Drugs
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LEADING ARTICLE

Targeting Bruton's Tyrosine Kinase Across B-Cell Malignancies

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Abstract
Bruton's tyrosine kinase (BTK) is crucial in B-cell development and survival. The role of BTK as a downstream kinase in the B-cell receptor (BCR) signaling pathway is well described. As a key player in the pathogenesis of B-cell malignancies, targeting of dysregulated BCR signaling has been explored by development of inhibitors of downstream mediators. Discovery of the biological function of BTK and the development of covalent inhibitors for clinical use, ibrutinib as the lead agent and acalabrutinib as the second clinically approved BTK inhibitor, have revolutionized the treatment options for B-cell malignancies. Currently, ibrutinib is approved for mantle cell lymphoma, chronic lymphocytic leukemia, lymphoplasmacytic lymphoma/Waldenström macroglobulinemia, small lymphocytic lymphoma, marginal zone lymphoma and chronic graft versus host disease, while acalabrutinib is approved for mantle cell lymphoma. Potential expansion of indications in other diseases is under investigation in several clinical trials, while combination of BTK inhibitors with either chemoimmunotherapy or other targeted agents is being systematically explored in B-cell malignancies.

Key Points

Inhibition of BTK is a highly efficient treatment option for numerous B-cell malignancies.

Combination of BTK inhibitors with standard chemo-immunotherapy regimens or other targeted agents may provide a shift in treatment paradigm.

1 Introduction

In 1952 Dr. Ogden Carr Bruton described the disease x-linked agammaglobulinemia in children, which four decades later in 1993 was linked to mutations in Bruton's tyrosine kinase (BTK), unravelling its crucial role in B-cell development and survival [1, 2]. Later, BTK's role as a downstream kinase in the B-cell receptor (BCR) signaling pathway was demonstrated. With the back-to-back papers on the prognostic impact of immunoglobulin heavy chain variable region genes (IGHV) mutated or unmuatated status in chronic lymphocytic leukemia (CLL) by Hamblin et al. and Damle et al., it became clear that BCR signaling plays a pivotal role in CLL [3, 4]. As the central role of dysregulated BCR signaling in the pathogenesis of a multitude of B-cell malignancies became clear, several inhibitors targeting downstream kinases in the pathway have been explored in preclinical and clinical settings [5, 6]. Currently, two drugs targeting BTK have been approved by the US Food and Drug Administration (FDA) [7, 8]. In November 2013 the first-generation BTK inhibitor ibrutinib, an irreversible inhibitor of BTK, was FDA approved for treatment of mantle cell lymphoma (MCL). In the following years the approval was expanded to chronic lymphocytic leukemia (CLL) (2014), lymphoplasmacytic lymphoma (LPL)/Waldenström macroglobulinemia (WM) (2015), small lymphocytic lymphoma (SLL) (2016), marginal zone lymphoma (MZL) (2017), and chronic graft versus host disease (cGvHD) (2017). More recently, the second-generation BTK inhibitor acalabrutinib was FDA approved in 2017 for the treatment of MCL [9]. In addition, the PI3Kδ inhibitor idelalisib has been approved for treatment of CLL and relapsed follicular lymphoma. Several other inhibitors of molecules in the BCR pathway are currently under preclinical and clinical testing in B-cell malignancies; however, these are outside the scope of this

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review. In the European Union and Japan, ibrutinib has been approved for the treatment of MCL, CLL, and WM.

Currently, numerous pre-clinical and clinical studies are evaluating the efficacy of BTK inhibitors as single agents and in combination with chemoimmunotherapy or other targeted agents in various B-cell malignancies. Here, the biological function of BTK and the major milestones in the development of ibrutinib and acalabrutinib are reviewed along with current clinical practice and the status for clinical trials with BTK inhibitors.

2 Bruton's Tyrosine Kinase (BTK) Background

2.1 Structure and Function

The structure of BTK has been well described. It consists of several domains also present in other kinases of the TEC and SRC families. In the N-terminal region, a pleckstrin homology (PH) domain represents the site of activation by phosphatidylinositol phosphates and inhibition by protein kinase C. This is followed by a TEC homology (TH) domain and two SRC homology (SH) domains, through which SH3 mediated-binding to proline-rich motifs and SH2 binding to tyrosine-phosphorylated peptide motifs on other molecules are mediated. A C-terminal tyrosine kinase domain represents the active site and the binding site for covalent, irreversible inhibitors [10, 11] (Fig. 1). Mutations in the kinase domain at C481 have been demonstrated on progression of CLL upon treatment with ibrutinib, thus causing resistance to covalent kinase inhibitors [12–14].

2.2 Activation

Following antigen binding to the extracellular part of the BCR, activation motifs are phosphorylated by SRC family protein tyrosine kinases (Lyn) leading to activation of kinases in SH2 domains (SYK) and PI3Kδ. Through downstream signaling, BTK and other BCR kinases are recruited upon phosphorylation of BLNK, which assembles the signaling molecules to the cell membrane. This leads to further activation through phospholipase C-γ2 (PLCγ2) phosphorylation and calcium mobilization [5, 15]. Activation of BCR can occur constitutively, proposedly mediated through ongoing crosslinking of the receptor with surface membrane immunoglobulins on the B-cell or through binding of antigens from microbes or apoptotic cells. Several studies also indicate that elements of the BCR pathway, such as p38, LYN, NF-AT, and NFkβ, may be constitutively activated in CLL cells [16–20] (Fig. 2).

3 Ibrutinib

Ibrutinib is a first-generation BTK inhibitor that irreversibly binds to the kinase domain including cysteine (Cys)-481, thereby potently blocking the enzymatic activity. In vitro treatment of activated CLL cells with ibrutinib resulted in inhibition of BTK tyrosine phosphorylation [21]. Ibrutinib was the first BTK inhibitor approved for clinical use, and is currently FDA approved for MCL, CLL, SLL, LPL/WM, MZL, and cGvHD. However, as evidence emerges, more indications will likely be added (see Table 1) [7, 22, 23]. In several clinical studies, ibrutinib has been demonstrated to decrease proliferation, induce apoptosis and cause downregulation of chemokines in vitro and in vivo. Additionally, prevention of interaction between CLL cells and the microenvironment, which is pivotal for CLL cell proliferation, has been demonstrated upon treatment with ibrutinib [21, 24–26]. Ibrutinib also inhibits several other kinases that contain cysteine residues homologous to Cys-481 in BTK, accounting for some of the off-target effects reported [27]. In this context, inhibition of Tec by ibrutinib is believed to contribute to platelet dysfunction and the increased risk of bleeding in the clinical setting. A recent study also found

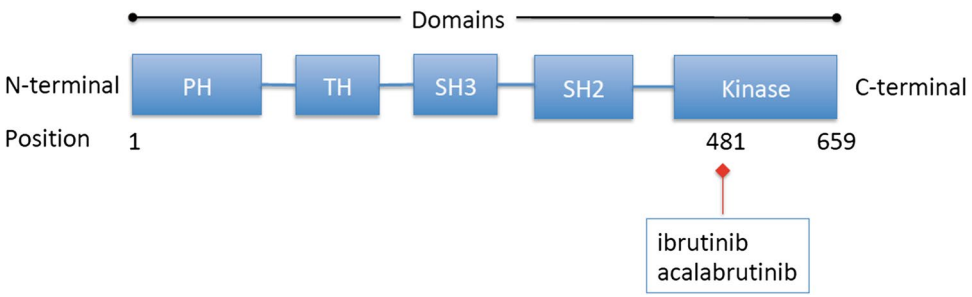


Fig. 1 Structure of Bruton's tyrosine kinase (BTK) from the N-terminal region pleckstrin homology (PH) domain followed by a TEC homology (TH) domain, two SRC homology (SH) domains (SH3 and SH2) and a C-terminal tyrosine kinase domain. Ibrutinib and acalabrutinib binds to the same part of the kinase domain, which includes amino acid 481; mutations of this residue renders CLL cells resistant to the inhibitors

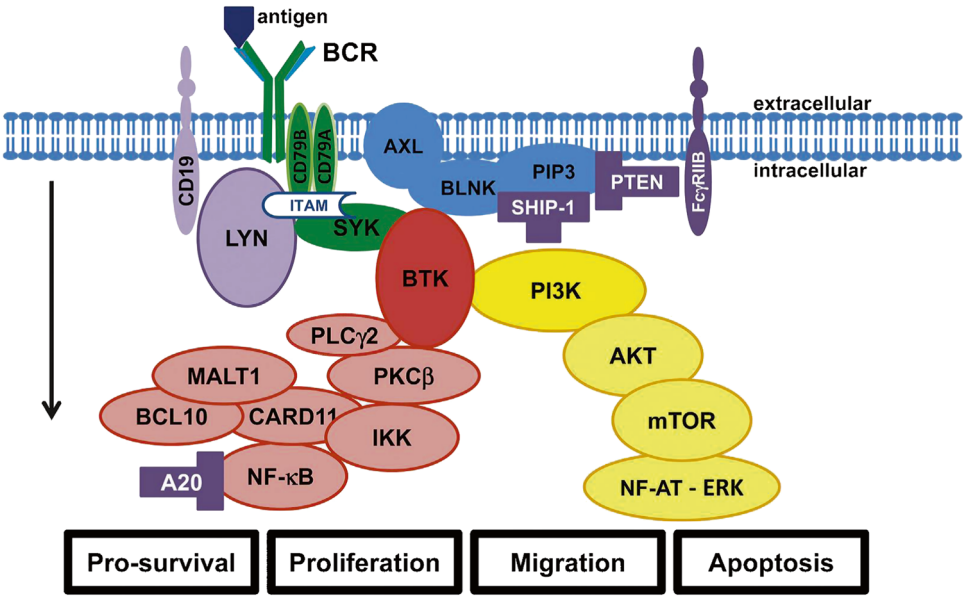


Fig. 2 The B-cell receptor (BCR) and its downstream pathways. The arrow indicates the direction of signaling from the plasma membrane towards the effectors. Antigen binding or cell autologous interaction activates BCR, resulting in phosphorylation of ITAMs in the cytoplasmic domains of CD79A and CD79B. SYK amplifies the initial signal by autophosphorylation and further phosphorylation of ITAMs (the initial amplifying complex is marked in green). LYN has a double function in initiating and terminating BCR signaling depending on interaction with CD19 (inhibitory molecules marked purple, bifunctional molecules light purple). SYK also activates the PI3K arm of the pathway (marked in yellow). Phosphatidylinositol 4,5-bisphosphate (PIP2) is phosphorylated by PI3K to phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3, AXL, and BLNK form a signaling hub that recruits the upper part of the BCR pathway to the plasma membrane. Inhibitory mechanisms include FcγRIIB, which inhibits BCR signaling upon binding to immune complexes at the BCR. SHIP-1 and PTEN phosphatases inhibit the PI3K arm of the pathway by hydrolysis of PIP3. AKT and mTOR relay PI3K activation further to downstream targets and cell-cycle regulation. The BTK arm of the pathway (marked in red) is initiated by recruitment of BTK to the plasma membrane-signaling hub. PLCγ2 is activated downstream of BTK, leading to subsequent activation of PKCβ. PKCβ phosphorylates IKK to activate NF-κB transcription factors that regulate gene expression of several survival factors. The complex of CARD11, MALT1, and BCL10 is an important part of the pathway activating NF-κB, whereas A20 is a negative regulator of NF-κB. The downstream effectors can be modulated towards the pro-apoptotic NF-AT–ERK arm or the pro-survival NF-κB arm, depending on balancing of the signaling cascades (reprinted from the original paper with permission: Niemann and Wiestner [5])

that ibrutinib inhibition of SRC family kinases, which have a critical role in platelet adhesion, may contribute to increased risk of bleeding [28]. Ibrutinib also impacts T-cell function directly and indirectly through disruption of the microenvironmental interaction. This leads to reduced T-cell proliferation and activation as well as decreased production of anti-inflammatory cytokines (IL-6, IL-10, and TNF-α) [21, 26, 29–32]. The impact on T-cell function in vitro and in vivo probably accounts in part for the effect of ibrutinib in cGvHD. The most commonly reported adverse reactions associated with ibrutinib include anemia, thrombocytopenia, neutropenia, headache, diarrhea, fatigue, myalgia, peripheral edema, bruising, and atrial fibrillation.

4 Acalabrutinib

The second-generation BTK inhibitor acalabrutinib is currently FDA approved for MCL. Similar to the case for ibrutinib, indications for acalabrutinib will likely be broadened as evidence emerges [9]. Acalabrutinib seems a more selective BTK inhibitor than ibrutinib, probably due to a unique reactive butynamide group, which forms a covalent bond with the Cys-481 residue in BTK, as the acrylamide group of ibrutinib does. In contrast to ibrutinib, acalabrutinib only inhibits BTK, BMX, and ERBB4 at clinically relevant concentrations, accounting for the higher specificity of this BTK inhibitor (Fig. 3). The pharmacokinetic potency of acalabrutinib is higher than for ibrutinib, probably in part due to lower protein binding, which also leads to higher levels of BTK occupancy in lymph nodes [27]. Recent clinical reports have demonstrated that toxicities and off-target activity, including atrial fibrillation and bleeding, which are associated with ibrutinib, may be lower in patients with relapsed/refractory CLL treated with acalabrutinib for a median of 14 months. However, further clinical data from head-to-head comparison of ibrutinib, acalabrutinib, and other BTK inhibitors are awaited before any conclusive statements on differences in adverse event profiles between BTK inhibitors can be made [33, 34]. The most commonly reported

Table 1 Bruton's tyrosine kinase inhibitors in clinical trials

Drug name	Number of ongoing clinical trials[a]	Published studies: indication and *clinical setting*	References
Ibrutinib (PCI-32765)	192	**Chronic lymphocytic leukemia**	
		Frontline	
		Single agent	[38, 39, 41, 65–68]
		Combination	
		Venetoclax, obintuzumab	[44]
		Rituximab	[59, 69]
		Relapsed/refractory	
		Single agent	[23, 38, 39, 41, 67, 70]
		Combination	
		Rituximab	[69]
		Rituximab, fludarabine, cyclophosphamide	[71]
		Rituximab, bendamustine	[60, 61, 71]
		Ofatumumab	[61, 72]
		Venetoclax	[56, 57]
		Follicular lymphoma	
		Frontline	
		Combination	
		Rituximab, lenalidomide	[73]
		Diffuse large B-cell lymphoma	
		Frontline	
		Combination	
		Rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone	[74]
		Relapsed/refractory	
		Combination	
		Rituximab, bendamustine	[75]
		LPL/Waldenström's macroglobulinemia	
		Relapsed/refractory	
		Single agent	[49]
		Combination	
		Rituximab	[24]
		Mantle cell lymphoma	
		Frontline	
		Combination	
		Rituximab	[76]
		Relapsed/refractory	
		Single agent	[77]
		Combination	
		Venetoclax	[47]
		Lenalidome Rituximab	[48]
		Marginal zone lymphoma	
		Relapsed/refractory	
		Single agent	[25]
Acalabrutinib (ACP-196)	25	**Chronic lymphocytic leukemia**	
		Relapsed/refractory	[33]
		Mantle cell lymphoma	
		Relapsed/refractory	[9]

Table 1 (continued)

Drug name	Number of ongoing clinical trials[a]	Published studies: indication and *clinical setting*	References
Spebrutinib (CC–292)	2	**Chronic lymphocytic leukemia**	
		Relapsed/refractory	
		Single agent	[78]
Tirabrutinib (ONO–4059)	5	**Chronic lymphocytic leukemia**	
		Relapsed/refractory	
		Single agent	[79]
		B-cell malignancies (broad)	
		Relapsed/refractory	
		Single agent	[80]
Vecabrutinib (SNS-062)	1	**Safety assessment**	[81]
Zanabrutinib (BGB-3111)	14	**None**	

[a]ClinicalTrials.gov was accessed on 14 September 2018

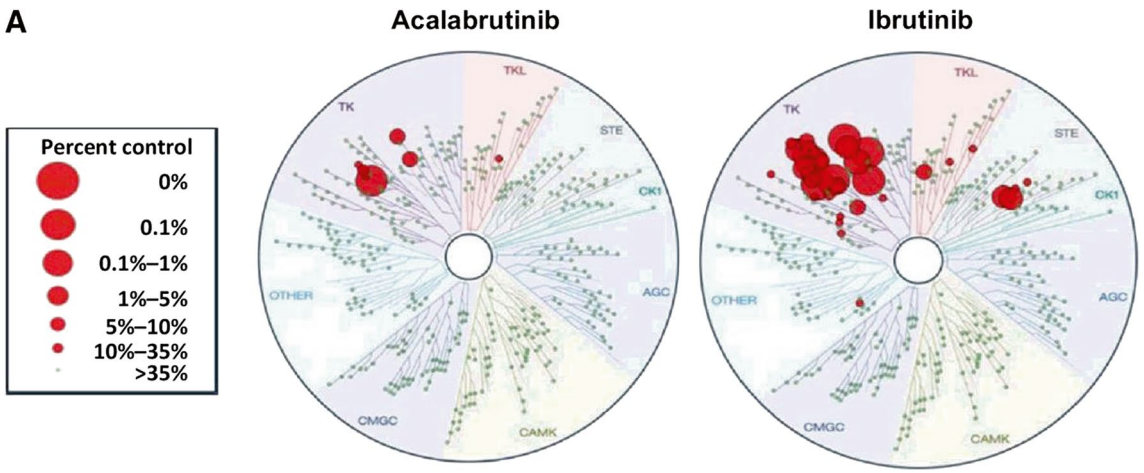


Fig. 3 Acalabrutinib is a potent and selective inhibitor of Bruton's tyrosine kinase (BTK). Acalabrutinib and ibrutinib were profiled at 1 μM over a panel of 395 wild-type human kinases, including mutants, at DiscoveRx kinase assays. The size of the red circles represents the extent of inhibition, with larger circles meaning stronger inhibition compared to the control signal as defined in the scale (reprinted from the original paper with permission: Herman et al. [64])

adverse reactions associated with acalabrutinib are anemia, thrombocytopenia, headache, neutropenia, diarrhea, fatigue, myalgia, atrial fibrillation, and bruising.

5 Clinical Use in Hematological Diseases

5.1 B-Cell Malignancies

5.1.1 Chronic Lymphocytic Leukemia

Early in the development of ibrutinib, the activity towards CLL cells was demonstrated in preclinical studies [35]. In early clinical assessment, efficacy in CLL patients was demonstrated by six out of seven patients showing gradually decreasing lymph node size. As seen with all BCR inhibitors in clinical use, an initial increase in peripheral blood CLL cell counts with a subsequent slow decrease in lymphocytosis towards the normal range over months to years was demonstrated. While effects on CLL apoptosis was more difficult to demonstrate in vivo (probably due to rapid clearance of apoptotic cells from the blood stream), proliferating ($Ki67^+$) CLL cells, which at baseline were up to 11%, disappeared from the blood during the first week of treatment [36].

Clinical efficacy has been clearly demonstrated in the relapsed/refractory setting as well as for first-line treatment as summarized in Table 1. Efficacy has been demonstrated through improvement of progression-free survival (PFS), overall survival (OS), and response rate. Superiority has been demonstrated versus chlorambucil and ofatumumab as

single agents for all parameters, while results from clinical trials with more effective comparator arms are still awaited [37].

With 5-year follow-up from the landmark studies, the overall response rate (ORR) on ibrutinib was 89%, with rates of complete response in 29% of treatment-naïve (TN) and 10% of relapsed or refractory (R/R) patients. The 5-year PFS was 92% and 44% in the TN and R/R groups, respectively [38]. Especially in the high-risk setting of del(17p) or *TP53* mutations, ibrutinib has proven to be a much longed-for treatment option. In a cohort of 144 R/R del(17p) patients, the investigator-assessed ORR was 86%, while in a cohort of 33 TN and 15 R/R *TP53* mutated patients, the objective response rates were as high as 97% and 80%, respectively [39, 40]. With long-term follow-up for this high-risk group, the 5-year PFS was 74% in the TN group and 10% among R/R patients, with OS of 85% and 54%, respectively [41] (Table 2).

Based on real-world data outside of clinical trials, the efficacy of ibrutinib for *TP53*-mutated patients has also been confirmed [42]. In a Swedish study including 95 R/R CLL patients treated with ibrutinib, the investigator-assessed ORR was 80%, with 61% of the cohort representing patients with del(17p)/*TP53* disruption [43].

Ongoing studies comparing standard chemoimmunotherapy versus ibrutinib in various combinations [44] are expected to further impact treatment guidelines in coming years, while the introduction of ibrutinib has already led to a shift of paradigm for treatment of CLL.

Although not a lot of clinical data are available yet, acalabrutinib is probably getting close to clinical use in CLL as well, as evidence from ongoing clinical trials is evolving. In an uncontrolled, phase 1–2, multicenter study including 61 R/R CLL patients treated with single-agent acalabrutinib, the ORR was 95%, while the remaining 5% of patients had stable disease. Among patients with del(17p), the ORR was 100%, providing another treatment option for this high-risk patient group as well [33]. Currently, two phase 3 studies with acalabrutinib single-agent treatment in TN and R/R CLL patients have completed recruitment with results expected to be presented in 2019 [45, 46].

5.1.2 Mantle Cell Lymphoma

Both ibrutinib and acalabrutinib were initially approved for MCL by the FDA. The approval of Ibrutinib was based on results from a phase 2 trial including 111 patients with R/R MCL treated with 560 mg daily. Here the ORR was 68%, with rates of complete remission (CR) of 21% and partial remission (PR) of 47%. The estimated median PFS was 13.9 months, indicating that a persisting response similar to CLL could not be obtained in MCL, while the median OS was still not reached [7]. In a phase 2 study including 124 R/R MCL patients treated with acalabrutinib, the ORR was 81% with CR rates of 40%. Here the estimated median PFS and OS were not reached, while the 12-month rates were 67% and 87%, respectively [9]. Combinations of BTK inhibitors with other novel agents have also been shown to be efficacious in MCL. In a recently published study including 24 R/R patients treated with a combination of ibrutinib and venetoclax, CR rates were as high as 71% [47]. Ongoing studies are exploring the effects of BTK inhibition in combination with CD20 antibodies, other targeted agents, and immunomodulatory drugs; thus combination treatments including BTK inhibitors are likely candidates for further improved treatment in MCL [48].

5.1.3 Marginal Zone Lymphoma

In this more indolent B-cell neoplasia arising from post-germinal center marginal zone B cells, inhibition of BTK by ibrutinib has also been shown to be efficacious. In a multicenter, phase 2 study including 63 previously treated patients, the ORR was 48% and the median PFS was 14.2 months [25]. Here ibrutinib may provide a treatment option for patients unfit for chemotherapy, rituximab, or splenectomy as well as for patients in the R/R setting.

5.1.4 Lymphoplasmacytic Lymphoma/Waldenström Macroglobulinemia

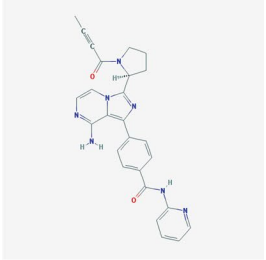
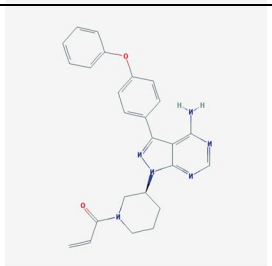
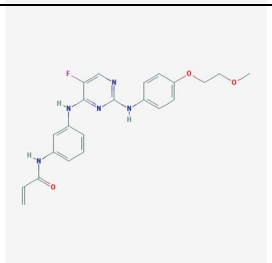
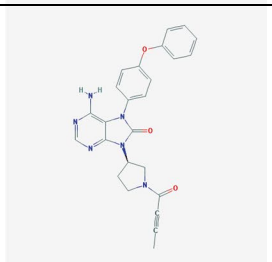
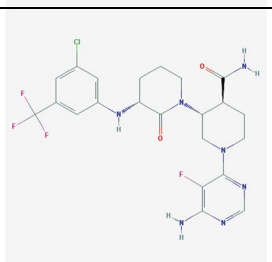
In LPL/WM, several studies have demonstrated high efficacy of BTK inhibition in previously treated and rituximab-resistant patients. Among 63 symptomatic, previously treated patients, IgM levels decreased while hemoglobin increased significantly upon ibrutinib treatment. In the analyses of bone marrow infiltration, a significant reduction from 60 to 25% median infiltration was demonstrated [49]. In the INNOVATE study including 31 rituximab-resistant LPL patients who were treated with ibrutinib until disease progression or unacceptable toxicity, the overall and major response rates were 84% and 65%, respectively. The median IgM level declined by more than 50% at the end of cycle 1 [24, 50].

5.2 Other Approaches

5.2.1 Chronic GvHD

In preclinical models, ibrutinib reduces the severity of cGvHD, which is proposedly mediated through effects on interleukin-2–inducible T-cell kinase (ITK) in T cells, which do not express BTK [26]. Other indirect effects of ibrutinib on interaction between immune effector cells may also contribute to the effect of ibrutinib in CGvHD. Efficacy of ibrutinib has been demonstrated in a multicenter open-label

Table 2 Bruton's tyrosine kinase inhibitors' molecular structure and IC50

Drug	Molecular structure	Mechanism of action	IC_{50} value (nM)
Acalabrutinib		Irreversible binding	3
Ibrutinib		Irreversible binding	0.5
Spebrutinib		Irreversible binding	< 0.5
Tirabrutinib		Reversible binding	2.2
Vecabrutinib		Reversible, function against C418S	2.9
Zanabrutinib	Not available	Irreversible binding	Not available

study evaluating the efficacy of ibrutinib in patients with inadequate response to corticosteroid-containing therapies. Among the 42 patients included, the ORR was 67%, with 71% of responders showing sustained response with a median corticosteroid dose decrease from 0.29 to 0.12 mg/kg per day. Based on these results, ibrutinib was approved by the FDA for treatment of adult patients with cGvHD after failure of one or more lines of systemic therapy [22].

6 Perspectives

Inhibition of BTK has proven to be a highly efficient treatment option for numerous B-cell malignancies. Several pre-clinical and clinical studies support the efficacy of BTK inhibitors as monotherapy and/or as combination therapy with other targeted agents, monoclonal antibodies, and chemotherapy in CLL, DLBCL, FL, MM, MCL, and WM

(Table 1). The potential expansion of indications for BTK inhibitors based on results from preclinical studies leading to trials in other hematological malignancies, immune disorders, and solid tumors broadens the perspectives for use of BTK inhibitors. Furthermore, trials combining BTK inhibitors with either chemoimmunotherapy or other targeted agents may open the way for a cure in B-lymphoproliferative malignancies.

The appearance of acquired resistance to ibrutinib in several patients due to mutations in BTK (Cys481 required for binding of ibrutinib to the kinase activation site) or in PLCgamma2 downstream of BTK emphasizes the on-target mechanism of action [14, 51, 52]. In a study including 84 CLL patients treated with single-agent ibrutinib, mutations in BTK (Cys481) and/or PLCG2 were found in 11% of patients, all of whom progressed. In retrospective analyses of samples, mutations could be detected up to 15 months prior to clinical progression [53]. Vecabrutinib (SNS-062), a noncovalent BTK inhibitor in development for B-cell malignancies with inhibitory activity towards BTK with C481S mutation, may hold promise for further treatment options for patients with acquired resistance to ibrutinib, thus addressing a current unmet need [54]. Another treatment option for patients progressing on ibrutinib is the BCL-2 inhibitor venetoclax. In this setting, a study including 91 CLL patients demonstrated an ORR of 59%. Despite limited follow-up, the durability of response to venetoclax after ibrutinib failure seemed somehow limited, thus for eligible patients posing the option as a bridge to allogeneic stem cell transplantation [55].

Improved efficacy with a longer response duration may be obtained through the combination of BTK inhibitors with other targeted agents. A recent phase 2 study including 24 R/R MCL patients treated with ibrutinib in combination with venetoclax demonstrated a CR rate of 71%, with 67% of patients obtaining bone marrow MRD negativity [47]. The first interim data from the Vision study on the combination of ibrutinib and venetoclax in patients with R/R CLL demonstrated 100% ORR already after 3 months of treatment, with a 56% clinical CR rate [56]. Another study including 50 patients with R/R CLL having either a short response duration or del(17p) reported similar promising results [57]. Another strategy for improvement of response duration is debulking with chemotherapy followed by combinations of targeted therapy; this is currently being tested by the German CLL study group [58].

Currently, BTK inhibitors are administered until progression or toxicity. However, in ongoing trials MRD-guided discontinuation of therapy with combination regimens including BTK inhibitors are being tested [56, 57]. These approaches likely represent the next shift in paradigm from the current indefinite treatment length towards time-limited regimens. Results from a number of on-going trials (FLAIR, VISION, CLARITY, GAIA) are awaited before such strategies can be translated into clinical practice [44, 57, 59].

Other options include the combination of BTK inhibitors with standard chemoimmunotherapy regimens such as fludarabine, cyclofosfamide, and rituximab (FCR) or bendamustine and rituximab (BR). In a randomized trial comparing ibrutinib or placebo combined with BR, the PFS was significantly improved in the ibrutinib group with a hazard ratio of 0.203 versus the placebo group [60, 61]. For 35 patients treated with a combination of ibrutininb and FCR, the ORR was 100%, with 39% obtaining CR and bone marrow MRD negativity in 39% of cases, which is superior to historic results with FCR [62].

The integration of clinical studies with translational studies allowing for subgroup analyses based on functional and genetic characterization of malignant B cells is crucial in investigating which patients will benefit the most from which drugs and drug combinations. Application of next-generation sequencing for concomitant assessment of MRD and subclonal development based on recurrent mutations will play a key role in this process. Moreover, the development of predictive in vitro screening methods for prediction of in vivo drug efficacy will allow for further personalization of treatment for lymphoproliferative malignancies [63]. This combination of highly effective targeted regimens with pretreatment in vitro drug-sensitivity testing and on-treatment MRD testing + assessment of subclonal development is expected to provide for individualized treatment resulting in long-lasting deep remissions, or even to provide a cure for these previously incurable diseases.

Compliance with Ethical Standards

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