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Bruton's Tyrosine Kinase: From X-Linked Agammaglobulinemia Toward Targeted Therapy for B-Cell Malignancies

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ABSTRACT

Discovery of Bruton's tyrosine kinase (BTK) mutations as the cause for X-linked agammaglobulinemia was a milestone in understanding the genetic basis of primary immunodeficiencies. Since then, studies have highlighted the critical role of this enzyme in B-cell development and function, and particularly in B-cell receptor signaling. Because its deletion affects mostly B cells, BTK has become an attractive therapeutic target in autoimmune disorders and B-cell malignancies. Ibrutinib (PCI-32765) is the most advanced BTK inhibitor in clinical testing, with ongoing phase III clinical trials in patients with chronic lymphocytic leukemia and mantle-cell lymphoma. In this article, we discuss key discoveries related to BTK and clinically relevant aspects of BTK inhibitors, and we provide an outlook into clinical development and open questions regarding BTK inhibitor therapy.

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INTRODUCTION

In 1952, Ogden Bruton, a pediatrician at the Walter Reed Army Hospital in Washington, DC (Fig 1), reported the first case of congenital agammaglobulinemia in an 8-year-old boy who suffered from recurrent pneumococcal sepsis. Protein electrophoresis revealed lack of the serum globulin fraction in this first patient[1] and in a subsequent series of patients analyzed in collaboration with Charles Janeway.[2] Immunoglobulin (Ig) replacement therapy was subsequently demonstrated to be effective in preventing infections and became central to the foundation of the discipline of clinical immunology.[3,4] Today, this primary immunologic deficiency (PID) is called X-linked agammaglobulinemia (XLA) or Bruton's agammaglobulinemia, and its estimated incidence is approximately 1:250,000.[3] After Bruton's and Janeway's discoveries in the 1950s, it was approximately four decades until the genetic basis of XLA was identified[5,6] (Fig 2). In 1993, two laboratories cloned *BTK* independently,[7,8] and deciphered the coding sequence and Bruton's tyrosine kinase (BTK) mutations.[7] Before that, the gene locus for XLA in the Xq22 region was already narrowed down with DNA probes,[13,14] which served as the basis for the cloning strategy. Because of its involvement in XLA, this kinase was named after Bruton. With these groundbreaking discoveries, XLA became the first example of mutations in a tyrosine kinase that cause a PID. Mutation analyses of larger series of patients with XLA detected a wide variety of BTK gene abnormalities (more than 800 different mutations, collected in a mutation database [BTKbase][15]) distributed across the entire BTK gene, which include promoter mutations and missense mutations in the Tec (tyrosine kinase expressed in hepatocellular carcinoma) homology and SH1 domains.[16,17] Importantly, no correlations between distinct genotypes and clinical phenotype were noted.[17]

As a consequence of functional null BTK mutations, B-lymphocyte precursors in the bone marrow fail to develop into mature B lymphocytes and, consequently, patients with XLA lack peripheral blood B cells and have markedly decreased or absent serum immunoglobulins of all isotypes.[18] Characteristically, XLA-related immunodeficiency manifests in young boys within their first 2 years of life, after depletion of protective maternal antibodies with recurrent bacterial and enteroviral infections. To prevent these opportunistic infections, patients with XLA typically are treated with intravenous or subcutaneous gamma globulin infusion, which reduces the number of and duration of infections and improves life expectancy. Alternatively, gene therapy strategies explore the transfer of normal *BTK* into Btk-deficient mice.[19,20] The advantage of this experimental approach is that it may offer a potential for cure, but technical problems and severe complications of this approach in patients with another type of PID—lymphoproliferative disorders resulting from insertional mutagenesis in patients with

Table 1. Phase III Clinical Trials of the BTK Inhibitor Ibrutinib

Study Title	Study Phase	Primary Objective	Secondary Objective	Estimated Enrollment	ClinicalTrials.gov Identifier	Status
A Phase 3 Study of Ibrutinib (PCI-32765) Versus Ofatumumab in Patients With Relapsed or Refractory Chronic Lymphocytic Leukemia (RESONATE)	III	PFS	OS, hematologic improvements, improvement of disease-related symptoms	350	NCT01578707	Finished accrual; estimated completion in July 2015
A Multicenter, Open-label, Phase 3 Study of the Bruton's Tyrosine Kinase Inhibitor PCI-32765 Versus Chlorambucil in Patients 65 Years or Older With Treatment-naive Chronic Lymphocytic Leukemia or Small Lymphocytic Lymphoma (RESONATE-2)	III	PFS	Efficacy ORR, safety	272	NCT01722487	Recruiting; estimated completion in February 2016
A Study of Ibrutinib in Combination With Bendamustine and Rituximab in Patients With Relapsed or Refractory Chronic Lymphocytic Leukemia or Small Lymphocytic Lymphoma	III	PFS	ORR, OS, adverse effects	580	NCT01611090	Recruiting; estimated completion in August 2015
Study of Ibrutinib (a Bruton's Tyrosine Kinase Inhibitor), Versus Temsirolimus in Patients With Relapsed or Refractory Mantle Cell Lymphoma Who Have Received at Least One Prior Therapy	III	PFS	ORR, OS, duration of response, time to next treatment, safety	280	NCT01646021	Recruiting; estimated completion in August 2014
A Study of the Bruton's Tyrosine Kinase Inhibitor Ibrutinib Given in Combination With Bendamustine and Rituximab in Patients With Newly Diagnosed Mantle Cell Lymphoma	III	PFS	ORR, OS, safety, MRD, response duration	520	NCT01776840	Recruiting; estimated completion in October 2019
Rituximab and Bendamustine Hydrochloride, Rituximab and Ibrutinib, or Ibrutinib Alone in Treating Older Patients With Previously Untreated Chronic Lymphocytic Leukemia	III	PFS	CR rate, MRD, toxicity and tolerability	523	NCT01886872	Not yet recruiting; estimated completion in March 2018
A Study of the Bruton's Tyrosine Kinase Inhibitor, PCI-32765 (Ibrutinib), in Combination With Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone in Patients With Newly Diagnosed Non-Germinal Center B-Cell Subtype of Diffuse Large B-Cell Lymphoma	III	EFS	PFS, OS, CR rate	800	NCT01855750	Not yet recruiting; estimated completion in June 2020

Abbreviations: BTK, Bruton's tyrosine kinase; CR, complete response; EFS, event-free survival; MRD, minimum residual disease; ORR, overall response rate; OS, overall survival; PFS, progression-free survival.

the X-linked form of severe combined immunodeficiency (XSCID)—have decelerated the clinical development of gene replacement therapy for XLA.[21]

Shortly after the discovery of the human *BTK* gene, a mutation of a single conserved residue within the pleckstrin homology (PH) domain of Btk was discovered as the genetic basis for murine X-linked immunodeficiency (xid).[22,23] Btk gene–deleted mice subsequently confirmed that Btk deficiency is the basis for xid.[24-26]

GENETICS AND BIOCHEMISTRY OF BTK

The BTK gene encodes a cytoplasmic nonreceptor protein tyrosine kinase, which belongs to the Tec kinase family, the second largest class of nonreceptor protein tyrosine kinases.[27,28] Tec kinases have four structural modules: the PH domain at their N terminus, a characteristic feature of these kinases, along with SH3 (Src homology 3) domain, SH2 (Src homology 2) domain, and kinase (Src homology 1) domain. In humans, members of this protein family are primarily expressed in hematopoietic cells, and their activation is one of the first steps in antigen-receptor signaling.[28] BTK is a 659-amino-acid protein that contains five signaling domains (Fig 3)—characteristic for members of the Tec family—and has diverse partner molecules.[29,30] The PH domain at the N terminus is essential for BTK membrane localizing and is followed by the proline-rich Tec homology domain, which is unique to the Tec family. The Tec homology domain comprises the BTK motif, a highly conserved zinc finger motif that mediates binding and coordination of BTK to zinc ions (Zn^{2+}). BTK requires Zn^{2+} for optimal activity and stability.[31] The Src homology domains SH3 and SH2 have binding functions and contain the autophosphorylation site tyrosine 223, whereas the SH1 kinase domain has a catalytic function

Fig 1. Ogden Bruton, MD. Photo with personalized autograph kindly provided by Billy F. Andrews, MD, Department of Pediatrics, University of Louisville, Louisville, KY.

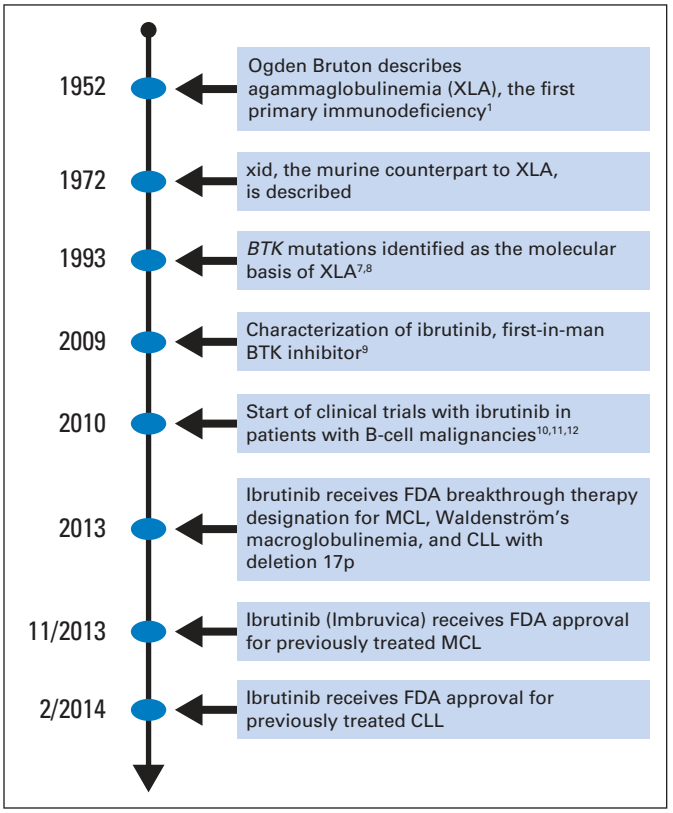

Fig 2. Milestones in Bruton's tyrosine kinase (BTK) research. CLL, chronic lymphocytic leukemia; FDA, US Food and Drug Administration; MCL, mantle-cell lymphoma; xid, X-linked immunodeficiency; XLA, X-linked agammaglobulinemia.

and contains the phosphorylation site tyrosine 551.[32] Detailed structure, function, and specific mutations of individual BTK domains and deep insights into the kinetics have been well characterized.[29,33-35]

DIVERSE BIOLOGIC ROLES OF BTK

BTK transmits, diversifies, and amplifies signals from a wide variety of surface molecules that cells use to communicate with their microenvironment. These include growth factor and cytokine receptors, G protein-coupled receptors such as chemokine receptors, antigen receptors (especially the B-cell receptor [BCR]), and integrins. BTK in turn activates many of major downstream signaling pathways, including the phosphoinositol-3 kinase (PI3K)-AKT pathway, phospholipase-C (PLC), protein kinase C, and nuclear factor kappa B (NF-κB).[36] Among these various functions, the involvement of BTK in BCR signaling and in cell migration has been well established, and these functions appear to be primary targets of BTK inhibitors.[37,38] In the following paragraphs, we will focus on the role of BTK in BCR signaling and in B-cell migration and homing.

BTK in BCR Signaling

Signaling through the BCR transmits not only signals for adaptive immune responses after contact with specific antigen, it also plays a fundamental role in B-cell development, promoting antigen-independent B-cell maturation and resulting in the presence of mature B cells in the peripheral blood. BTK plays an important role in antigen-induced BCR signaling (Fig 4).[39,40] BTK activation in response to BCR engagement by antigens induces a range of protein interactions and the recruitment of signaling molecules, resulting in B-cell survival, proliferation, differentiation, and the production of antibodies.[41-43] One of the first steps after BCR engagement is clustering of the signal transduction molecules Igα and Igβ (CD79a and CD79b) and phosphorylation within the cytoplasmic tails of their immunoreceptor tyrosine [kinase] activation motifs (ITAMs). This phosphorylation is mediated by nonreceptor tyrosine kinases of the Src family, such as Lyn. Subsequently, the spleen tyrosine kinase (SYK) binds to the ITAM motifs, in which it is activated in a multistep process. SYK, in turn, phosphorylates multiple tyrosine residues in the B-cell linker scaffold protein (BLNK, also known as SLP65 or BASH). Lyn also phosphorylates BTK and CD19, which leads to the activation of PI3K and consequently to increased phosphatidylinositol 4,5-triphosphate (PIP_3) levels on the cytoplasmic side of the plasma membrane. The first key regulatory step in BTK activation on antigen receptor stimulation is its localization to the plasma membrane. This is mediated by interaction of the PH domain with PIP_3 and is generated by PI3K activity.[44] BTK has been shown to target to specific membrane microdomains (lipid rafts or glycolipid-enriched membrane

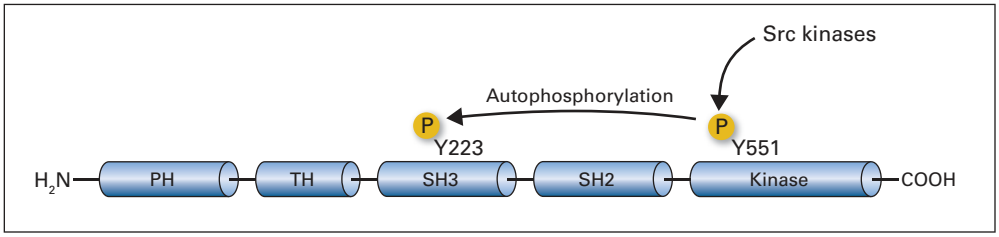

Fig 3. Schematic domain structure of Bruton's tyrosine kinase (Btk). PH, Pleckstrin homology domain; TH, Tec homology domain; SH2 and SH3, Src homology domains.

microdomains).[45] PIP_3 serves as a docking site for the PH domains of PLCγ2 and BTK, whereas their SH2 domains bind to BLNK. Phosphorylation of BTK at tyrosine 551 within the activation loop of the kinase domain leads to its autophosphorylation at tyrosine 223 in its SH3 domain, constituting the second key regulatory step in BTK activation on BCR stimulation and inducing the full activation of BTK. This allows BTK to phosphorylate PLCγ2, which triggers the conversion of PIP_2 into the second messenger molecules inositol 1,4,5-triphosphate and diacylglycerol, inducing an increase in intracellular Ca^{2+} concentrations. Btk is also critically important for activating the NF-κB transcription factor in response to BCR[46,47] and Toll-like receptor 9 (TLR9)[48] activation.

In the absence of BTK, BCR signaling is insufficient to induce late transitional B cells to differentiate into mature peripheral B cells.[49] BTK mutant cells and cell lines are defective in their response to BCR signaling, resulting in impaired Ca^{2+} mobilization, activation of MAP kinases, cytoskeleton rearrangements, and transcriptional activation.[41,42] This leads to altered development and defects in functional responses, including cellular proliferation, expression of activation markers, cytokine and antibody production, and responses to infectious diseases. Interestingly, and consistent with these results from *BTK* deletion, BTK overexpression in B cells results in the spontaneous formation of germinal centers, increased plasma cell numbers, antinuclear autoantibody production, and a systemic lupus erythematosus (SLE)–like autoimmune disease. These changes were the result of hyper-responsive BCR signaling and increased NF-κB activation and could be reversed by treatment with the BTK inhibitor ibrutinib (PCI-32765).[50]

BTK Effects on Other B-Cell Functions

Besides its role in BCR signaling, BTK also plays a role in the signaling of a wide variety of B-cell molecules such as cytokine receptors, CD19, CD38, CD40,[45,51] G protein-coupled receptors[52] such as the CXCR4 chemokine receptor,[53] tumor necrosis factor receptors, and TLRs. For example, BTK can form complexes with endosomal major histocompatibility complex class II molecules, CD40, and MyD88,[54] promoting TLR signaling. TLR9-induced BTK activation in turn can provoke excessive autoantibody production and autoimmunity.[55]

Of particular clinical and translational interest are the effects of BTK on cell motility and tissue homing, given that the BTK inhibitor ibrutinib causes redistribution of tissue-resident chronic lymphocytic

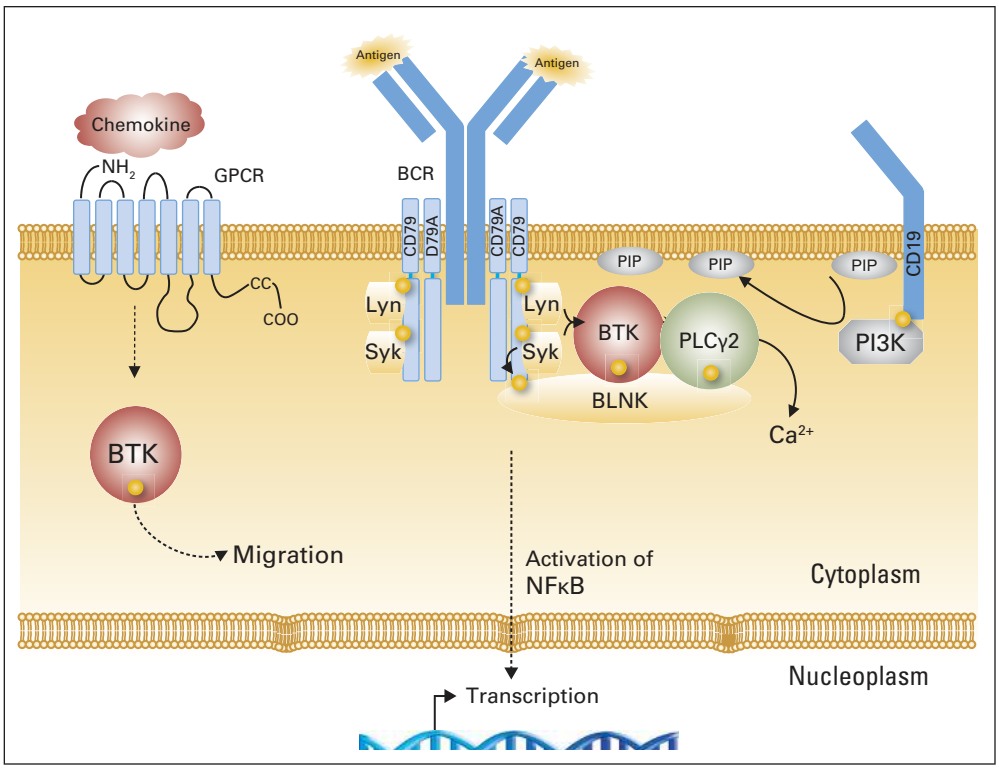

Fig 4. Role of Bruton's tyrosine kinase (BTK) in the B cell signaling. By signaling through the B-cell receptor (BCR), complex signaling cascades are initiated that recruit BTK to the cell membrane and activate other kinases, which leads to an increase in intracellular Ca2+ and activation of transcription κB (NF-κB). BTK also plays a role in chemokine receptor and adhesion molecule (integrin) signaling pathways and in signaling of multiple other surface receptors. BLNK, B-cell linker scaffold protein; GPCR, G protein-coupled receptor; PI3K, phosphoinositol-3 kinase; PIP, phosphatidylinositol; PLCγ2, phospholipase-C-γ2.

leukemia (CLL)[10] and mantle-cell lymphoma (MCL)[56] B cells into the peripheral blood, causing lymphocytosis that depends on the continuous presence of the BTK inhibitor.[10] The molecular basis for this striking activity could have been predicted from earlier work on the role of BTK in chemokine receptor and integrin signaling in normal B cells.[53,57] G$\beta\gamma$ subunits of G proteins can bind directly to the PHTH domain of BTK, consequently increasing its activity. In addition, the chemokine CXCL12 (SDF-1) as well as the neutrophil chemotactic factor fMet-Leu-Phe and a wide range of receptors can activate PI3Ks, which induce translocation of BTK to the plasma membrane. The chemokine CXCL12 induces BTK activation; in Btk-deficient murine (pre) B cells, human B cells, B-cell lines, and DT40 cells, the integrin-mediated adhesion and migration (as a response to stimulation with CXCL12 or CXCL13) and in vivo homing to lymphoid organs are impaired.[53] Chemokine-controlled B-cell migration, trafficking, and homing to lymphoid organs play an important role in the pathogenesis of B-cell malignancies as well as chronic inflammatory or autoimmune diseases. BTK is also an essential mediator in BCR-controlled adhesion of B cells to vascular cell adhesion molecule-1 (VCAM-1) and fibronectin.[57]

BTK Expression and Function in Other Hematopoietic Cells

BTK is expressed in most cells of the hematopoietic system, especially in B cells, myeloid cells, and platelets, whereas T lymphocytes and plasma cells have low or undetectable BTK levels.[58,59] B lymphocytes are the only cells known to be affected in XLA, and therefore the physiologic importance of BTK expression in other cell types remains to be established. Nonetheless, multiple reports have described effects of BTK in the development of other cell types such as platelets, macrophages,[60,61] and osteoclasts.[62] BTK may play a role in platelet aggregation[63] by transmitting signals from platelet membrane glycoprotein Ib. Quek et al[64] reported that BTK is important for signaling via the collagen receptor glycoprotein VI in platelets. However, the findings of this in vitro study need to be interpreted with caution, as emphasized by Jackson et al,[65] and patients with XLA who have defective BTK do not have an increased risk for bleeding events.[18] Farooqui et al[66] presented data about platelet numbers and function in 25 patients treated with ibrutinib. Their analysis indicates that ibrutinib does not have any significant effects on platelet function, and platelet counts improved rapidly in the majority of patients.

BTK in Inflammation, Especially in Autoimmune Diseases

Autoimmunity is thought to be related to extensive innate immune system activation by bacterial or viral pathogens, involving leukocyte activation via TLRs. TLRs are pattern recognition receptors essential for the detection of specific viral and bacterial components. For example, bacterial lipopolysaccharides initiate a proinflammatory response via recognition by TLRs. BTK has a central role in TLR signaling, in which it is part of a signaling cascade leading to the activation of the transcription factor NF-κB[55,67-70] as well as the regulation of pro- and anti-inflammatory cytokine production.[71] Experimental overexpression of Btk in mouse B cells causes antinuclear autoantibody production and SLE-like autoimmunity, which could be reversed by the BTK inhibitor ibrutinib, and which was absent in Btk transgenic mice overexpressing a kinase-inactive Btk mutant.[50] Pan et al[72] reported that the ibrutinib-related Celera compound 4 significantly inhibited arthritis development in a dose-dependent manner, with more than 95% inhibition of disease development in a murine rheumatoid arthritis (RA) model. Honigberg et al[9] reported that ibrutinib inhibited collagen-induced arthritis (CIA) as well as autoantibody production and development of kidney disease in the MRL-Fas(lpr) lupus model. Along the same lines, BTK blockade with a different inhibitor (CGI1746) inhibited BCR-dependent B-cell proliferation and reduced autoantibody levels in CIA.[73] Moreover, in this mouse model, BTK inhibition diminished FcγRIII-induced production of proinflammatory cytokines (TNF-α, interleukin-1β [IL-1β], IL-6),[73] suggesting multiple targets of BTK inhibition in RA. Chang et al[74] tested ibrutinib in a series of arthritis and immune complex animal models including CIA, collagen antibody-induced arthritis, reversed passive anaphylactic reaction, and passive cutaneous anaphylaxis. The authors reported the high efficacy of ibrutinib in CIA and in immune complex models that do not depend on autoantibody production, indicating again that ibrutinib targets not only B cells but also other proinflammatory cells, such as monocytes, macrophages, and mast cells. The complex role of BTK in autoimmunity is further highlighted in an elegant mouse model reported by Kubo et al,[55] demonstrating a link between augmented TLR9-induced BTK activation in PIR-B–deficient B-1 cells, causing excessive autoantibody production and autoimmunity. Kil et al[50] reported about a mouse model in which Btk was overexpressed in B cells, resulting in spontaneous formation of germinal centers, increased numbers of plasma cells, antinuclear autoantibody production, and SLE-like autoimmune disease affecting kidneys, lungs, and salivary glands. These pathologic changes were absent in Btk transgenic mice overexpressing a kinase-inactive Btk mutant, and ibrutinib decreased germinal center B cells and plasma cells and normalized B-cell activation and differentiation. Finally, in lupus-prone B6.Sle1 and B6.Sle1.Sle3 mice, ibrutinib dampens humoral and cellular autoimmunity as well as lupus nephritis.[75] BTK also plays a role in bone metabolism by transmitting signals in osteoclasts downstream of receptor activator of NF-κB and ITAM. Mice lacking Btk and Tec show severe osteopetrosis caused by a defect in bone resorption.[62] At this time, there are no clinical trials using BTK inhibition in autoimmune diseases, but it is expected that these will commence within the near future.

BTK AND ITS ROLE IN B-CELL MALIGNANCIES

On the basis of the phenotype in XLA and xid, B lymphocytes appear to be the most vulnerable cell type regarding the functional integrity of BTK. Consequently, research to explore function and targeting of BTK in cancers has largely focused on B-cell malignancies. Similar to its role in normal B cells, BTK also plays a role in signaling of critical receptors of malignant B cells, especially in BCR signaling[37,76] and signaling of B-cell homing receptors.[37,77] Chronic active BCR signaling in activated B-cell–like diffuse large B-cell lymphoma (DLBCL) can be induced by activating mutations in the BCR signaling pathway (eg, CD79A and CD79B mutations[76]), and it activates multiple downstream pathways, including NF-κB, which normally are activated by antigen. Tonic BCR signaling (eg, in Burkitt's lymphoma) typically engages the PI3K pathway.[78] In contrast, antigen-induced BCR activation appears to play a role in other lymphomas and in CLL, in which BCR activation can be triggered via binding to autoantigens and other environmental or microbial antigens. For example, CLL BCRs can

bind cytoskeletal nonmuscle myosin heavy chain IIA and vimentin, as well as the Fc tail of IgG, single-stranded DNA, double-stranded DNA, lipopolysaccharides, apoptotic cells, insulin, and oxidized lactate dehydrogenase.[79-87] A small subset of patients with CLL have high-affinity BCRs for an antigenic determinant of yeasts and filamentous fungi, β-1,6-glucan,[88] suggesting that ubiquitous antigens, such as β-1,6-glucan and autoantigens could promote the expansion of certain CLL clones via antigen-specific BCR signaling. In addition, a recent study demonstrated an interesting additional mechanism of BCR activation in CLL, best characterized as autonomous BCR signaling, in which the HCDR3 component of the BCR binds to an epitope in the second framework region, leading to Ca^{2+} signaling.[89] Along the same lines, Binder et al[90] reported an alternative epitope for CLL BCR self-recognition located in third framework region of Igs. This feature of BCR self-reactivity appears to be characteristic of Igs made by CLL cells[89] and is consistent with the ability of CLL BCRs to bind Ig components and act like rheumatoid factors.[79,80,87]

Increased levels of BTK protein have been detected in B-cell CLL cells,[91,92] and BTK activation in CLL can be activated via BCR and CD40 triggering.[92] BTK inhibition using the BTK inhibitor ibrutinib decreased DNA synthesis and prosurvival signal from stromal cells, CD154, and cytokines.[92] Moreover, ibrutinib abrogated BCR- and nurse-like cell-derived survival signals and decelerated disease progression in an adoptive transfer TCL1 mouse model of CLL.[37] Ibrutinib also inhibited CLL cell adhesion and migration toward the chemokines CXCL12 and CXCL13,[37,77] which are critical for tissue homing of CLL cells. These ibrutinib effects mirror the function of BTK in normal B-cell migration and homing[53] and appear to be the basis for tissue redistribution of CLL cells during therapy with ibrutinib and other related kinase inhibitors.[38] In another mouse model of CLL, the IgH.ETμ model, Btk deletion abrogated CLL formation, and Btk overexpression accelerated disease onset and mortality.[93] In multiple myeloma (MM) models, ibrutinib inhibited receptor activator of NF-κB ligand/macrophage colony-stimulating factor–induced phosphorylation of BTK and downstream signaling in osteoclasts, resulting in diminished bone resorption. Ibrutinib also inhibited secretion of cytokines and chemokines from osteoclasts and stromal cells, CXCL12-induced migration of MM cells, IL-6– and stroma-supported growth of MM cells, and in vivo MM cell growth and MM cell–induced osteolysis of implanted human bone chips in SCID mice.[94] In DLBCL, ibrutinib demonstrated selective toxicity in cell lines with chronic active BCR signaling,[76] it down regulates IRF4, and synergizes with lenalidomide in killing of activated B-cell–like DLBCL cells.[95] In contrast to mature B-cell malignancies, especially in CLL and MCL in which BTK generally is accepted as a lymphoma/leukemia-promoting kinase, its role in immature B-cell malignancies is controversial. BTK is generally expressed in childhood B-cell acute lymphoblastic leukemia (B-ALL),[96] but it can be altered by kinase-deficient BTK splice variants, which can provide survival advantage to B-ALL cells,[97] or it can be silenced.[98] In mouse models of B-cell development, Btk promotes B-cell differentiation and functions as a tumor suppressor in pre-B cells, which is independent of its catalytic activity.[99,100] The role of BTK in leukemias with constitutively active BCR-ABL kinase is also controversial. BTK can physically interact with c-Abl,[101] and can become activated by BCR-ABL1, mimicking constitutively active pre-BCR survival signaling.[98] c-Abl can phosphorylate tyrosine 223 in the SH3 domain of BTK,[101] but the deletion of BTK in such leukemia cells had no significant effects on cell growth; thus, other investigators concluded that BTK does not play a critical role in BCR-ABL–mediated leukemogenesis.[102]

BTK INHIBITORS

The crucial role of BTK in B-cell malignancies makes this protein an interesting therapeutic target. Knowledge of structural and functional details of the BTK molecule led to the design of effective inhibitors with a broad range of kinase selectivity profiles.[72,103,104] Kinase inhibitors can function as reversible adenosine triphosphate (ATP) –competitive agents that target the ATP binding site of protein kinases. The advantage of such reversible inhibitors is lack of irreversible modifications of off-target proteins, but poor selectivity and binding site competition with endogenous ATP remain key challenges for this class of inhibitors. In contrast, irreversible kinase inhibitors covalently bond to their target and exhibit high selectivity, prolonged pharmacodynamics, and potency in overcoming endogenous ATP competition.[105] The following paragraphs discuss some of the most promising compounds established during the last few years.

LFM-A13 (leflunomide metabolite analog α-cyano-β-hydroxy-β-methyl-*N*-[2,5-dibromophenyl]-propenamide) is the first BTK-specific tyrosine kinase inhibitor.[106] LFM-A13 binds to the catalytic site within the BTK kinase domain (IC_{50} [concentration that inhibits 50%], 2.5 μmol/L), inhibiting its activity without affecting the enzymatic activity of other protein tyrosine kinases.[107] Despite promising antileukemia activity in B-ALL cells[106] and lack of any major toxicity in preclincal studies,[107] LFM-A13 has not yet entered clinical development.

Ibrutinib is a potent (IC_{50}, 0.5 nmol/L), selective BTK inhibitor that inactivates BTK through irreversible covalent bonding to Cys-481 in the ATP binding domain of BTK.[9,72,108] Only a small subset of tyrosine kinases in the human genome is predicted to contain a modifiable cysteine residue homologous to Cys-481 in BTK, and only this subset is thought to be susceptible to irreversible and durable inhibition by ibrutinib. The Cys-containing kinases include EGFR, HER2, HER4, ITK, BMX, JAK3, TEC, and BLK. The extent to which inhibition of one or more of these alternate kinases contributes to the efficacy or toxicity of ibrutinib is largely unknown. Dubovsky et al[109] provided compelling evidence, however, that ITK functions as an additional target of ibrutinib in T cells. The initial development at Celera and subsequently at Pharmacyclics was focused on RA and, consequently, ibrutinib initially was tested in RA using in vivo models.[9,72] The in vivo activity of ibrutinib in B-cell lymphoma was first demonstrated in spontaneous canine B-cell lymphomas.[9] The most mature clinical data about effects of ibrutinib on B-cell malignancies are available for patients with CLL,[11] MCL,[12] and DLBCL.[10] For CLL, ibrutinib is given orally as a once-per-day fixed dose of 420 mg on a continuous schedule until progression or toxicity. At this dose, ibrutinib induces full BTK target occupancy, based on probe (fluorescently tagged derivative of ibrutinib) assays of peripheral blood mononuclear cell samples from patients with CLL who were treated with ibrutinib.[10] Ibrutinib is rapidly absorbed and rapidly eliminated after oral administration. The effective half-life of ibrutinib following oral dosing in humans is 2 to 3 hours (as measured post time to maximum concentration [T_{max}] to 6 hours), and pharmacokinetics appear to be

the same in patients with CLL and MCL. Ibrutinib is almost exclusively metabolized by CYP3A4/5, and polymorphisms could potentially influence ibrutinib metabolism. Ibrutinib is metabolized to the dihydrodiol metabolite (PCI-45227), which is monitored in clinical studies. Despite rapid clearance from plasma, BTK remains covalently bound to ibrutinib for at least 24 hours. Byrd et al[11] reported that ibrutinib induces high rates of durable remissions in patients with CLL and small lymphocytic lymphoma, including patients with high-risk disease. This was based on data from a phase Ib/II multicenter study of ibrutinib in 85 patients with relapsed or refractory CLL or small lymphocytic lymphoma. The authors report an overall response rate of 71%, and an additional 15% to 20% of patients had a partial response with lymphocytosis. The response was independent of clinical and genomic risk factors present before treatment, including advanced-stage disease, the number of previous therapies, and the 17p13.1 deletion. At 26 months, the estimated progression-free survival rate was 75% and the overall survival rate was 83%. Tissue redistribution of CLL cells from the tissue compartments into the peripheral blood is a characteristic early response to ibrutinib therapy, resulting in a surge in lymphocyte counts and resolution of enlarged lymph nodes and organs. This lymphocytosis typically is transient and must not be confused with disease progression according to currently accepted guidelines.[110]

Even with encouraging early clinical data, which are the basis for several ongoing phase III clinical trials in CLL, MCL, and other B-cell malignancies (Table 1), we need to keep in mind that the follow-up of clinical studies with ibrutinib still remains relatively short, and therefore we do not have a mature understanding of longer-term durability of responses to ibrutinib therapy. To date, untreated patients with CLL and low-risk patients seem to have the most durable remissions, whereas high-risk patients with CLL, especially patients with del17p, more frequently progress on therapy, either with development of Richter's transformation, or with more indolent progression of their CLL. This latter type of relapse during ibrutinib therapy appears to be associated with mutations within the ibrutinib binding site of BTK (C481S mutation), or in a downstream pathway molecule PLCγ2 (R665W mutation), suggesting that continuous therapeutic pressure favors the emergence of clones that are more ibrutinib resistant.[111] The fact that the majority of ibrutinib-treated patients with CLL do not achieve complete remissions (CRs) likely promotes the development of ibrutinib resistance, and therefore additional therapeutic strategies are needed to increase the numbers of patients that achieve CR, especially in high-risk patients with CLL. Combination therapy with established CLL treatment modalities (monoclonal antibodies, chemoimmunotherapy) likely will achieve the goal of higher CR rates, although not necessarily in patients with del17p who typically have inferior responses to established CLL therapies. Likely, only long-term follow-up will tell whether the risk-benefit ratio favors combination over single-agent ibrutinib therapy in defined CLL subgroups. Another important factor, age, also needs to be taken into consideration. Elderly patients older than age 70 years typically do not tolerate chemoimmunotherapy as well as younger patients and, consequently, ibrutinib monotherapy or combinations with antibodies are more appropriate in this population. More intensive chemoimmunotherapy-based combinations are used in younger patients, and in younger high-risk patients, debulking of the disease with ibrutinib followed by cellular therapies (allogeneic stem-cell transplantation, chimeric antigen receptor T-cell therapy) is discussed.

Immunosuppression may have been anticipated as a consequence of continuous BTK inhibition, given the clinical presentation of patients with XLA. However, in patients with CLL who are treated with ibrutinib, the average rate of infection declined from 7.1 per 100 patient-months during the first 6 months to 2.6 per 100 patient-months thereafter; IgG and IgM levels remained relatively stable throughout treatment, whereas IgA levels increased,[11] suggesting that CLL disease control outweighs immunosuppressive effects and that BTK inhibition in adults does not result in Ig depletion.

Wang et al[12] reported single-agent efficacy of ibrutinib in MCL on the basis of phase II study data on patients with relapsed or refractory MCL, in which ibrutinib was given at a daily dose of 560 mg. In 111 patients, the investigators noted a response rate of 68%, with a CR rate of 21% and a partial response rate of 47%. The most common treatment-related adverse events were mild or moderate diarrhea, fatigue, and nausea. Grade 3 or higher hematologic events were infrequent and included neutropenia, thrombocytopenia, and anemia. The estimated median response duration was 17.5 months, the estimated median progression-free survival was 13.9 months, and the estimated overall survival rate was 58% at 18 months.

CC-292 is another covalent irreversible BTK inhibitor developed by Celgene that is currently in phase I clinical trials for hematologic malignancies. CC-292 binds to BTK protein with high specificity and effectively inhibits constitutive and induced BTK and PLCγ2 phosphorylation at low nanomolar concentrations. It is, however, not fully BTK-specific and also targets other kinases containing homologous cysteine residue, such as JAK3 and TEC.[112] A first-in-human trial with healthy volunteers demonstrated that a single oral dose of 2 mg/kg CC-292 consistently engaged all circulating BTK protein,[112] thus providing the basis for dose selection in the ongoing clinical trials in patients with hematologic malignancies.

In conclusion, BTK gene mutations lead to XLA, the first identified PID caused by a mutated protein tyrosine kinase. Subsequent investigations established a crucial role for BTK in several signaling pathways that are critical in B-cell development and function. Intensive efforts during the last decade led to the generation of specific agents that target the kinase activity of BTK, consequently impairing B-cell proliferation, differentiation, and survival. Their target sites and modes of action have been characterized in biochemical and cellular assays, and their beneficial effects have been confirmed in animal models and in first clinical trials.[11,12] The highly encouraging clinical results led to breakthrough therapy designation for ibrutinib by the US Food and Drug Administration for patients with CLL, MCL, and Waldenström macroglobulinemia, and the recent US Food and Drug Administration approval of ibrutinib (Imbruvica) for previously treated patients with MCL (in November 2013) and CLL (in February 2014). With these exciting new developments, BTK has become a role model for translational research, in which basic research defined the genetic and molecular basis of XLA. These developments allowed for innovative development of BTK inhibitors that already have an impact on the lives of many patients suffering from B-cell malignancies. Reflecting on the clinical developments of BTK inhibitors over the last few years, we come to appreciate the decades of diligent clinical and basic research on XLA and BTK that laid the

foundation for these novel therapies, demonstrating that substantial translational discoveries often take decades of work to transition into clinical practice.

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