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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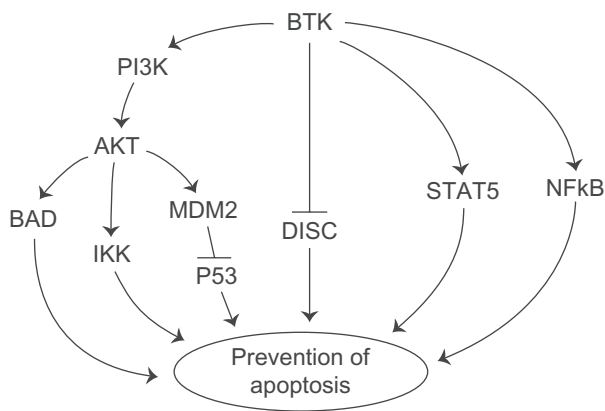
Figure 1 Btk activates antiapoptotic pathways. Btk is an upstream regulator of multiple antiapoptotic pathways, including the PI3K–AKT pathway, STAT5 pathway, and NFκB pathway. BTK also blocks the Fas-mediated apoptosis. See text for further discussion and references.
Abbreviations: Btk, Bruton's tyrosine 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-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.

Ibrutinib (PCI-32765)   **LFM-A13**   **GDC-0834**
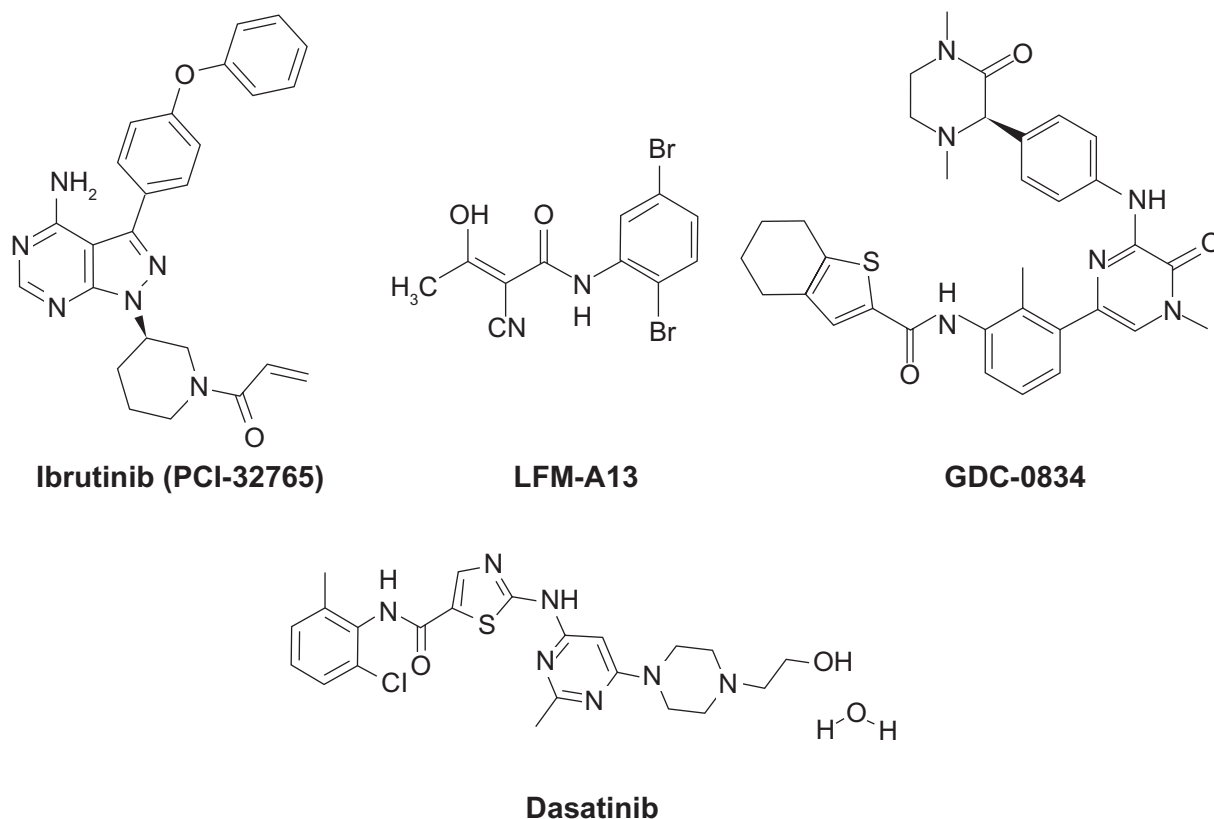
Dasatinib

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-[(3R)-3-[4-amino-3-(4-phenoxy-phenyl)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

a CD5^{+} B-cell leukemia with unmutated immunoglobulin heavy-chain variable-region (IgVH) resembling unmutated CLL.[113] PCI-32765 affected leukemia cell homing and disease progression. In this model, PCI-32765 caused a transient early lymphocytosis and profoundly inhibited CLL progression, as assessed by weight, development of hepatosplenomegaly, and survival.[108] PCI-32765 induced objective clinical responses in dogs with spontaneous B-cell NHL.[101] Active site occupancy of Btk was tightly correlated with the blockade of BCR signaling and in vivo efficacy.

Clinical studies

Ibrutinib/PCI-32765 is being investigated in patients with B-cell malignancies that include CLL/small lymphocytic lymphoma (SLL), MCL, DLBCL, FL, and MM (Tables 1 and 2). In phase I/II studies, PCI-32765 showed encouraging clinical activity in patients with several types of B-cell lymphoma.[114]

Relapsed/refractory B-cell malignancies

Advani et al[115–117] reported a dose-escalating phase I study of PCI-32765 (1.25 mg/kg/day to 12.5 mg/kg in a 28-day cycle) in 56 patients with multiple histologic subtypes of B-cell NHL (NCT00849654). The overall response rate was achieved in 30 (62%) of 50 evaluable patients, including seven complete responses (CRs) and 23 partial responses (PRs). Responses were achieved in eleven (79%) patients with CLL/SLL, seven (78%) with MCL, six (46%) with FL, three (75%) with WM (Waldenström macroglobulinemia), two (29%) with DLBCL, and one (33%) with Marginal zone lymphoma (MZL). Median progression-free survival in all patients was 13.6 months. Therapy was well tolerated, and grade 3 or higher adverse events (AEs) observed were neutropenia, thrombocytopenia, and anemia (<20%). In a second phase I study by Fowler et al[118,119] in 47 patients with various relapsed or refractory B-cell malignancies, a dose of 420 mg daily was established based on >90% occupancy of Btk. In this study, PCI-32765 induced a durable objective response (CR + PR) in various relapsed or refractory B-cell malignancies.

Relapsed/refractory MCL

An interim analysis of a phase II study in patients with relapsed or refractory MCL, Staudt et al,[120] showed that PCI-32765 (560 mg daily in 28-day cycles) produced an objective response of 67%; in patients pretreated with bortezomib (Velcade) the overall response rate was 75%, compared with 58% in bortezomib-naive patients.[121] PCI-32765 was generally well tolerated, with hematologic grade 3/4 AEs occurring in <5% of patients. A unique observation of marked lymphocytosis, termed a "compartment shift," was observed where the lymphocyte count doubled from about day 8 to 28 of cycle 1, and then slowly declined to about 40% above baseline with subsequent cycles. These lymphocytes were identified as being CD5^{+} and CD45^{+}, consistent with circulating, mobilized lymphoma cells. The mechanism may be related to inhibition of tissue-homing cytokines CXCL12 and CXCL13, and inhibition of adhesion molecule functioning. Wang et al[122] reported the results of a phase II study of PCI-32765 in relapsed or refractory MCL patients. In this study, 111 patients (bortezomib-naive and bortezomib-exposed) received oral PCI-32765 (560 mg daily). The primary end point of the study was overall response rate. Duration of response and safety evaluation were secondary end points. The median follow-up time was 9.2 months, with a range of time to response to treatment of 1.4 to 16.4 months. The study showed an overall response rate of 68% (22% CR and 46% PR), with a median progression-free survival

Table 1 Small-molecule Bruton's tyrosine kinase inhibitors in development

Company	Compound	Indications	Stage
Pharmacyclics, Janssen	Ibrutinib/ PCL-32765	R/R CLL/SLL, MCL, FL, DLBCL, MM, indolent NHL, MALT/MZL	Phase Ib/II Phase III (CLL/NHL)
Pharmacyclics, Janssen	PCL-32765 + rituximab	R/R CLL/SLL	Phase II
	PCL-32765 + ofatumumab	High-risk CLL	Phase III
	PCL-32765 + bendamustine and rituximab		
Avila Therapeutics, Celgene	AVL-292	CLL, B-cell NHL, WM	Phase Ib
Bristol-Myers Squibb	Dasatinib + fludarabine	R/R CLL/SLL	Phase II
University of Southern California	LFM-A13	B-cell malignancies	Preclinical
Ono Pharmaceutical	ONO-WG-307	B-cell malignancies	Preclinical
Genentech, Gilead	GDC-0834	B-cell malignancies	Preclinical

Abbreviations: DLBCL, diffuse large B-cell lymphoma; R/R CLL/SLL, relapsed/refractory chronic lymphocytic leukemia/small lymphocytic lymphoma; MCL, mantle-cell lymphoma; FL, follicular lymphoma; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma; MALT/MZL, mucosa-associated lymphoid tissue/marginal zone lymphoma; WM, Waldenström macroglobulinemia.

Table 2 Summary of Bruton's tyrosine kinase inhibitor candidates and their stage of clinical development

Compound	Indication	Trial identifier	Phase I	Phase II	Phase III
Ibrutinib/	B-cell lymphoma	NCT00849654	X		
PCI-32765	B-cell lymphoma and CLL	NCT01109069	X		
	Newly diagnosed B-cell NHL	NCT01569750	X		
	CLL/SLL	NCT01292135	X		
	CLL/SLL	NCT01105247	X	X	
	CLL/SLL and prolymphocytic leukemia	NCT01217749	X	X	
	CLL/SLL	NCT01744691		X	
	MCL	NCT01236391		X	
	DLBCL	NCT01325701		X	
	R/R MM	NCT01478581		X	
	MCL	NCT01599949		X	
	R or R MCL	NCT01646021			X
	R or R CLL or SLL	NCT01611090			X
	R or R CLL or SLL	NCT01578707			X
	Treatment-naive CLL or SLL	NCT01722487			X
AVL-292	R or R B-cell NHL, CLL, WM	NCT01351935	X		
Dasatinib/ Sprycel	R or R CLL/SLL	NCT00438854		X	

Abbreviations: DLBCL, diffuse large B-cell lymphoma; R/R CLL/SLL, relapsed/refractory chronic lymphocytic leukemia/small lymphocytic lymphoma; MCL, mantle-cell lymphoma; FL, follicular lymphoma; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma; WM, Waldenström macroglobulinemia.

estimated at 13.9 months. Results were similar between the bortezomib-naive and bortezomib-exposed patients. Long-term follow-up of the initial 51 patients showed an incremental improvement in the response rate over time. The overall response rate increased for this patient subset to 75%. PCI-32765 resulted in high and durable responses and was generally well tolerated. Pneumonia was the only grade 3 or higher treatment-emergent AE occurring in ≥5% of patients. Recently, Janssen has initated a randomized, multicenter phase III trial comparing PCI-32765 versus temsirolimus as a monotherapy in relapsed or refractory MCL patients who had a prior rituximab-containing chemotherapy regimen (NCT01646021). The primary end point of the study is progression-free survival when compared to temsirolimus. The plan is to enroll 280 patients outside the US.

MCL cells express surface IgM and Btk and exhibit constitutive Btk autophosphorylation, which is inhibited by PCI-32765. Ponader et al[123] tested the comparative sensitivity of PCI-32765 to inhibit BCR-signaling pathways in nine MCL cell lines, including phosphorylation of phospholipase C (PLC)-γ that leads to calcium flux. In resistant cells, IgM stimulation induces p-Btk but not pPLC-γ2 or calcium flux. PCI-32765-sensitive cell lines were shown to display constitutive p-ERK activity, which was increased on IgM stimulation and completely abrogated by PCI-32765. The more resistant cell lines had low endogenous levels of p-ERK, which was induced strongly by IgM and only partially blocked by PCI-32765. Sensitive cell lines showed a dose-dependent inhibition of IgM-induced p-ERK. These data

suggested the existence of additional Btk-dependent pathways (perhaps leading to p-ERK) in MCL that regulate their growth and phosphorylation of downstream kinase, supporting ERK as a marker of sensitivity to PCI-32765.

Relapsed/refractory DLBCL

Wilson et al[124] reported the results of a phase II study of PCI-32765 in 70 patients with relapsed or refractory DLBCL in two genetically distinct subtypes of DLBCL: the ABC subtype and the germinal center (GC) subtype. The ABC subtype appears to be more driven by BCR signaling. The overall response rate in the heavily pretreated population was 23% (16 of 70 patients). Responses were primarily in the ABC subtype, with twelve of 29 patients (41%) responding (five CR and seven PR). In the 20 GC patients, only one patient (5%) had a PR. This study supports the use of ABC DLBCL molecular subtype as a biomarker for enrichment of patients for future PCI-32765 studies. Grade 3 or higher AEs were 5%–10% of the patients.

Relapsed/refractory CLL or SLL

Daily oral administration of PCI-32765 has been evaluated in patients with R/R CLL or SLL that had been treated with at least two prior therapies, including fludarabine.[125] In an ongoing phase Ib/II study by O'Brien et al,[126,127] 61 patients with R/R CLL enrolled at two dose levels: 420 mg (n = 27) or 840 mg (n = 34). Serious grade 3 or 4 infections were noted in 25% of patients in the 420 mg cohort and 29% patients in the 840 mg cohort. Treatment was associated with an early

increase in lymphocytosis that began around 1 week and persisted for several months in the majority of patients. With a median follow-up of 12.6 months in the 420 mg cohort and 9.3 months in the 840 mg cohort, the overall response rate for the 420 mg dose level was 67%, and for the 840 mg dose 68%. In addition, six (22%) and eight (24%) of patients in the 420 mg and 840 mg cohorts had a nodal response with persistent lymphocytosis. The 1-year estimated progression-free survival for the patients enrolled on this study is 86%.[125] These data indicate that PCI-32765 is highly active and well tolerated in CLL/SLL patients, irrespective of high-risk genomic abnormalities, and suggest that this drug may be an important new targeted treatment approach for CLL, particularly in combination with other agents.

Recently, O'Brien et al[128] reported the updated results of a phase Ib/II trial (NCT01105247) in R/R CLL (n = 61) and TN CLL (n = 31) patients enrolled at two fixed continuous-dose levels of PCI-32765 (420 mg and 840 mg). In addition to the data reported by Byrd et al,[129,130] on the treatment-naive patients, this report provided updated progression-free survival data in the R/R patient population. With a median follow-up of 17.5-months, progression-free survival in the 420 mg cohort was 87.7%. High risk R/R patients with 17p deletion (n = 20) and IgVH unmutated status (n = 42) had estimated 18-month progression-free survival of >70% and >80%, respectively. In the treatment-naive 420 mg dose cohort patients (≥65 years old) the overall response rate was 81%, which included a 12% complete response rate. Fifty percent of patients with pretreatment of cytopenias experienced sustained improvement in hemoglobin and/or platelet levels. The estimated progression-free survival in the treatment naive patients at 15 months was 96% in the 420 mg cohort. Recently, Byrd et al[129,130] reported the results of a phase Ib/II study of PCI-32765 in subjects with CLL/SLL either R/R (n = 85) or treatment-naive (n = 31) patients (≥65 years) designed to assess safety, tolerability, and efficacy at two dose levels (420 mg or 840 mg daily). Among 116 patients, the overall response rate was 68% in treatment-naive patients, with an estimated 96% progression-free survival rate at 26 months (NCT01105247). In patients with R/R CLL/SLL, the overall response rate was 71%, with an estimated progression-free survival at 26 months of 75%. Responses were independent of high-risk clinical or genetic features, such as a deletion of part of chromosome 17 (del17p). Grade 3–4 toxicities (10%–13%) included diarrhea, infection, and hematologic toxicity (anemia and thrombocytopenia). There was no evidence of cumulative toxicity or long-term safety concerns. With a maximum follow up of 26 months, it was estimated that 96% of the treatment-naive and 75% of the R/R high-risk patients were without progression.

Combination clinical trials of PCI-32765

Currently, PCI-32765 is being tested in phase Ib/II combination with ofatumumab (NCT01217749), fludarabine, cyclophosphamide and rituximab or bendamustine and rituximab (NCT01292135) in CLL patients in the relapsed setting. Data from phase I/II trials demonstrated that high-risk CLL patients respond equally as well as low-risk patients to PCI-32765-treated CLL patients with characteristically delayed responses or stable disease due to persistent lymphocytosis, caused by redistribution of tissue-resident CLL cells into the peripheral blood. To accelerate and improve responses and to expand upon the PCI-32765 experience in high-risk CLL patients, Burger et al[131] reported results of an ongoing phase II study of PCI-32765 plus rituximab in 40 patients (median age 65 years). The high-risk patients had one of the following characteristics, all predictive of poor outcome to standard chemotherapy: deletion in chromosome 17p, mutation in the tumor-suppressor gene *TP53*, deletion in chromosome 11q or relapse < 36 months after chemoimmunotherapy. Patients were treated with PCI-32765 420 mg daily in combination with weekly rituximab (375 mg/m^2) for weeks 1–4 (cycle 1), then daily PCI-32765 plus monthly rituximab until cycle 6, followed by daily single-agent PCI-32765. After a follow-up of 6 months, the overall response rate was 83%, and 38 of 40 patients continued on therapy without disease progression with 95% of all patients and 90% of patients with del17p not progressing. There was a large and rapid reduction in lymph-node and spleen sizes, with 84% of patients (26/31) experiencing more than a 50% decrease in lymph-node size. Treatment was well tolerated overall, with grade 3 or grade 4 toxicities infrequent and transient in nature, including febrile neutropenia, anemia, mucositis, and pneumonia, in this clinical study.

Brown et al[132] and O'Brien et al[133] reported the interim phase Ib/II study data on the combination of PCI-32765 with bendamustine/rituximab (BR) in patients with R/R CLL (median age 65 years). BR produced an overall response rate of 59% in R/R CLL. This combination trial (NCT01292135) enrolled a total of 30 patients in the BR cohort; 37% were refractory to a purine analogue-containing regimen and 13% refractory to bendamustine. Twenty-three percent of the patients had deletion 17p, and 43% had deletion 11q. R/R CLL patients received 420 mg of PCI-32765 daily for 28-day cycles until disease progression. Bendamustine 70 mg/m^2 was administered on days 1 and 2

combined with rituximab 375 mg/m^2 on day 0 for cycle 1, escalating to rituximab 500 mg/m^2 for cycles 2–6. Response was evaluated according to the International Workshop on Chronic Lymphocytic Leukemia criteria. Grade 3/4 neutropenia and thrombocytopenia were noted in 47% and 10% of patients, respectively. At 8.5 months of median follow-up, the overall response rate with BR was 93%, with 13% of patients achieving a complete response with no morphologic evidence of CLL. Responses appeared independent of high-risk clinical or genomic features. No new safety signals with the combination of PCI-32765 and BR were identified. The estimated 11-month progression-free survival was 90%. The high overall response rate, rapid onset of response, low rate of progressive disease, and good tolerability compared favorably with historical controls and prompted a randomized phase III study of PCI-32765 in combination with BR (Table 2).

Jaglowski et al[134] reported the results of a phase Ib/II study of a combination of PCI-32765 with ofatumumab, an anti-CD20 monoclonal antibody. Patients with R/R CLL/SLL following two or more therapies were treated with PCI-32765 first given as a single agent, 420 mg/day, 4 weeks prior to the addition of ofatumumab on day 1 of cycle 2 (initial dose 300 mg; 2000 mg on days 8, 15, and 22 of cycle 2, weekly in cycle 3, and day 1 of cycles 5–8). Interim data from 27 patients (median age 65 years) with either CLL/SLL (n = 24) or Richter's transformation (RT = 3) underwent at least six cycles of treatment. All 24/24 CLL/SLL/PLL patients achieved PR (100% overall response rate) within six cycles; two of three RT patients had a PR. The majority of AEs were grade 1/2. Grade 3/4 AEs included anemia, pneumonia, urinary tract infection, and hyponatremia (7%–11%). PCI-32765 combined with ofatumumab was well tolerated and highly active in patients with heavily pretreated R/R CLL/SLL. Rapid onset of response, low relapse rate, and a favorable safety profile make this combination worthy of further study.

Pharmacyclics has initiated randomized phase III clinical trials (NCT01646021, NCT01611090) of PCI-32765 in combination with bendamustine and rituximab in patients with relapsed or refractory R/R CLL/SLL (Table 2). Another trial is designed to demonstrate superiority of PCI-32765 versus ofatumumab (NCT01578707). The primary end point of the latter study is to demonstrate a clinically significant improvement in progression-free survival in relapsed or refractory CLL/SLL patients. This global study is open, and Pharmacyclics plans to enroll 350 patients worldwide. Pharmacyclics has also initiated the Resonate-17p study

(NCT01744691), a randomized, phase II trial using PCI-32765 as a monotherapy in patients who have deletion 17p and who did not respond to or relapsed after at least one prior treatment with chemoimmunotherapy. The primary outcome of the study is overall response rate. The key secondary end points are duration of response and other measures of clinical benefit. This study plans to enroll 111 patients worldwide. In addition, Pharmacyclics and Janssen have initiated a pivotal phase III trial of PCI-32765 in combination with bendamustine and rituximab in R/R CLL/SLL patients who received at least one line of prior systemic therapy. The primary end point of the study is to demonstrate a clinically significant improvement in progression-free survival versus bendamustine and rituximab therapy alone. The key secondary end points include overall response rates, overall survival, and other measures of clinical benefit. This global study is expected to enroll 580 patients worldwide.

Ibrutinib/PCI-32765 inhibits 22 tyrosine kinases other than Btk at nanomolar concentrations.[101] Furthermore, it exhibited antileukemic activity only at micromolar concentrations (EC$_{50}$ 10 µM, EC$_{90}$ 100 µM), at which it has been shown to inhibit almost every tyrosine kinase it was tested against. Several kinases with homology to Btk in the Tec kinase family, including Bmx and Itk, have similar cysteine residues that might also be reversibly or irreversibly inhibited by PCI-32765. The fact that ibrutinib/PCI-32765 inhibits many other protein tyrosine kinases also at nanomolar concentrations demonstrates that its proposed mechanism of action is not sufficient to explain its experimentally documented promiscuity towards virtually all tyrosine kinases. Its interaction with multiple protein tyrosine kinases would be expected to result in poor intracellular pharmacokinetics, due to undesired binding to a large pool of "competing" kinases leading to reduced occupancy of the Btk-active site. That is likely the cause for the mg/kg dose levels and >100 nM plasma concentrations of ibrutinib/PCI-32765 required for Btk active-site occupancy in clinical settings.[115,117] Furthermore, many of the protein tyrosine kinases it inhibits at nanomolar concentrations, including Lyn and C-terminal Src kinase (Csk)[101] are inhibitory protein tyrosine kinases that counterbalance Btk action. Therefore, its promiscuity would be expected to significantly compromise its net impact on leukemia/lymphoma cell survival. These predictions were confirmed in recently published preclinical and clinical data on ibrutinib/PCI-32765.[65,101,135] In the only animal model in which its antineoplastic activity was evaluated, ibrutinib/PCI-32765 showed activity at mg/kg dose levels that yield concentrations far above those inhibitory to

multiple protein tyrosine kinases.[101] Therefore, neither the in vitro antileukemic activity nor the promising clinical activity of PCI-32765 can be attributed to its Btk-inhibitory activity alone.

AVL-101, AVL-291, and AVL-292

AVL-101, AVL-291, and AVL-292 (Avila Therapeutics/ Celgene Corporation) are orally active dianilinopyrimidine-based irreversible Btk inhibitors with micromolar activity against lymphoma cells.[136] AVL-101/291/292 covalently modify their targets, leading to nonspecific and durable silencing of the targeted proteins, including PDGFR, c-Kit, EGFR, and Btk, in vitro, ex vivo and in vivo. At low micromolar concentrations, AVL-101 inhibited the Tec (tyrosine kinase expressed in hepatocellular carcinoma) family of kinases (Bmx, Btk, Itk) as well as JAK 2, Aurora A, fibroblast growth factor receptor (FGFR)-1, Flt4, Ret, and TrkA. AVL-291/292 also inhibited the Tec family of kinases (Bmx, Btk, Tec) as well as JAK2, JAK3, Txk, Flt4.[136] AVL-101 disrupts the BCR-signaling pathway, as measured by the inhibition of both Btk Y[223] autophosphorylation and Btk substrate PLC-γ2 phosphorylation.[137,138] AVL-101 inhibited the proliferation of the B-cell lymphoma cell lines DOHH2, DHLA4, and WSU-DLCL2 in vitro. Moreover, AVL-101 inhibited Btk-dependent B-cell function in vivo. These properties support the potential of AVL-101/291/292 in B-cell malignancies.

In preclinical studies, AVL-291 selectively and potently inhibited Btk and BCR signaling in vitro and was efficacious in a variety of animal disease models. Moreover, AVL-292 inhibited osteoclast function and reduced osteoclast-stimulated proliferation of MM cells.[139] AVL-291 modified the course of rheumatoid arthritis (RA) in animal models, with 75% inhibition of the clinical score at an oral dose of 3 mg/kg that correlated directly with 75% Btk target occupancy.[140] AVL-291 reduced clinical arthritis scores, as well as inflammation, joint damage, cartilage damage, and bone erosion in the collagen-induced arthritis (CIA) models of RA. Complete inhibition of the disease correlated with complete target occupancy at 10 mg/kg. Thus, AVL-291 is potentially useful for patients with lymphoid malignancies and RA and possibly in other autoimmune diseases characterized by aberrant B-cell activation. AVL-292 is currently being investigated in autoimmune diseases as well as in B-cell malignancies.[141] AVL-292 has demonstrated biological activity in patients suffering from CLL as well as preclinical data on its therapeutic potential in the prevention of bone destruction

by abrogating osteoclast sealing zone formation caused by MM and MM-related bone disease.

Clinical studies

AVL-292 has successfully completed two phase Ia clinical studies to date. Westlin et al[143] assessed the safety and pharmacokinetics of AVL-292 in a double-blind, placebo-controlled, single-ascending-dose study in healthy adult subjects. Single oral doses of 0.5–7.0 mg/kg AVL-292 were safe and well tolerated. Subjects that received 1–2 mg/kg of AVL-292 achieved > 80% Btk occupancy, which was sustained through 24 hours even after plasma levels of AVL-292 had declined.[143] The minimum dose that achieved complete target occupancy in this study (125 mg) was carried forward as the starting dose in a phase Ib study of hematological malignancies of B-cell origin. Subjects diagnosed with B-cell malignancies received single daily oral doses of AVL-292 in continuous 28-day cycles. Btk occupancy in this phase Ib clinical study confirmed observations in healthy volunteer subjects. The ongoing phase Ib clinical trial of AVL-292 is being conducted in patients with B-cell malignancies, including B-cell NHL, CLL, and WM. An escalating-dose study (125–625 mg) in patients with relapsed or refractory B-cell NHL, CLL, and WM is ongoing (NCT01351935).

Noncovalent Btk inhibitors
Dasatinib (Sprycel)

Dasatinib (Sprycel/BMS-354825, Bristol-Myers Squibb) [*N*-(2-chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl) piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide], is a potent, orally active, multikinase BCR/Abl and Src family TKI that is FDA-approved for treatment of CML and is a potent inhibitor of Btk.[105,144] A chemical proteomics-profiling approach identified the Tec kinases Btk and Tec to be very prominent targets of dasatinib.[105,145] Structure-based mutagenesis experiments by Hantschel et al[105] identified the gatekeeper residue as a critical determinant for dasatinib sensitivity. Mutation of Thr-474 in Btk to Ile conferred resistance to dasatinib. Small gatekeeper residues like Thr in Btk allow for dasatinib binding, whereas bulky gatekeeper residues block dasatinib binding.

Inhibition of Btk by dasatinib might be of clinical benefit in pre-B lymphoblastic leukemia. In the LN microenvironment, CLL cells are protected from apoptosis by upregulation of antiapoptotic proteins. The situation in CLL LN could be mimicked by prolonged in vitro CD40 stimulation of CLL cells, which results in upregulation of antiapoptotic Bcl-xL,

A1/Bfl-1, and Mcl-1 proteins, and affords resistance to various chemotherapeutics (fludarabine, bortezomib, roscovitine). The antiapoptotic profile of CD40-triggered CLL resembles BCR-Abl-dependent changes seen in CML. Dasatinib prevented CD40-mediated antiapoptotic changes in CLL with Abl and Btk as dominant targets.[145] Functional analysis revealed that CD40-mediated antiapoptotic signals and drug resistance could be overcome both by dasatinib and the Abl inhibitor imatinib, but not by the Btk inhibitor ibrutinib/PCI-32765. BCR- and chemokine-controlled adhesion can be abolished by dasatinib and ibrutinib, but not by imatinib.[146] Thus, dasatinib combines two key aspects that are clinically relevant: inhibition of Abl overrides chemoprotective survival signals, whereas inhibition of Btk impairs integrin-mediated adhesion of CLL cells in the microenvironmental niche.

A phase II trial evaluated the combined inhibition of Abl and Btk by dasatinib and fludarabine in 20 refractory CLL patients.[147] In agreement with the in vitro data, reductions in LN size ($\geq$20%) were observed in most patients that provided a significant improved progression-free survival (256 days) and overall survival (510 days), as compared to nonresponders (80 days and 158 days, respectively). Thus, in agreement with in vitro molecular studies, dasatinib seems to have clinical efficacy in heavily pretreated refractory CLL patients. These data encourage further studies on the use of dasatinib in combination with other classes of drugs in R/R CLL.

LFM-A13

LFM-A13 (alpha-cyano-beta-hydroxy-beta-methyl-*N*-(2,5-ibromophenyl) propenamide) is a rationally designed selective Tec family kinase inhibitor.[148,149] The selectivity of LFM-A13 against Tec family kinases has been extensively tested in diverse preclinical models by several research groups. LFM-A13 binds to the catalytic pocket of the kinase domain. In vitro, LFM-A13 inhibited recombinant Btk with an IC_{50} value of 2.5 μM, but it did not affect the enzymatic activity of other protein tyrosine kinases, including JAK1 and JAK2, Src family kinase Hck, and receptor family tyrosine kinases, EGF-receptor kinase and insulin-receptor kinase (Irk), at concentrations as high as 278 μm.[148] Kim et al[150] and Heinonen et al[151] further demonstrated that its effects are virtually identical to those of Btk-specific siRNA duplexes that target human Btk mRNA. Among serine/threonine kinases, LFM-A13 inhibited only polo-like kinase (Plk), but none of the remaining twelve kinases.[152] Both targets of LFM-A13 (viz Btk and Plk) are expressed in B-lineage lymphoid malignancies, including DLBCL and B-ALL.[152,153]

In vivo, LFM-A13 at nontoxic dose levels exhibited antineoplastic, antithrombotic, and immunosuppressive activity in various mouse models.[151–156] Unlike the covalent inhibitors, LFM-A13 exhibits proapoptotic activity against mature B cells with an intact BCR signaling apparatus as well as BCR-negative leukemic B-cell precursors, and it is the only Btk inhibitor with experimentally documented in vitro and in vivo antineoplastic activity against human B-ALL cells.[154]

Good manufacturing practice-grade LFM-A13 exhibited potent single-agent activity in a nonobese diabetic/severe combined immunodeficient (NOD/SCID) mouse model of chemotherapy-resistant B-ALL that employs a highly aggressive and chemotherapy-resistant ALL cell line (viz NALM-6). When used for only 4 consecutive treatment days at 1 mg/kg/day, a dose level that corresponds to 1% of single-dose no observed adverse effect level (NOAEL) and 0.09% of multiple-dose cumulative NOAEL; this therapeutic margin and potency against B-ALL is unprecedented among other drug candidates in development.[11,155] LFM-A13 plus vincristine (VCR) combinations also were significantly more effective than vehicle or VCR alone, as evidenced by the significantly lower human leukemia burden in LFM-A13 plus VCR vs vehicle/VCR-treated control mice.[156] Thus, the chemoresistance of relapsed B-cell precursor ALL can be overcome by using LFM-A13 in combination with chemotherapy. LFM-A13 also inhibited MM cells in vitro and prevented in a SCID mouse xenograft model of MM, the formation of osteolytic lesions in implanted bones.[156]

ONO-WG-307

ONO-WG-307 (Ono Pharmaceutical) is a multikinase inhibitor with greater selectivity for Btk (IC_{50} = 2 nM) than Lyk, Fyn, or Lyn A in the in vitro kinase assay. In vitro, ONO-WG-307 exhibited differential antiproliferative effects in the nanomicromolar range against multiple NHL/CLL cell lines.[158] Quantitative phosphoproteome studies with both sensitive and nonsensitive cells revealed selective ONO-WG-307 suppression of Akt-mediated signaling and cellular protein kinase D activity in sensitive compared to nonsensitive cells.[159] These findings suggest a critical role of Btk-mediated signaling through Akt and protein kinase D. Orally administered ONO-WG-307 (50 mg/kg) induced a dose-dependent inhibition of tumor growth in an ABC-DLBCL xenograft (TMD-8) model and moderate antitumor activity in an FL xenograft model.[158] Based on these preliminary preclinical results, ONO-WG-307 is being developed for the treatment of B-cell lymphoproliferative diseases.

GDC-0834

GDC-0834 (Genentech, Gilead): 2.2 GDC-0834 GDC-0834 [R-N-(3-(6-(4-(1,4-dimethyl-3-oxopiperazin-2-yl) phenylamino)-4-methyl-5-oxo-4,5-dihydropyrazin-2-yl)-2-methylphenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-2-carboxamide], is a reversible, adenosine triphosphate-competitive small-molecule inhibitor of Btk potentially useful in the treatment of RA and lymphoid malignancies.[160,161] GDC-0834 inhibited Btk phosphorylation (p-Btk) in in vitro assays and in vivo in rodents. GDC-0834 inhibited p-Btk-Y223 with an in vitro IC_{50} of 5.9 nM and 6.4 nM in biochemical and cellular assays, respectively, and in vivo IC_{50} of 1.1 and 5.6 µM in mouse and rat, respectively. GDC-0834 prevented murine and human B-cell proliferation in response to BCR or CD40 stimulation. GDC-0834 suppressed arthritis in the prophylactic rat CIA model in a dose-dependent manner. Administration of GDC-0834 (30–100 mg/kg) in the rat CIA model resulted in a dose-dependent decrease of ankle swelling and reduction of morphologic pathology. In addition, GDC-0834 significantly inhibited joint inflammation, cartilage damage, and bone resorption. However, GDC-0834 has been discontinued due to poor pharmacokinetics in humans.[161]

Conclusion

Inhibition of Btk is emerging as a promising mechanism for targeting B-cell malignancies. Ibrutinib/PCI-32765, a covalent multikinase inhibitor, is undergoing late-stage efficacy studies in patients with various B-cell malignancies. PCI-32765 induced a durable objective response in various relapsed or refractory B-cell malignancies. PCI-32765 is highly active and well tolerated in CLL/SLL patients, irrespective of high-risk genomic abnormalities, which suggests that this drug candidate may be an important new targeted treatment approach for CLL, particularly in combination with other agents. Data from several phase I/II trials demonstrated that high-risk CLL patients respond equally as well as low-risk patients to PCI-32765. This compound has shown an interesting pattern of response, in which very rapid nodal shrinkage occurs at the same time as the lymphocyte count rises, suggesting redistribution of CLL cells from the LNs and BM to the peripheral blood. Ibrutinib in combination with bendamustine/rituximab or ofatumumab had high overall response rates, rapid onset of response, low rate of progressive disease, and good tolerability in patients with heavily pretreated R/R CLL/SLL.

A major drawback of the long-term covalent inhibition of Btk function is the potential to inhibit a number of kinases in addition to crucial functions of the nonlymphoid lineages, especially the normal functioning of platelets. The most prominent dose-limiting toxicity observed with covalent Btk inhibitors was thrombocytopenia. It remains to be seen whether long-term Btk suppression by covalent Btk inhibitors is a viable therapeutic strategy in indolent diseases like CLL/SLL, which could result in hematological toxicity as well as infectious complications. In contrast, rationally designed novel noncovalent Btk inhibitors show potential as antileukemia/antilymphoma drug candidates with apoptosis-promoting and chemosensitizing properties. The incorporation of the apoptosis-promoting Btk inhibitors into the treatment regimens of patients with recurrent or high-risk B-lineage lymphoid malignancies may help overcome the chemotherapy resistance of their neoplastic B cells/B-cell precursors and thereby improve their treatment response and survival outcome.

Disclosure

The authors report no conflicts of interest in this work.

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