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Targeting Bruton Tyrosine Kinase: A novel strategy in the treatment of B-cell lymphomas

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Abstract: In normal B-cells, Bruton tyrosine kinase (Btk), a non-receptor tyrosine kinase involved in B-cell receptor (BCR) signalling, is essential for cell survival and maturation. Not surprisingly, Btk is also implicated in the pathogenesis of B-cell lymphomas, like Chronic Lymphocytic Leukaemia/Small Lymphocytic Lymphoma (CLL/SLL), Mantle Cell Lymphoma (MCL) and Waldenström's Macroglobulinemia (WM), which are driven by aberrant BCR signalling. Thus, targeting Btk represents a promising therapeutic strategy in the treatment of B-cell lymphoma patients. Ibrutinib, a selective Btk inhibitor, has already been approved as second-line treatment of CLL/SLL, MCL and WM patients, while more clinical studies of ibrutinib and novel Btk inhibitors are currently under way. In light of results of the RESONATE-2 trial, the approval of ibrutinib as a first-line treatment of CLL/SLL may well be approaching. Herein, we review Btk's role in normal and malignant BCR signalling, as well as ibrutinib's performance in B-cell lymphoma treatment and prognosis.

Keywords: *Bruton • tyrosine • kinase • inhibitor • ibrutinib • BCR • lymphoma • CLL • treatment*

List of Abbreviations (alphabetically)

ABC DLBCL: Activated B-cell Diffuse Large B-cell Lymphoma
BCR: B-cell Receptor
BLNK: B-cell Linker Protein
Btk: Bruton Tyrosine Kinase
CLL/SLL: Chronic Lymphocytic Leukaemia/Small Lymphocytic Lymphoma
CR: Complete Response
DAG: Diacylglycerol
DLBCL: Diffuse Large B-cell Lymphoma
FL: Follicular Lymphoma
HL: Hodgkin lymphoma
IGHV: Ig Heavy Variable Gene
IP_3: Inositol Triphosphate
ITAM: Immunoreceptor Tyrosine-based Activation Motif
M-CLL: Mutated Chronic Lymphocytic Leukaemia
MCL: Mantle Cell Lymphoma
mIg: Membrane Immunoglobulin
MYD-88: Myeloid Differentiation 88 Signalling Adaptor
MZL: Marginal Zone Lymphoma
NF-κB: Nuclear Factor Kappa B
NHL: Non-Hodgkin Lymphoma
OS: Overall Survival
PFS: Progression-Free Survival
PI3K: Phosphoinositide 3-Kinase
PIP_2: Phosphatidylinositol 4,5-Biphosphate
PIP_3: Phosphatidylinositol 3,4,5-Triphosphate
PIP5K: Phosphatidylinositol 5-Phosphate Kinase
PKC: Protein Kinase C
PLCγ2: Phospholipase C γ-2
PTK: Protein Tyrosine Kinase
U-CLL: Unmutated Chronic Lymphocytic Leukaemia
WM: Waldenström's Macroglobulinemia
Xid: X-linked Immunodeficiency
XLA: X-linked Agammaglobulinemia

Introduction

B cell receptor (BCR) signalling is necessary not only for effective antigen-specific humoral immunity, but also for B-cell maturation and survival. In 1993, it was discovered that mutations in a protein tyrosine kinase (PTK) involved in BCR signalling were the cause of primary

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immunodeficiencies in both men (1,2) and mice (3,4); X-linked Agammaglobulinemia (XLA) in men and X-linked Immunodeficiency (Xid) in mice are both characterized by a block in B-cell development at the pro-B cell stage, lack of mature B-cells, antibody deficiency and recurrent bacterial infections.[5] The kinase involved was named Bruton tyrosine kinase (Btk) after Dr. Ogden Bruton who first described XLA. The discovery that impaired PTK function could compromise B-cell maturation and survival hinted at the importance of BCR signalling in B-cell fate decisions and prompted the evaluation of the role of other signalling components in B-cell activation. Various knockout mice, including knockouts of Phosphoinositide 3-Kinase (PI3K), B-cell Linker (BLNK), Protein Kinase C (PKC) and Phospholipase C γ-2 (PLCγ2), all of them components of the B-cell receptor signalling machinery, exhibited Xid-like phenotype.[6] This led to the realization that they all must be part of a common signalling pathway; that there must be a B-cell 'signalosome' at work, a molecular scaffold responsible for transducing the signal, which would fall apart for the lack of a single component. So far, Btk is the only one of those signalling components whose inherited absence causes disease in humans, and is increasingly recognized as a major mediator of B-cell malignancy pathogenesis.

Btk in normal BCR signalling

Btk is a member of the Tec family of non-receptor tyrosine kinases, predominantly expressed in B-lymphocytes and cells of the myeloid lineage, but not in plasma cells or T-lymphocytes.[7] Nevertheless, B-cells are the ones that are primarily affected in patients with loss-of-function Btk mutations; thus, for the sake of simplicity, Btk can be considered a B-cell-specific molecule. It contains the following domains, each of which can interact with other proteins for signalling purposes: PH, TH, SH2, SH3 and SH1 (kinase domain).[8] PH is a pleckstrin homology domain used for binding to Phosphatidylinositol 3,4,5-triphosphate (PIP_3), TH is a Tec-homology domain that contains a zinc-finger motif, SH2 is an Src homology domain that allows docking to phosphorylated tyrosine residues on other proteins and SH3 is an Src homology domain used for protein-protein interactions that contains an activating site of autophosphorylation (Figure 1).

PH: Pleckstrin homology domain. TH: Tec homology domain. SH2, SH3: Src homology domains. SH1/TK: tyrosine kinase domain. PIP_3: Phosphatidylinositol 3,4,5-triphosphate. PKC: Protein kinase C. Lyn, Syk: Src-family tyrosine kinases. BLNK: B-cell linker protein. PLCγ2: Phospholipase C γ-2. NF-κB: Nuclear factor kappa B.

Btk contains five domains through which it interacts with other proteins of the B-cell receptor signalling pathway, leading to PLCγ2 and eventually, NF-κB activation. PH is used for binding to PIP_3, which recruits Btk to the plasma membrane; TH contains a zinc-finger motif; SH2 is used for docking to phosphorylated tyrosine residues on other proteins, allowing for interaction with proteins, such as BLNK and Lyn/Syk tyrosine kinases; SH3 contains a site of autophosphorylation, necessary for Btk activation; SH1 is a tyrosine kinase domain, used for PLCγ2 activation.

The B-cell receptor consists of a membrane immunoglobulin (mIg) produced through V(D)J recombination, associated with two other proteins, Ig-α (CD79a) and Ig-β (CD79b), which contain immunoreceptor tyrosine-based activation motif (ITAM) regions in their cytoplasmic terminal domains.[9] BCR signalling is outlined graphically in Figure 2. The pathway is initiated by antigen-binding to mIg, although

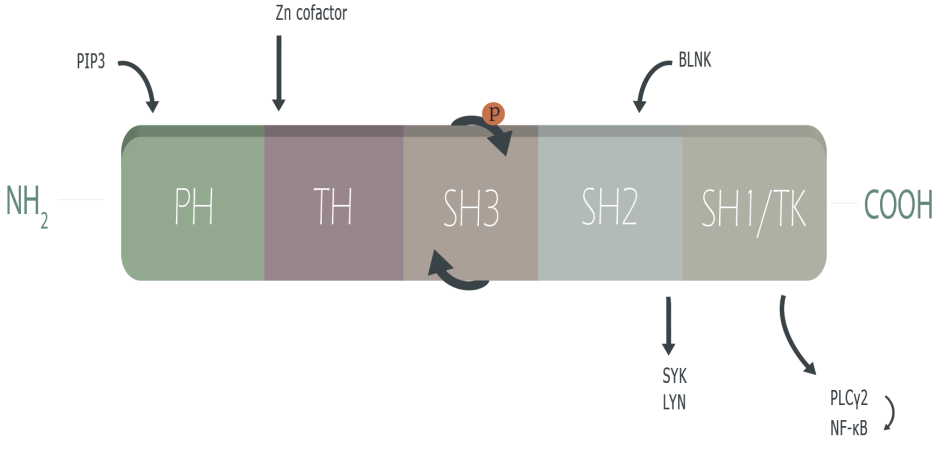

Figure 1. Bruton tyrosine kinase (Btk) molecular structure.

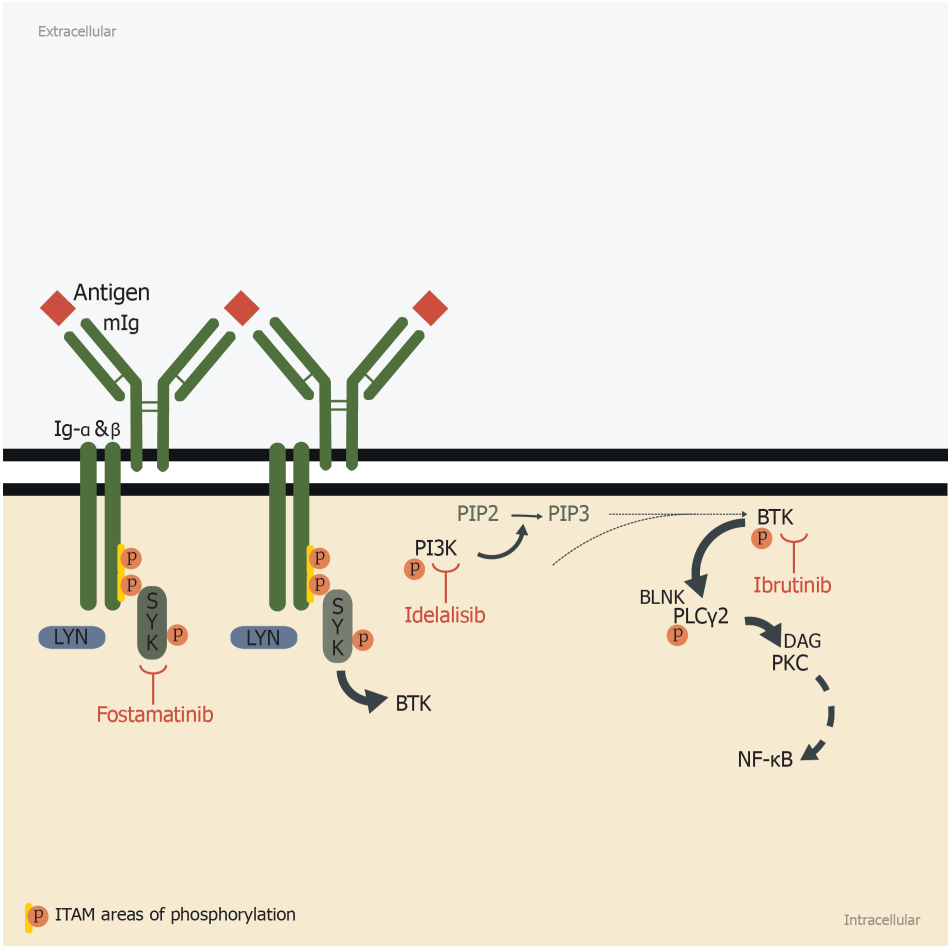

Figure 2. B-cell Receptor (BCR) signalling pathway.

a basal antigen-independent signal is also generated to allow for cell development and survival.[10] Following antigen-binding, the ITAM regions of Ig-α and Ig-β are phosphorylated by the Src family kinases Lyn, Fyn and Blk.[11] Those kinases also increase the activity of the Syk kinase,[12] which − via its own SH2 domain − binds to phosphorylated Ig-α and Ig-β.[13] As a result of BCR ligation, PI3K is also activated; it then phosphorylates phosphatidylinositol 4,5-biphosphate (PIP_2), producing phosphatidylinositol 3,4,5-triphosphate (PIP_3), which in turn recruits cytoplasmic Btk to the plasma membrane through binding to its PH domain.[14]Once there, Btk is phosphorylated by Lyn and Syk at tyrosine residue 551[15] and then autophosphorylated at tyrosine residue 223 within its own SH3 domain,[16] rendering itself active. Subsequently, Btk interacts with adaptor protein BLNK through its SH2 domain and the two together activate PLCγ2.[17] PLCγ2 utilizing PIP_2 − the same substrate as PI3K − produces Inositol Triphosphate (IP_3) and Diacylglycerol (DAG), which in turn mobilize intracellular calcium and activate PKC, respectively.[5,18] These intracellular changes culminate in the activation of Nuclear Factor kappa-B (NF-κB)[19] and other transcription factors, which then regulate the expression of genes related to B-cell survival, development and proliferation, such as Bcl-X_L.[5] Of note, Btk upregulates phosphatidylinositol 5-phosphate kinase (PIP5K), the enzyme responsible for producing PIP_2, which is a substrate for both PI3K and PLCγ2, thus enhancing their activity,[20] while it also plays a role in B-cell migration and adhesion.[21,22]

Lyn, Syk: Src-family tyrosine kinases. PI3K: Phosphoinositide 3-kinase. PIP_2: Phosphatidylinositol 4,5-biphosphate. PIP_3: Phosphatidylinositol 3,4,5-triphosphate. BLNK: B-cell linker protein. PLCγ2: Phospholipase C γ-2. DAG: Diacylglycerol. PKC: Protein kinase C. NF-κB: Nuclear factor kappa B.

BCR consists of a membrane immunoglobulin (mIg), noncovalently associated with Ig-α (CD79a) and Ig-β (CD79b), which contain immunoreceptor tyrosine-

based activation motif (ITAM) regions. Following antigen-binding, the ITAM regions of Ig-α and Ig-β are phosphorylated by Lyn, an Src-family kinase. Syk, another Src-family kinase, then binds to phosphorylated Ig-α and Ig-β; while PI3K, another kinase activated through BCR ligation, phosphorylates PIP_2, producing PIP_3, in turn recruits cytoplasmic Btk to the plasma membrane. Once there, Btk is phosphorylated by Lyn and Syk and then autophosphorylated, rendering itself active. Btk then interacts with BLNK and, together, they activate PLCγ2. PLCγ2 produces DAG, which in turn activates PKC. These intracellular changes culminate in the activation of NF-κB and other transcription factors, which then regulate the expression of genes related to B-cell survival, development and proliferation.

Btk in B-cell malignancies (CLL, MCL, DLBCL, WM, FL, MZL, HL)

Aberrant BCR signalling has been linked to a number of B-cell malignancies, but most commonly Chronic Lymphocytic Leukaemia (CLL), where it is now considered a major promoting factor.

Chronic Lymphocytic Leukaemia is strongly dependent on microenvironmental cross-talk. CLL cells undergo apoptosis when cultured *in vitro*, unless they are co-cultured with nurse-like cells or stromal cells (i.e., cells from the leukemic microenvironment), which are apparently required for leukemic cell survival.[23] In fact, there are multiple cellular (stromal cells, T-cells, nurse-like cells) and molecular (CXCR4, CXCR5, etc.) interactions at play,[24] but BCR signalling and antigen stimulation in particular lie at the centre of disease pathogenesis.

In 1998, it was discovered that CLL B-cells express a restricted Ig heavy variable (IGHV) gene repertoire.[25] More specifically, CLL B-cells are biased towards the use of certain V_H genes, that preferentially combine with certain D or J_H segments, creating similar antigen-binding pockets.[26] Furthermore, the BCRs of CLL cells from various patients exhibit similar somatic hypermutation patterns in their IGHV genes, typical of antigen receptors that have undergone selection by antigen.[27] Thus, it appears that the BCRs of CLL cells from different patients are often structurally similar or 'stereotyped', exhibiting little variation among different subsets of patients, despite the very many possible VDJ combinations.[28-31] This phenomenon is disease-restricted,[32,33] as it differs from the wider diversity of BCRs found in normal B-cells, and it suggests a limited set of (auto)antigens stimulating clonal evolution in CLL pathogenesis.[34,35,36]

B-cell receptor signalling in CLL is heterogeneous, depends on the levels of surface IgM (37) and correlates with tumour behaviour.[38] Generally, the two subtypes of CLL are recognized, mutated CLL (M-CLL) and unmutated CLL (U-CLL), depending on whether the B-cell mIg has undergone somatic hypermutation or not. In M-CLL, following somatic hypermutation, the surface IgM either exhibits avid affinity to antigen or no affinity at all; either way, the surface IgM is eventually down-regulated and the B-cell is rendered anergic over time.[38] On the contrary, in U-CLL, the surface IgM that has not undergone hypermutation retains its polyreactivity and, upon ligation to various (auto)antigens, propagates B-cell clone expansion.[38] Hence, BCR signalling is most important in U-CLL,[39] which is related to more aggressive disease and has a significantly worse prognosis. Apparently, BCR signalling is not only implicated in CLL pathogenesis, but it is also related to the outcome.

There are multiple B-cell malignancies, though, Btk might be involved in the pathogenesis of these malignancies. For example, a distinct Ig heavy variable gene repertoire with somatic hypermutation (again, indicative of antigenic selection)[40] and constitutively active BCR downstream signalling[41] were discovered in Mantle Cell Lymphoma (MCL) B-cells, indicating a role for BCR signalling in MCL pathogenesis as well. What is more, chronic BCR activation is required for cell survival in Activated B-cell Diffuse Large B-cell Lymphoma (ABC DLBCL),[42], making Btk a promising therapeutic target in that subset of DLBCL patients, while the effectiveness of Btk targeting in relapsed/refractory follicular lymphoma (FL) and marginal-zone lymphoma (MZL) suggests a role for Btk there, too.[43] Another example is Waldenström's Macroglobulinemia (WM); Btk is necessary for lymphoplasmacytic cell survival, as it is a downstream target of the Myeloid Differentiation 88 signalling adaptor (MYD88), which carries the most prevalent somatic mutation in WM patients, L265P.[44] And last but not least, although Btk is expressed in only 20% of patients with Hodgkin Lymphoma (HL),[45] targeting it has been shown effective in refractory classic HL.[46]

Targeting Btk in B-cell malignancies

Since aberrant BCR signalling is implicated in the pathogenesis of B-cell malignancies, such as CLL, MCL, ABC DLBCL, FL, MZL, WM and HL, it was only reasonable to evaluate the efficacy of protein kinase inhibitors in their treatment. Thus, a number of PTK inhibitors, such as PI3K and SYK inhibitors, were

developed and are being tested. Idelalisib, an oral PI3K inhibitor, has progressed into phase III trials in patients with advanced Non-Hodgkin's Lymphoma (NHL), CLL and MCL,[47] while fostamatinib, an oral SYK inhibitor, has induced partial responses in relapsed CLL patients in a phase I/II study.[48] Recently, ibrutinib (PCI-32765), a selective Btk inhibitor, was approved for use in CLL, MCL and WM.

In preclinical studies, ibrutinib was shown to actively inhibit Btk in CLL cells, abrogating BCR-induced survival signals and hindering B-cell migration and homing.[49,50,51] A phase 1 study of ibrutinib in patients with relapsed or refractory B-cell malignancies followed. And for a price of only mild/moderate toxicity, ibrutinib showed potent clinical antitumor activity, with objective responses in 60% of patients, complete response (CR) in 16% and a median progression-free survival (PFS) of 13.6 months.[43] Subsequently, a phase 1b-2 study of ibrutinib in relapsed CLL patients registered an overall response rate of 71% with 83% overall survival (OS); the responses were sustained and only varied according to IGHV mutation status (77% response in U-CLL and 33% in M-CLL).[52] Another phase 1b-2 study in elderly treatment-naive patients with CLL confirmed the mild/moderate toxicity profile and showed a 71% objective response rate.[53] The phase 3 RESONATE trial of ibrutinib versus ofatumumab (anti-CD20 mAb), for previously treated CLL patients, favoured ibrutinib in terms of response rate, PFS and OS, with a 57% reduction in the rate of death versus ofatumumab.[54] Based on these results, ibrutinib was FDA-approved for CLL patients who have received at least one prior therapy, as well as for all Del(17p) CLL patients, considered to have the poorest prognosis. Three-year follow-up data of ibrutinib in CLL showed acceptable toxicity and continued activity with sustained responses that improve in quality over time.[55] Recently, results from RESONATE-2, the phase 3 study of ibrutinib versus chlorambucil, in patients 65 years or older with treatment-naive CLL/SLL, showed increased overall response rate and improved overall survival with ibrutinib.[56] Ibrutinib was thus deemed superior to chlorambucil in previously untreated patients over 65 years old with CLL/SLL, setting the ground for the incorporation of ibrutinib in first-line treatment of CLL. Nevertheless, more data are needed, as chlorambucil is seldom used alone in clinical practice; it is usually combined with rituximab (anti-CD20 mAb) and fludarabine – a purine analogue – if the patient is fit and negative for Del(17p) [Del(17p) confers resistance to standard chemotherapy]. What is more, cyclophosphamide, another alkylating agent, is often preferred over chlorambucil, again, in combination with fludarabine and rituximab (FCR regimen). Thus, as far as the first-line treatment is concerned, it still remains to be seen whether ibrutinib is as good as FCR or if, perhaps, a combination of ibrutinib and rituximab can improve the outcome and prognosis compared to FCR.

Ibrutinib was also FDA-approved for patients with MCL, who have received at least one previous therapy, based on the results of a phase 2 study in relapsed or refractory MCL that showed a 68% response rate[57] and median OS of 22.5 months after an extended 2-year follow-up period.[58] Ibrutinib combinations with other drugs, such as rituximab, are also under study in a similar setting.[59]

Finally, following a study of ibrutinib in previously treated patients with WM that showed sustained responses and limited toxicity,[60] the drug was also FDA-approved as a second-line treatment for WM patients or even first-line for those unsuitable for chemo-immunotherapy. The responses achieved with ibrutinib in patients with ABC DLBCL were good,[61] but the drug has not been approved for use in those patients yet.

Finally, novel Btk inhibitors, more selective and more potent than ibrutinib, are under clinical studies, promising more therapeutic choices for B-cell lymphomas.[62]

Conclusion

Btk is a non-receptor protein kinase involved in BCR signalling, necessary not only for antigen-specific humoral immunity, but also cell survival and maturation. As it is linked to various B-cell malignancies, including CLL, MCL and WM, it represents a prime therapeutic target in their treatment. Ibrutinib, a selective Btk inhibitor, has already been shown effective in selected patients with CLL, MCL and WM, but it is currently being tested for the treatment of other B-cell malignancies as well. Recent results from RESONATE-2, the phase 3 study of ibrutinib versus chlorambucil in patients 65 years or older with treatment-naive CLL/SLL, favoured ibrutinib over chlorambucil, laying the groundwork for the incorporation of ibrutinib in the first-line treatment of CLL patients. Ibrutinib has already improved CLL therapeutics, in the sense that it constitutes an effective, well-tolerated alternative for patients refractory to classic treatment, and it might advance it even further, perhaps as part of a new combination regimen, as more and more data are gathered.

Conflict of Interest

The authors declare that there is no conflict of interest.

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