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(54) **BRUTON'S TYROSINE KINASE INHIBITORS FOR HEMATOPOIETIC MOBILZATION**

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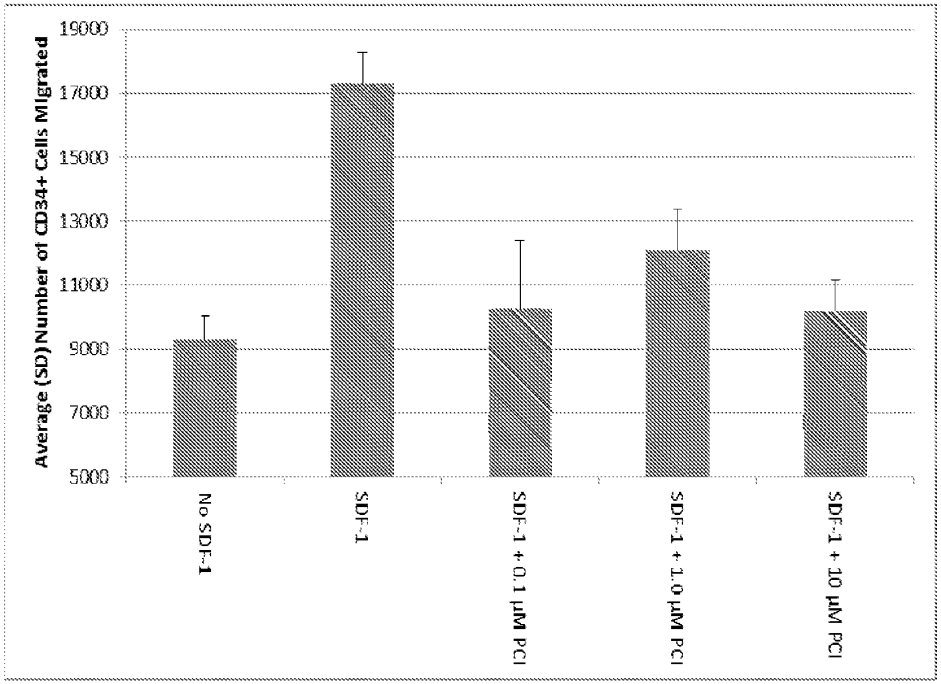
(57) **ABSTRACT**

Methods to improve hematopoiesis and increase white blood cell counts in subjects and patients using pyrimidine-based inhibitors of Bruton's tyrosine kinase (Btk) are disclosed.

Figure 1



BRUTON'S TYROSINE KINASE INHIBITORS FOR HEMATOPOIETIC MOBILZATION

FIELD OF INVENTION

[0001] The invention is in the field of therapeutics and medicinal chemistry. The present invention relates generally to the administration of a Bruton's tyrosine kinase inhibitor to mobilize hematopoietic stem and progenitor cells from the bone marrow into the peripheral blood and the use of such hematopoietic cells to improve hematopoiesis and/or in the treatment of various disorders.

BACKGROUND OF THE INVENTION

[0002] Bruton's tyrosine kinase (Btk) is a member of the Tec family of non-receptor tyrosine kinases and plays a role in several 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., Jeffries, et al. (2003) *J. Biol. Chem.* 278:26258-26264; Horwood et al. (2003) *J. Exp. Med.* 197: 1603-1611; Iwaki et al. (2005) *J. Biol. Chem.* 280(48):40261-40270; Vassilev et al. (1999) *J. Biol. Chem.* 274(3):1646-1656, and Quek et al. (1998), *Curr. Bio.* 8(20):1137-1140. It is a particularly important in the signaling pathway initiated upon stimulation of the B cell receptor and during B cell development. Mutations in the Btk gene result in X-linked agammaglobulinemia, an immunodeficiency characterized by failure to produce mature B lymphocytes and associated with a failure of Ig heavy chain rearrangement. Rawlings and Witte (1994) *Immun. Rev.* 138:105-119. In the mouse, point mutation or deletion of Btk causes X-linked immunodeficiency (xid), with about 50% fewer conventional B2 B cells, absent B1 B cells, and reduced serum Ig levels. Khan et al (1995) *Immunity* 3:283-99; Rawlings et al (1993) *Science* 261:358-61. Btk is also expressed in specific cells of the myeloid lineage, and evidence suggests that it contributes to immune-complex mediated activation of the FcγR and FcεR signaling pathways in monocytes/macrophages, neutrophils, and mast cells. See, e.g., Jongstra-Bilen et al. (2008) *J. Immunol.* 181:288-298; Wang et al. (2007) *Int. Immunopharmacol.* 7:541-546; Hata et al. (1998) *J Exp Med.* 187:1235-1247.

[0003] Due to the role of Btk in inhibiting Fas/APO-1 apoptotic signals in the B cell lineage, inhibitors of Btk, also referred to as Btk inhibitors, have been evaluated as agents for treating hematopoietic malignancies (e.g., B cell lymphoma). Additionally, due to the role of Btk in the signaling pathways of other immune cells, Btk inhibitors have also been evaluated as agents for suppressing the immune system, e.g., in patients with autoimmune disorders or organ transplants. See, e.g., Honinberg et al. (2010) *Proc. Natl. Acad. Sci. USA* 107:13075-80; Chang et al. (2011) *Arthr. Res. & Ther.* 13:R115. Evidence for the role of Btk in autoimmune and/or inflammatory disease has been established in Btk-deficient mouse models. For example, in standard murine preclinical models of systemic lupus erythematosus (SLE), Btk deficiency has been shown to result in a marked amelioration of disease progression. Moreover, Btk deficient mice are also resistant to developing collagen-induced arthritis and are less susceptible to Staphylococcus-induced arthritis Inhibition of Btk activity is useful for the treatment of autoimmune and/or inflammatory diseases such as: SLE, rheumatoid arthritis, multiple vasculitides, idiopathic thrombocytopenic purpura (ITP), myasthenia gravis, and asthma. See, e.g., U.S. Pat. No. 7,393,848.

[0004] Btk inhibitors have also been shown useful in preventing or reducing the risk of thromboembolism. See, e.g., Uckun (2008) *Int. Rev. Immunol.* 27:43-69.

SUMMARY OF INVENTION

[0005] In contrast to the prior art uses of Bruton's Tyrosine Kinase inhibitors to suppress immune cells and/or the immune system, disclosed herein is the surprising discovery that Btk inhibitors can mobilize hematopoietic stem cells and progenitor cells to the peripheral blood of a subject, e.g., to increase the white blood cell count in the subject. Accordingly, provided herein are methods and compositions for improving hematopoiesis and increasing the white blood cell count in a subject in need thereof, including patients undergoing chemotherapy, radiation therapy and/or bone marrow transplantation. Also provided herein are methods of determining whether a Btk inhibitor is a "mobilizing Btk inhibitor" capable of mobilizing hematopoietic stem and/or progenitor cells to the peripheral blood of a subject; and methods of using a mobilizing Btk inhibitor to mobilize such cells, including harvesting such cells for subsequent reinfusion into the same or a different subject.

[0006] In one aspect, the invention provides methods for mobilizing hematopoietic stem and/or progenitor cells in a subject in need thereof comprising administering to said subject a pharmaceutical composition comprising a mobilizing Bruton's Tyrosine Kinase (Btk) inhibitor in an amount effective to mobilize said cells into the peripheral blood of said subject. The inventive methods and uses can be advantageously employed in conjunction with bone marrow transplantation procedures, and/or subsequent to chemotherapy and/or radiation exposure to address leukopenia, neutropenia, granulocytopenia and/or thrombocytopenia in such patients. Accordingly, in some embodiments the subject may be a bone marrow transplantation patient, and/or a leukopenic or neutropenic patient or a patient at risk of impaired hematopoiesis due to prior chemotherapy and/or radiation therapy.

[0007] As compounds that increase the white blood cell count in a subject, the instant mobilizing Btk inhibitors may be administered as part of any therapeutic protocol aiming to restore or improve hematopoiesis in a patient in need thereof, e.g., to enhance the success of bone marrow transplantation, to reduce the extent or duration of leukopenia and neutropenia resulting from chemotherapy, radiation therapy or accidental radiation exposure, to enhance wound healing and burn treatment, and/or to aid in restoration of damaged organ tissue. They may also combat bacterial infections that are prevalent in leukemia.

[0008] In another aspect, the invention provides methods of obtaining mobilized hematopoietic stem and progenitor cells and uses thereof. The subject methods comprise administering to a subject a mobilizing Btk inhibitor in an amount effective to increase the number of such cells in the subject, preferably in the peripheral blood of the subject. In one embodiment, the administering step comprises administration of a mobilizing Btk inhibitor alone. In another embodiment, the step comprises administration of a mobilizing Btk inhibitor in combination with other compounds, e.g., cytokines, that also increase the white blood cell count in the peripheral blood of a subject. Suitable compounds may be selected from the group consisting of granulocyte-macroph-

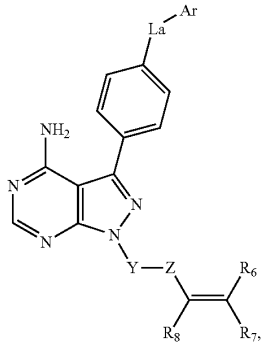
age colony stimulating factor (GM-CSF), Interleukin-1 (IL-1), Interleukin-3 (IL-3), Interleukin-8 (IL-8), PIXY-321 (GM-CSF/IL-3 fusion protein), macrophage inflammatory protein, stem cell factor, plerixafor, thrombopoietin, growth related oncogene, and/or combinations thereof. Thus, the subject methods comprise administering to a subject a mobilizing Btk inhibitor (with or without other mobilizing factors) in an amount effective to increase the number of hematopoietic stem and progenitor and/or white blood cells in the peripheral blood of the subject, and obtaining the immune cells so mobilized, e.g., by apheresis.

[0009] The harvested cells may be used therapeutically, e.g., in hematopoietic stem and/or progenitor cell transplantation. Accordingly, in another aspect the invention provides methods of treating a patient in need of improved hematopoiesis comprising administering to a subject a mobilizing Btk inhibitor (with or without other mobilizing factors) in an amount effective to increase the number of hematopoietic stem, progenitor and/or white blood cells in the peripheral blood of the subject, obtaining the cells so mobilized, and introducing the cells into the patient. Preferably, the subject and the patient are histocompatible. In one embodiment, the histocompatible subject and the patient are syngeneic. In another embodiment, the histocompatible subject and the patient are allogeneic.

[0010] In another embodiment, the harvested cells are enriched and/or cultured ex vivo prior to introduction into the patient. Such ex vivo culture comprises differentiating the obtained cells into or enriching for myeloid cells, lymphoid cells, and common progenitors thereof etc. Accordingly, in one embodiment, a method of treating a patient in need thereof further comprises culturing the obtained hematopoietic cells in one or more differentiation factors prior and/or enriching the obtained hematopoietic cells for a common progenitor cell or cells prior to introducing the cells into the patient.

[0011] Mobilizing Btk inhibitors may be administered to any animal subject in order to mobilize hematopoietic stem and progenitor cells. In a preferred embodiment, the mobilizing Btk inhibitor is administered to a mammal, and more preferably to a human.

[0012] Preferred mobilizing Btk inhibitors suitable for use in the subject invention comprise a pyrimidine ring, i.e., a 1,3 diazine. In one embodiment, the mobilizing Btk inhibitor is selected from a compound of structural Formula I:



wherein:

[0013] La is CH_2, O, NH or S;

[0014] Ar is a substituted or unsubstituted aryl, unsubstituted phenyl, or a substituted or unsubstituted heteroaryl;

[0015] Y is a 4-, 5-, 6-, or 7-membered cycloalkyl ring, or

[0016] Y is a 4-, 5-, 6-, or 7-membered monocyclic nitrogen containing heterocycloalkyl ring; or

[0017] Y is an optionally substituted group selected from among alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl; or

[0018] Y is selected form the group consisting of azetidinyl, pyrrolidinyl, piperidinyl, and azepanyl;

[0019] Z is C(═O), OC(═O), NHC(═O), NRC(═O), C(═S), S(═O)x, OS(═O)x, NHS(═O)x, where x is 1 or 2;

[0020] R_7 and R_8 are independently selected from among H, unsubstituted C_1-C_4alkyl, substituted C_1-C_4 alkyl, C_1-C_6 alkoxyalkyl, C_1-C_8 alkylamino alkyl, C_1-C_4 alkyl(phenyl), unsubstituted C_1-C_4heteroalkyl, substituted C_1-C_4heteroalkyl, unsubstituted C_3-C_6cycloalkyl, substituted C_3-C_6cycloalkyl, unsubstituted C_2-C_6heterocycloalkyl, and substituted C_2-C_6heterocycloalkyl; or

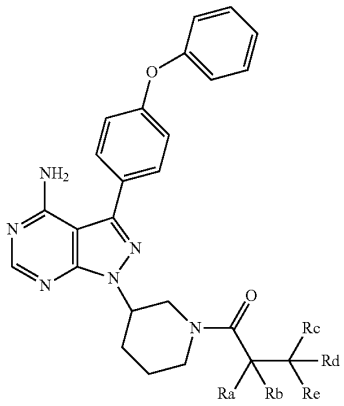
[0021] R_7 and R_8 taken together form a bond;

[0022] 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_8alkylaminoalkyl, substituted or unsubstituted C_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(phenyl), C_1-C_4alkyl(heteroaryl), C_1-C_4alkyl(C_3-C_8cycloalkyl), or C_1-C_4alkyl(C_2-C_8heterocycloalkyl), or C_1-C_8 alkylamino alkyl;

[0023] R is H, or C_1-C_6alkyl; and

pharmaceutically acceptable solvates or pharmaceutically acceptable salts thereof.

[0024] In another embodiment, the mobilizing Btk inhibitor for use in the subject invention is selected from a compound of structural Formula II:



wherein:

[0025] Ra, Rb, Rc, Rd, and Re, are each independently selected from H, F, Cl, Br, I, —CN, —SR_2, —OR_3, CO_2R_3; or

[0026] Ra, or Rb together with one of Rc, Rd and Re, and the carbon atoms to which they are attached form an epoxide;

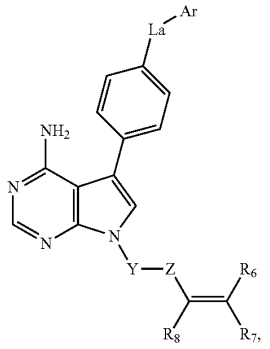
[0027] wherein Ra, Rb, Rc, Rd, and Re, cannot all be H;

[0028] R_2 is selected from H, methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, tert-butyl, a cysteinyl, a glutathionyl, C_1-C_4alkyl, a cysteinyl, or a glutathionyl;

[0029] R_3 is selected from H, C_1-C_4alkyl, phenyl, or benzyl; and

pharmaceutically acceptable solvates or pharmaceutically acceptable salts thereof.

[0030] In another embodiment, the mobilizing Btk inhibitor for use in the subject invention is selected from a compound of structural Formula III:



wherein;

[0031] La is O or S;

[0032] Ar is an unsubstituted phenyl;

[0033] Y is a 4-, 5-, 6-, or 7-membered cycloalkyl ring, or

[0034] Y is a 4-, 5-, 6-, or 7-membered monocyclic nitrogen containing heterocyclic ring;

[0035] Z is C(═O), OC(═O), NHC(═O), $S(═O)_x$, or $NHS(═O)_x$, where x is 2;

[0036] R_8 is H; R_7 is H, unsubstituted C_1-C_4 alkyl, C_1-C_6 alkoxyalkyl, C_1-C_8 alkylaminoalkyl, or C_1-C_4 alkyl(phenyl); or

[0037] R_7 and R_8 taken together form a bond;

[0038] R_6 is H, unsubstituted C_1-C_4 alkyl, C_1-C_6 alkoxyalkyl, C_1-C_8 alkylaminoalkyl, or C_1-C_4 alkyl(phenyl); and

pharmaceutically acceptable solvates or pharmaceutically acceptable salts thereof.

[0039] In certain embodiments, the compounds of Formulas I-III may include an asymmetric center or centers, and may be in the form of a composition of a racemic mixture, a diastereoisomeric mixture, a single enantiomer, an enantiomeric diastereomer, a meso compound, a pure epimer, or a mixture of epimers thereof, etc. Further, the compounds of Formulas I or II may have one or more double bonds, and may be in a form of a cis/trans, E/Z mixture or an E or Z geometric isomer thereof.

[0040] The compounds of Formulas I, II and III may also be prepared as a salt form, e.g., pharmaceutically acceptable salts, including suitable acid forms, e.g., salt forms selected from hydrochloride, hydrobromide, acetate, propionate, butyrate, sulphate, hydrogen sulphate, sulphite, carbonate, hydrogen carbonate, phosphate, phosphinate, oxalate, hemi-oxalate, malonate, hemi-malonate, fumarate, hemi-fumarate, maleate, hemi-maleate, citrate, hemi-citrate, tartrate, hemi-tartrate, aspartate, glutamate, etc.

BRIEF DESCRIPTION OF THE FIGURES

[0041] FIG. **1** shows the results of an in vitro transwell assay using human $CD34^+$ cells and migration towards an SDF-1 gradient with varying concentrations of a pyrimidine-based Btk inhibitor.

DETAILED DESCRIPTION

[0042] Blood cells play a crucial part in maintaining the health and viability of animals, including humans. White blood cells include neutrophils, macrophages, eosinophils, basophils, mast cells, and the B and T cells of the immune system. White blood cells are continuously replaced via the hematopoietic system, by the action of colony stimulating factors (CSF) and various cytokines on progenitor cells in hematopoietic tissues. The nucleotide sequences encoding a number of these growth factors have been cloned and sequenced. Perhaps the most widely known of these is granulocyte colony stimulating factor (G-CSF) which has been approved for use in counteracting the negative effects of chemotherapy by stimulating the production of white blood cells and progenitor cells (peripheral blood stem cell mobilization). A discussion of the hematopoietic effects of this factor can be found, for example, in U.S. Pat. No. 5,582,823, incorporated herein by reference.

[0043] Several other factors have been reported to increase white blood cells and progenitor cells in both human and animal subjects. These agents include granulocyte-macrophage colony stimulating factor (GM-CSF), Interleukin-1 (IL-1), Interleukin-3 (IL-3), Interleukin-8 (IL-8), PIXY-321 (GM-CSF/IL-3 fusion protein), macrophage inflammatory protein, stem cell factor, thrombopoietin and growth related oncogene, as single agents or in combination. Dale et al. (1998) *Am. J. of Hematol.* 57:7-15; Rosenfeld et al. (1997) *Bone Marrow Transplantation* 17:179-183; Pruijt et al., (1999) *Cur. Op. in Hematol.* 6:152-158; Broxmeye et al. (1995) *Exp. Hematol.* 23:335-340; Broxmeyer et al. (1998) *Blood Cells, Molecules and Diseases* 24:14-30; Glaspy et al. (1996) *Cancer Chemother. Pharmacol.* 38 (suppl): S53-S57; Vadhan-Raj et al. (1997) *Ann. Intern. Med.* 126:673-81; King et al. (2001) *Blood* 97:1534-1542; Glaspy et al. (1997) Blood 90:2939-2951.

[0044] While endogenous growth factors are pharmacologically effective, the well-known disadvantages of employing proteins and peptides as pharmaceuticals underlies the need to add to the repertoire of such growth factors with agents that are small molecules. In another aspect, such small molecules are advantageous over proteins and peptides where production in large quantities are desired.

[0045] As used herein, the term "progenitor cell" refers to a cell that, in response to certain stimuli, can form differentiated hematopoietic or myeloid cells. The presence of progenitor cells can be assessed by the ability of the cells in a sample to form colony-forming units of various types, including, for example, CFU-GM (colony-forming units, granulocyte-macrophage); CFU-GEMM (colony-forming units, multipotential); BFU-E (burst-forming units, erythroid); HPP-CFC (high proliferative potential colony-forming cells); or other types of differentiated colonies which can be obtained in culture using known protocols.

[0046] As used herein, "stem cells" are less differentiated forms of progenitor cells. Typically, such cells are often positive for CD34. Some stem cells do not contain this marker, however. These CD34+ cells can be assayed using fluorescence activated cell sorting (FACS) and thus their presence can be assessed in a sample using this technique.

[0047] In general, CD34+ cells are present only in low levels in the blood, but are present in large numbers in bone marrow. While other types of cells such as endothelial cells and mast cells also may exhibit this marker, CD34 is considered an index of stem cell presence.

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