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REVIEW

Novel Bruton's tyrosine kinase inhibitors currently in development

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Abstract: Bruton's tyrosine kinase (Btk) is intimately involved in multiple signal-transduction pathways regulating survival, activation, proliferation, and differentiation of B-lineage lymphoid cells. Btk is overexpressed and constitutively active in several B-lineage lymphoid malignancies. Btk has emerged as a new antiapoptotic molecular target for treatment of B-lineage leukemias and lymphomas. Preclinical and early clinical results indicate that Btk inhibitors may be useful in the treatment of leukemias and lymphomas.

Keywords: tyrosine kinase, personalized therapy, kinase inhibitors, Btk, leukemia, lymphoma

Introduction

Bruton's tyrosine kinase (Btk) is intimately involved in multiple signal-transduction pathways regulating survival, activation, proliferation, and differentiation of B-lineage lymphoid cells.[1,2] Btk is an upstream activator of multiple antiapoptotic signaling molecules and networks, including the signal transducer and activator of transcription 5 (STAT5) protein,[3] phosphatidylinositol (PI) 3-kinase/AKT/mammalian target of rapamycin (mTOR) pathway,[4] and nuclear factor kappa B (NF-κB) (Figure 1).[5,6] Further, Btk associates with the death receptor Fas via its kinase and pleckstrin homology (PH) domains and prevents the interaction of Fas with Fas-associated protein with death domain (FADD), which is essential for the recruitment and activation of caspase-8/FLICE by Fas during the apoptotic signal (Figure 1). This impairment by Btk prevents the assembly of a proapoptotic death-inducing signaling complex (DISC) after Fas ligation.[7]

Btk is abundantly expressed in malignant cells from patients with B-cell precursor (BCP)-acute lymphoblastic leukemia (ALL, the most common form of cancer in children and adolescents), chronic lymphocytic leukemia (CLL), and non-Hodgkin's lymphoma (NHL).[8–11] A meta-analysis of cancer-associated gene expression changes utilizing the Oncomine database revealed a marked enrichment of the most discriminating Btk-dependent antiapoptotic gene targets in 17 comparisons for diagnostic classes of human leukemias and lymphomas obtained from eight studies.[11] Consequently, Btk has emerged as a new molecular target for treatment of B-lineage leukemias and lymphomas.

Btk disease targets

Lymphohematopoietic malignancies

B-lineage ALL (B-ALL) and B-cell CLL (B-CLL) are the most common childhood and adult leukemias, respectively. In both ALL and CLL, the resistance of leukemia

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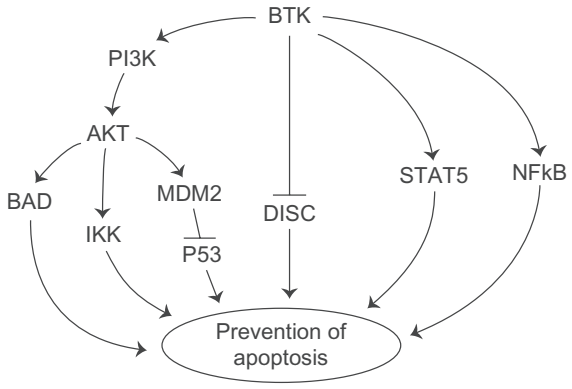

Figure 1 Btk activates antiapoptotic pathways. Btk is an upstream regulator of multiple antiapoptotic pathways, including the PI3K–AKT pathway, STAT5 pathway, and NFκB pathway. BTK also blocks the Fas-mediated apoptosis. See text for further discussion and references.
Abbreviations: Btk, Bruton's tyrosine kinase; PI3K, phosphatidylinositol 3-kinase; BAD, B-cell lymphoma 2-associated death promoter; IKK, IκB kinase; DISC, death-inducing signaling complex; STAT, signal transducer and activator of transcription; NFκB, nuclear factor kappa B.

cells to apoptosis-inducing chemotherapeutic agents hampers a more successful outcome.[12–15]

B-cell chronic lymphocytic leukemia

CLL is the most common leukemia in adults, accounting for 25% of all leukemias, with approximately 8000 new cases diagnosed each year.[16–19] CLL is characterized by the accumulation of mature, $CD5^+/CD23^+$ monoclonal B lymphocytes in the blood, secondary lymphatic tissues, and bone marrow (BM).[20] It is well established that the tumor microenvironment plays a major role in the pathogenesis of CLL: various cytokines, chemokines, and adhesion molecules provided within the lymph nodes (LNs), spleen, and BM microenvironment, as well as signaling by the B-cell receptor (BCR), play a critical role in the localization, growth, survival, and drug resistance of CLL cells.[21–26] Proliferating CLL cells, which account for approximately 0.1%–1% of the CLL clone,[21] are typically found within microanatomical structures called proliferation centers or pseudofollicles,[22] where CLL cells interact with accessory cells (ie, stromal cells or T cells), thereby receiving survival and growth signals.[23] Such external signals from the leukemia microenvironment can supplement intrinsic oncogenic lesions, thereby promoting maintenance and expansion of the CLL clone.[22] Among the various external stimuli in the tissue microenvironments, BCR activation and signaling, particularly in lymphatic tissues, is a central pathologic mechanism, even though the precise mechanism of BCR stimulation and the nature of the antigen(s) that activate the BCRs remain obscure.[20,24,25] The most direct evidence for the importance of BCR signaling in CLL comes from recent comparative gene-expression profiling data that revealed BCR signaling as the most prominent pathway activated in CLL cells isolated from lymphatic tissues.[24] Because either tonic, chronic, or antigen-driven BCR signaling is involved in the pathogenesis of most types of B-cell malignancies, the BCR signalosome provides a rational therapeutic target, including CLL.[26]

Although CLL is a disease that is considered to be incurable with currently available therapy, its clinical course is heterogeneous: some patients have a more stable disease and die after many years from unrelated causes, whereas others progress very quickly and die within a few years. This variability has stimulated the search for prognostic markers with which to predict the outcome of patients and to allow treatments to be adapted to the specific risk. There is an urgent need for apoptosis-promoting new antileukemic agents against B-CLL. Several kinases are expressed at elevated levels in CLL cells, including Btk, Zeta-chain-associated protein (ZAP), and Lyn, and therefore have emerged as potential molecular targets.[27]

B-lineage acute lymphoblastic leukemia

B-ALL is the most common form of cancer in children and adolescents.[28] Current treatment with ALL can cure approximately 80% of children with the disease.[29,30] Currently, the major challenge in the treatment of B-ALL is to cure patients who have relapsed (~20%) despite intensive multiagent chemotherapy.[31–35] The standard approach to the treatment of these high-risk patients has been salvage chemotherapy to achieve a second remission and subsequent use of very intensive treatment regimens, including high-dose "supralethal" chemotherapy, often combined with total-body irradiation (TBI), followed by hematopoietic stem cell transplantation (SCT). Laboratory evidence indicates that primary clonogenic blasts from a significant portion of B-ALL patients are among those reported as the most radiation-resistant human tumor cells.[36,37] Furthermore, clonogenic leukemia cells from over two-thirds of the ALL patients exhibit a substantial capacity to repair sublethal radiation damage.[38] Resistance of B-leukemia cells to the proapoptotic effects of radiation-induced oxidative stress hampers the attempts to improve the survival outcome of patients with B-ALL undergoing TBI and SCT and only $< 20\%$ of high-risk B-ALL patients become long-term disease-free survivors even after TBI/SCT, with substantial short-term and long-term morbidity and mortality.[38,39] These preclinical and clinical observations in B-ALL emphasize

the urgent need for identification of new drug candidates capable of potentiating the antileukemic potency of pre-SCT radiochemotherapy regimens.

In contrast with the marked improvements achieved in the treatment outcome of pediatric ALL patients, adult patients with ALL continue to have a poor outcome, with long-term leukemia-free survival rates of less than 50%.[39–42] This poor outcome has been attributed in part to an increased frequency of high-risk leukemia with greater drug resistance.[2,40–44] A more rapid and complete reduction of the leukemia cell burden by upfront induction chemotherapy is likely to prevent drug resistance and improve the survival outcome in ALL.[8,9,43] Furthermore, a more effective postinduction intensification therapy may eliminate a higher fraction of residual leukemia cells, thereby improving the duration of remission.[9,13,15,43] Consequently, the development of new potent anti-ALL drugs and the design of combinative treatment protocols using these new agents have emerged as exceptional focal points for leukemia research.[8,13,24]

Btk is the first cytoplasmic non-Janus kinase (JAK) to be identified as a positive regulator of STAT5A in neoplastic B cells and B-cell precursors (BCPs). STAT5, a direct substrate of Btk that is activated by Btk-mediated tyrosine phosphorylation of its Y^{694} residue,[40] is an important regulator of survival[17,44–46] and proliferation[27,44,47–49] of BCPs at various stages of B-cell ontogeny. Recent studies have further revealed a nucleocytoplasmic shuttling system for Btk, which has implications regarding potential targets inside the nucleus and which may be critical in gene regulation during B-cell development and differentiation as well as apoptosis.[50] Btk is essential for BCR-mediated activation of the NF-κB/Rel family of transcription factors, which in turn regulates genes controlling B-cell growth.[51–53] STAT5 knockout mice suffer from leukopenia/lymphopenia and an accelerated rate of lymphohematopoietic cell apoptosis in the BM.[17] Conversely, constitutive activation of STAT5 is capable of causing leukemic transformation of lymphohematopoietic cells[19] and development of BCP leukemia in mice.[45] Dominant-negative forms of STAT5 induce massive apoptosis in BCP-ALL cells.[46] Btk is required for pre-BCR-dependent survival signals in BCP-ALL cells, including STAT5 activation and STAT5-mediated upregulation of BCL-xL, which rescues $BCR\text{-}ABL^{+}$ BCP-ALL cells from apoptosis. The Btk-linked NF-κB and phosphatidylinositol 3-kinase (PI3K)/Akt survival pathways are activated by chemotherapeutic agents and also contribute to drug resistance of BCP-ALL cells.[54,55] Tyrosine kinase inhibitors (TKIs) targeting Btk are likely to act as antileukemic agents with apoptosis-promoting and chemosensitizing properties and enhance the chemosensitivity of ALL cells.[2,56]

B-lineage non-Hodgkin's lymphoma

NHL is the third most common group of malignancies in children and adolescents in the US and accounts for approximately 7% of newly diagnosed cancers.[57,58] NHL and Hodgkin's lymphomas are represented prominently in the adolescent and young adult population. NHL constitutes 6%–10% of all pediatric malignancies in different parts of the world. NHL is the fifth most common cause of cancer deaths in young adult women aged 20–39 years. Although age-adjusted incidence rates of NHL increase with age, the more aggressive lymphomas are seen more commonly in the younger population, with a transition to low-grade, indolent subtypes as the population ages. Burkitt lymphoma, diffuse large B-cell lymphoma (DLBCL), lymphoblastic lymphoma, and anaplastic large-cell lymphoma make up the most common subtypes in the young adult population, although within the subgroup aged 30–39 years, follicular lymphoma (FL) becomes more prominent. Btk inhibitors have demonstrated clinical activity against a variety of B-cell malignancies in ongoing phase I/II trials, including mantle-cell lymphoma (MCL), CLL, FL, and DLBCL, with good tolerability.

DLBCL is one of the most common types of aggressive B-cell NHL. Pathologically, the tumor has a fast growth rate with a high Ki67 index. Without treatment, patients usually die within 6–24 months, and with current immunochemotherapeutic regimens 50%–60% of patients can be cured. However, 40%–50% of patients remain refractory to the therapy. Recently, BCR signaling has been recognized as a key pathway in the pathogenesis of DLBCL.[59] Gene-expression profiling and unsupervised consensus clustering for analysis studies have identified a subset of lymphoma that demonstrates a BCR/proliferation signature (BCR-type DLBCL).[60] In activated B-cell-like (ABC) DLBCL, NF-κB activity relies upon chronic active BCR signaling, which can be potentially blocked by kinase inhibitors targeting Btk. Btk inhibitors are highly active against ABC DLBCL cells in vitro, and demonstrated clinical activity in a subset of patients with relapsed/refractory (R/R) ABC DLBCL.[61]

Follicular NHL is the most common of the indolent lymphomas, accounting for about 70% of them and about 22% of all lymphomas in North America and Europe. One of the primary concerns for any patient with follicular or other indolent lymphoma is transformation to a more aggressive lymphoma, such as DLBCL.[62,63] Similarly, patients with

recurrent B-lineage NHL or NHL that shows progression on standard chemotherapy regimens have a dismal prognosis and are in urgent need for innovative treatments capable of overcoming the multidrug resistance of their NHL cells. In the US, approximately 70,000 people will be diagnosed with NHL and 19,000 people will die of NHL during 2013.

MCL is a malignancy of mature B cells characterized by the translocation t(11;14) that leads to aberrant expression of cyclin D1.[64] Response to first-line chemotherapy is good, but most patients relapse, resulting in a median survival of 5–7 years. Clinical studies with Btk inhibitors suggest that BCR signaling could play a role.[65]

Multiple myeloma

Multiple myeloma (MM) is a clonal malignancy of plasma cells accumulating in the BM. Myeloma cells have a high capacity to induce osteolytic bone lesions in patients, especially in the advanced stages.[66] One key clinical feature of this cancer is the hyperactive bone resorption and minimal bone regeneration, partly due to overactive osteoclasts and inactive osteoblasts via unbalanced regulation of cytokines and chemokines in the BM microenvironment.[67] MM cells are highly dependent on the BM microenvironment for growth and survival through interactions, particularly with BM stromal cells, osteoclasts, and osteoblasts, all of which secrete important MM growth factors and cytokines.

Btk protein is expressed in plasma cell cancers, including MM and Waldenström's macroglobulinemia (WM), with baseline activation correlating with levels of protein expression. Btk plays a crucial role in myeloma-associated osteolysis via regulating a broad panel of cytokines and chemokines both at transcriptional and protein levels in osteoclast lineage cells and BMSCs, which are in close contact with MM cells within the BM microenvironment. These Btk-targeted cytokines and chemokines include macrophage inflammatory protein (MIP)-1α,[68] MIP-1β,[69] stromal cell-derived factor (SDF)-1,[69] transforming growth factor (TGF)-β1,[70] activin A,[71] acidic protein rich in leucines (APRIL),[72] B-cell-activating factor (BAFF),[73] and interleukin (IL)-8,[74] which have been shown to contribute to MM-related bone lesions and disease progression. Btk activation in the BM milieu promotes MM cell growth, survival, and interaction with other BM stromal components, in addition to triggering MM-induced bone lysis. In a genome-wide screening of mRNA for nonreceptor TKs expressed during osteoclast and osteoblast differentiation in mice, high expression of Lyn and Syk, which are upstream of Btk, as well as Src, were identified in osteoclasts. In addition, Btk was shown to regulate osteoclast maturation by modulating the activity of NFATc1, the major osteoclast transcriptional factor activated following receptor activator of NF-κB ligand (RANKL) stimulation.[75] These findings suggest a potential role of Btk in mediating osteolytic bone disease in MM and a framework for the clinical development of Btk inhibitors as a novel therapeutic strategy in MM.[76]

Solid tumors

Recently, it was discovered that Btk is expressed not only in malignant lymphohematopoietic cells but in solid tumor cells as well.[77–79] Eifert used an RNA interference (RNAi) screen to perform a large-scale loss-of-function analysis to facilitate the identification of individual factors necessary for the survival of an ErbB2/human epidermal growth factor receptor (HER)-2-positive breast cancer cell line.[77,78] Notably, Btk short interfering RNA (siRNA) caused apoptosis in breast cancer cells.[77,78] An aberrantly spliced 80 kDa Btk product with an amino-terminal extension was abundantly expressed in breast cancer cells as a potential molecular target.[77,78] Likewise, Lavitrano et al[79] discovered a functional isoform of Btk in colorectal cancer cells and showed that its inhibition enhances cancer cell chemosensitivity.

Btk inhibitor pipeline

Several Btk inhibitors have been reported by us and others, and are being developed as therapeutic agents for various indications (Figure 2).[80–104] Among these, the covalent inhibitor ibrutinib/PCI-32765 (Pharmacyclics) was developed as a selective and irreversible inhibitor of Btk, targeting the cysteine-481 residue in the active site.[100] PCI-32765 is a potent nanomolar inhibitor of Btk, and exhibited promising activity in preclinical models of BCR-driven B-lineage lymphoma[101] and clinical testing in lymphoma patients.[102] Likewise, dianilinopyrimidine-based irreversible Btk inhibitors with micromolar activity were developed, and two lead compounds, AVL-101 and AVL-291 (Avila Therapeutics) showed promising in vitro activity against lymphoma cells.[103] Dasatinib/Sprycel, a breakpoint cluster region–Abelson (BCR–Abl) kinase inhibitor that is FDA-approved for the treatment of chronic myelogenous leukemia (CML), is a potent inhibitor of Btk.[104,105] The availability of the coordinates of the Btk kinase domain X-ray crystal structures will continue to support further development of rationally designed Btk inhibitors.[104,106]

Covalent Btk inhibitors

Conventional small-molecule inhibitors interact with high affinity in the binding site of a protein target, and the drug-target complex is favored when plasma drug concentration is high.

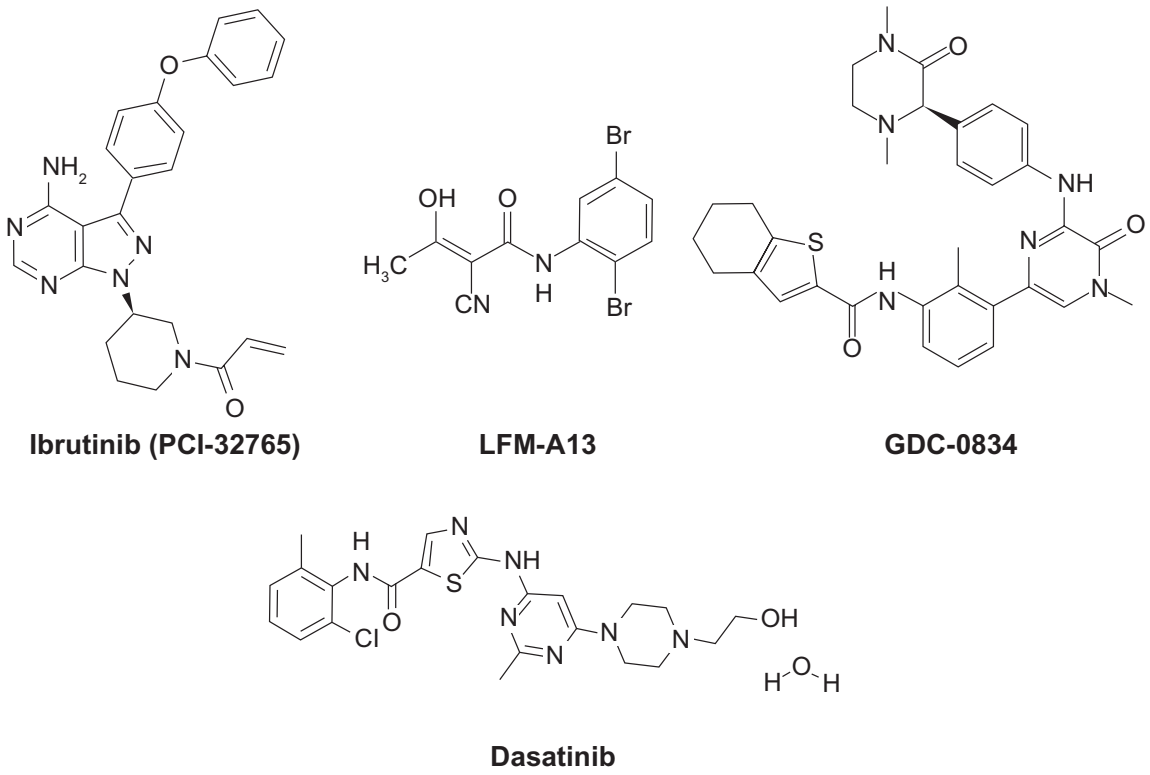

Figure 2 Chemical structures of Bruton's tyrosine kinase inhibitors. Ibrutinib (PCI-32765) is a covalent inhibitor currently under phase II and III clinical development for B-cell malignancies.
Note: LFM-A13, GDC-0834, and dasatinib are noncovalent adenosine triphosphate-competitive Bruton's tyrosine kinase inhibitors.

Covalent inhibitors also form high-affinity interactions with the binding site of the protein target and bring a low-reactivity warhead in close proximity to a structurally unique amino acid. Through covalent bonding, the compound is locked to its target, thereby silencing the target's activity. As a result, the pharmacodynamic behavior of a compound is coupled to protein half-life and turnover rather than pharmacokinetic properties. The covalent kinase inhibitors include ibrutinib/PCI-32765 (Figure 2), AVL-101, and AVL-291/292.

Ibrutinib/PCI-32765

Ibrutinib/PCI-32765 (1-[(3*R*)-3-[4-amino-3-(4-phenoxyphenyl)pyrazolo[3,4-d]pyrimidin-1-yl]piperidin-1-yl]prop-2-en-1-one) is an orally active, small-molecule inhibitor that forms an irreversible bond with cysteine-481 in the active site of Btk and inhibits Btk phosphorylation on Tyr^{223}, resulting in Btk inhibition (half maximal inhibitory concentration [IC_{50}] = 0.5 nM).[100,101] However, the cysteine residue is also present in a small subset of kinases with the potential for irreversible binding by PCI-32765. In vitro preclinical studies demonstrated that PCI-32765 blocks BCR-stimulated activation of ERK1/2, PI3K, and NF-κB, inhibits growth, and induces apoptosis in B-cell lymphoma cell lines.[107] PCI-32765 inhibits activation-induced proliferation of CLL cells and effectively blocks survival signals provided externally to CLL cells from the microenvironment (CD40L, BAFF, IL-6, IL-4, and tumor necrosis factor [TNF]-α), fibronectin engagement, and stromal cell contact, as well as migration in response to tissue-homing chemokines (CXCL12, CXCL13).[108,109] PCI-32765 targets not only B lymphocytes but also monocytes, macrophages, and mast cells, which are important Btk-expressing effector cells in arthritis.[110] PCI-32765 inhibited TNF-α, IL-1β, and IL-6 production in primary monocytes.[110]

PCI-32765 is active in multiple in vivo models: (1) transgenic murine model of BCR-driven lymphoma, (2) spontaneous B-cell lymphoma in canines, and (3) mouse models of autoimmune disease. Once-daily dosing resulted in 24-hour sustained target inhibition. PCI-32765 affected disease progression in an adoptive transfer T-cell leukemia/lymphoma 1 (TCL1) mouse model of CLL.[108] The TCL1 transgenic mouse model of CLL has been validated as a model similar to human CLL, providing an avenue to extend study of BCR signaling in the in vivo setting.[111,112] These mice spontaneously develop

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