The one sample t-test was used to determine the significance of differences between means and normalized values (100%). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Results

Transient increase in absolute lymphocyte count (ALC) following ibrutinib administration to MCL patients

[00767] In a Phase I study that enrolled patients with various B cell malignancies, MCL patients (n=9) were treated with ibrutinib in 35-day cycles where the drug was administered once-a-day for 28 days with a 7-day drug holiday between cycles. Under these conditions, a cyclical pattern of increasing and decreasing ALC was observed. This was demonstrated by an increase in ALC following the first few weeks of treatment followed by a return to baseline after the 7-day drug holiday. This cyclic ALC pattern continued for the duration of the treatment. During the course of ibrutinib treatments, the tumor volumes as determined by Sum of Perpendicular Diameters (SPD) were reduced on average by 80% during evaluations following 2, 4 and 6 cycles of treatments. Therefore, during the first 6 cycles of treatment, we observed a sawtooth pattern of increasing and decreasing peripheral blood ALC concomitant with a nodal response in these patients. The same increase in ALC was observed in a subsequent (Phase 2) study, where MCL patients were treated with a fixed dose of 560 mg per day without drug holiday. In this trial, the ALC increased by 100-150% following 2-4 weeks of drug treatment. The increase in ALC was transient with notable reductions in ALC observed by the end of the second cycle. A continued decrease in ALC was observed until tapering off in cycle 4-5.

Elevated ALC is due to an increase of light-chain restricted $CD19^+CD5^+$ cells

[00768] In order to define the population of lymphocytes increased by ibrutinib, the PBMCs of patients isolated before (D1) and after one week of treatment (D8) were stained with CD3, CD19, CD5 and analyzed by flow cytometry. The increased lymphocytes were characterized as $CD3^-CD19^+CD5^+$, both the absolute count and the percentage of $CD19^+CD5^+$ cells in the lymphocyte population were significantly increased after one week of ibrutinib treatment ($p<0.05$) whereas the $CD19^+CD5^-$ population was not. An illustrative patient is shown in Figure 35, where the $CD19^+CD3^-$ and $CD19^+CD5^+$ populations before drug treatments were 9.29% and 84.4%, respectively, and increased to 63% and 98.8%, after one week of treatment. The $CD19^+CD3^-CD5^+$ cells were light-chain restricted (data not shown), likely reflecting increased circulating MCL cells in the periphery following one week of drug treatment. In some cases, the mobilized cells comprised a distinct subset of $CD45^{dim}$ small cells, which is also consistent with MCL.

[00769] In order to confirm that full inhibition of the target BTK was achieved in these patients, occupancy of the BTK active site by ibrutinib was assessed in PBMCs from MCL patients using a competitive binding fluorescent probe assay. On average over 90% target occupancy was observed in patients following 1 week of treatment.

The peripheral $CD19^+CD5^+$ population is $CXCR4^{lo}CD38^{lo}$ and decreased in Ki67 following drug treatment.

[00770] Next we analyzed the $CD19^+CD5^+$ cells for expression of CXCR4, a chemokine receptor known to be involved in migration and homing to tissues. Surface expression of CXCR4 was significantly reduced ($p<0.05$) in the $CD19^+CD5^+$ population following one week of drug treatment (Figure 36A). CXCR4 and CD38 expression in CLL patients has been reported to be lower in lymph node resident cells compared to CLL cells in peripheral blood. Because of this, we analyzed CXCR4 and CD38 expression on patient-matched lymph nodes and peripheral blood resident MCL cells. CXCR4 expression was lower in MCL cells isolated from LNs compared to peripheral blood in all 3 patients examined (Figure 36D). This is consistent with the observed newly circulating $CXCR4^{lo}$ MCL cell population and is consistent with the mobilized cells originating from tissues such as LNs. This notion is further supported by the notable reduction in lymphadenopathy observed during the same period. Further study of the mobilized population disclosed high $CD38^+$ expression corresponding to the increased CD38 expression found in LN-resident MCL cells (Figure 36B/D). This initial increase in CD38+ cells was significantly decreased following treatment in $CD19^+CD5^+$ cells ($p<0.01$), while the $CD19^+CD5^-$ cells (which had consistently low CD38 expression) were not significantly altered (Figure 36B/C).

[00771] Next we examined changes to markers of proliferative capacity as well as homing and migration in the mobilized fraction. Intracellular Ki67 expression was significantly reduced after treatment ($p<0.05$) (Figure 36C). Phosphorylated ERK was also reduced, as demonstrated by phospho-flow cytometry, in the $CD20^+CD5^+$ subpopulation from patients before and after treatment. pErk expression was generally higher in MCL patients' $CD20^+CD5^+$ cells compared to healthy volunteers and was reduced by ibrutinib treatment (Figure 36C, lower panel) although these differences were not statistically significant.

[00772] In addition, chemokines important in homing (MDC, MIP-1β, CXCL13 and CXCL17) were reduced on average by more than 50% following one week of treatment. By the end of the first cycle of treatment, in addition to the decrease of MIP-1 β and MDC, IL-10 and TNF-α were also reduced by 50% (Figure 36E).

Ibrutinib inhibits pseudoemperipoliesis in MCL/stromal co-culture

[00773] The transient increase of ALC in MCL patients treated with ibrutinib may be due to a disruption in cellular adhesion and migration within the lymph node or tissue compartment. To investigate this, we established MCL-stromal cell co-cultures to determine the effect of drug in vitro. Primary MCL cells or the Mino cell line were grown in co-culture with murine bone marrow stromal cells M2-10B4. We found that primary MCL cells or Mino cells both adhered

and quickly migrated beneath the M2-10B4 cells (pseudoemperipoliesis). Significant inhibition of pseudoemperipoliesis by ibrutinib was observed, as demonstrated by light microscopy and the number of Mino cells or primary MCL remaining in the co-culture were quantified by flow cytometry of $hCD19^+$ cells harvested by gentle washing following 4 hrs of co-culture (Figure 37 A and 37B, left panels). Ibrutinib dose-dependently inhibited migration of Mino cells beneath the stromal cells, and the inhibition was significant at 100 nM ($p<0.01$) and 1000 nM ($p<0.001$). Pertussin toxin, a well-studied GPCR inhibitor used as a positive control for inhibition of Mino cell migration significantly inhibited migration at 200 ng/mL ($p<0.001$). In addition, CXCL12, an important chemokine for B cell homing and produced by stromal cells, increased cortical actin of Mino cells, as assessed by Phalloidin fluorescence microscopy, and this response was also dose-dependently and significantly inhibited by ibrutinib treatments at 10 and 100 nM ($p<0.001$) (Figure 37A right panel). Ibrutinib also suppressed actin polymerization of primary MCL in co-culture at 100 nM ($p<0.001$) (Figure 37B, right panel).

Ibrutinib inhibits Btk activity in MCL/stromal co-culture and suppresses stromal cell induced chemokine and cytokine secretion

[00774] To further understand the effect of the drug on MCL cells in co-culture with stromal cells, Mino cells were treated with drug and co-cultured with murine stromal cells (M2-10B4) or stimulated with anti-IgM. Ibrutinib dose-dependently inhibited pBtk, pPLCγ2 and pAkt in Mino cells alone, or in co-culture with M2 cells. Chemokine and cytokine concentrations of conditioned media were determined from ibrutinib treated MCL cell lines alone or in co-culture with M2 stromal cells or stimulated with anti-IgM. Despite lack of detectable activation of signaling proteins upon co-culture with M2, Mino cells increased chemokine and cytokine secretions following BCR stimulations or co-culture. Similar results were observed with the Jeko cell line. Ibrutinib dose dependently and potently suppressed production of human IL-10, MDC, MIP-1α, MIP-1β, TNFα, CCL17 and CCL21 following BCR activation or in co-culture whereas the murine stromal cells alone did not produce human chemokines or cytokines. Similarly, ibrutinib suppressed the production of IL-10, MDC, MIP-1α, MIP-1β, TNFα of Jeko1 cells in co-culture with M2-10B4 or human stromal cell line HS-5. These *in vitro* results with MCL cell lines correlate well with the plasma chemokine/cytokine reduction in ibrutinib treated patients.

Ibrutinib inhibits BCR- and chemokine mediated adhesion and migration in vitro

[00775] We measured the direct effect of ibrutinib on migration and adhesion of the MCL cell lines Mino, Jeko1 and JVM-1. First, the effect of ibrutinib on Btk signaling was determined in these MCL cells. As expected, ibrutinib inhibited phosphorylation of Btk and downstream signaling proteins PLCγ2, MAP kinases Erk, JNK and Akt following anti-IgM and chemokine

CXCL12 and CXCL13 stimulations. Cell surface expression of CXCR4, CXCR5, CCR7, surface IgM, and α4β1 integrin was confirmed by flow cytometry, and subsequent *in vitro* adhesion and chemotaxis assays were performed with drug. Ibrutinib significantly inhibited anti-IgM stimulated adhesion of Jeko1 and HBL1 cells onto fibronectin or VCAM1 at 100 nM (a clinically relevant concentration of ibrutinib) with over 50-70% inhibition. The inhibition of adhesion by ibrutinib was also dose-dependent. Similarly, the adhesion of both Mino and Jeko1 cells to VCAM1 or fibronectin was inhibited by ibrutinib at 100 nM following CXCL12 or CXCL13 activations. The extent of inhibition was greater in Mino cells (50-70%) than in Jeko1 cells (20-30%). In addition to changes in adhesion, we found that ibrutinib dose-dependently inhibited CXCL12-induced migration of Mino, Jeko1 and JVM-1 cells with Mino and Jeko1 cells being more sensitive to drug than JVM-1 cells. ibrutinib also significantly inhibited CXCL13 stimulated migration of Mino cells dose-dependently from 1 nM to 1 μM.

[00776] Next, we examined the effect of ibrutinib on signaling and adhesion in primary MCL cells. Phosphorylation of Y223 was increased in MCL cells compared to normal B lymphocytes, consistent with elevated BCR signaling in malignant B cells. Ibrutinib inhibited pBtk in both primary MCL and normal B cells on Y223, the autophosphorylation site of Btk, and Y551 (a tyrosine phosphorylated by Src-family kinases) and reduced pPLCγ2 on Y759 and Y1217 at concentrations of 10 nM and above. These results demonstrate ibrutinib directly inhibits Btk activity in MCL primary cells. Importantly, ibrutinib also inhibited CXCL12 or CXCL13 activated adhesion to VCAM1 as well as BCR stimulated adhesion to fibronectin at 100 nM in primary MCL cells. The degree of inhibition in these primary cells was about 10-20%, and the magnitude was less impressive compared to the MCL cell lines but the inhibition was statistically significant.

[00777] These studies collectively demonstrate ibrutinib inhibits BCR and CXCL12, CXCL13 activated adhesion and migration in MCL cell lines as well as primary MCL cells which is associated with the Btk inhibition in these cells.

WHAT IS CLAIMED IS:

1. A method for treating a hematological malignancy in an individual in need thereof, comprising administering to the individual an anti-cancer treatment, 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 method of claim 1, wherein the irreversible Btk inhibitor covalently binds to Cys 481 of Btk.

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

4. The method 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 method of claim 1, wherein the hematological malignancy is a B-cell malignancy.

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

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

8. The method 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 method 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 method of claim 1, wherein the mobilized cells are myeloid cells or lymphoid cells.

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

14. The method 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 method 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. 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.

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

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

20. The method 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 method of claim 16, wherein the hematological malignancy is a B-cell malignancy.

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

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

24. The method 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 method 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 method of claim 16, wherein the mobilized cells are myeloid cells or lymphoid cells.

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

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

35. The method 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. 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; and

b. preparing a biomarker profile for a population of cells isolated from the plurality of cells.

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

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

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

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

48. The method 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.