Inhibition of the Bruton Tyrosine Kinase Pathway in B-Cell Lymphoproliferative Disorders

Jorge J. Castillo, MD, Steven P. Treon, MD, PhD,† and Matthew S. Davids, MD‡*

Abstract: Activation of the Bruton tyrosine kinase (BTK) pathway plays an important role in the pathophysiology of a number of B-cell lymphoproliferative disorders (LPDs). A number of preclinical studies support inhibiting BTK as a mechanism to treat LPDs. Clinically, BTK inhibitors, specifically ibrutinib, have shown to be safe and effective on treating patients with indolent B-cell lymphomas and chronic lymphocytic leukemia (CLL). Ibrutinib has recently gained approval for the treatment of patients with mantle cell lymphoma, Waldenström macroglobulinemia, and CLL. Ongoing clinical trials are investigating ibrutinib and other BTK inhibitors alone or in combination for the treatment of mantle cell lymphoma, Waldenström macroglobulinemia, CLL, activated B-cell–type diffuse large B-cell lymphoma, and follicular lymphoma, among others. The objective of this review is to succinctly summarize the current body of evidence on BTK inhibition in patients with B-cell LPDs.

Key Words: Bruton tyrosine kinase, chronic lymphocytic leukemia, ibrutinib, mantle cell lymphoma, Waldenström macroglobulinemia

(*Cancer J* 2016;22: 34–39)

The B-cell receptor (BCR) pathway is critical for the development, maturation, and survival of normal B cells.[1] In lymphoid malignancies, chronicx antigenic stimulation, autostimulatory activation, and/or antigen-independent signaling can activate the BCR pathway and promote malignant cell survival.[2]

Binding of antigen, self or foreign, to the extracellular part of the BCR activates kinases that subsequently phosphorylate immunoreceptor tyrosine–based activation motifs.[3] Immunoreceptor tyrosine–based activation motifs are crucial for intracellular signaling initiation mediated through LYN, FYN, and BLK. LYN then phosphorylates SYK, a nonreceptor tyrosine kinase, which amplifies BCR signaling.[4] Two parallel but probably interrelated pathways are then activated, the phosphatidylinositol 3 kinase (PI3K) and the Bruton tyrosine kinase (BTK) pathways. PI3K activates the mammalian target of rapamycin, a cell cycle regulator.[5] Bruton tyrosine kinase activates nuclear factor κB (NF-κB), favoring cell survival and proliferation by up-regulating, among others, BCL-2.[6]

Several components of the BCR pathway have been described and are currently used as therapeutic targets. In this review, we will specifically focus on the BTK pathway and mechanisms of BTK activation in non-Hodgkin lymphoma (NHL), Waldenström macroglobulinemia (WM), and chronic lymphocytic leukemia (CLL), as well as the clinical experience with the BTK inhibitor ibrutinib.

From the Bing Center for Waldenström's Macroglobulinemia and Division of Hematologic Malignancies, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.

The authors have disclosed that they have no signif icant relationships with, or financial interest in, any commercial companies pertaining to this article.

Correspondence to: Jorge J. Castillo, MD, 450 Brookline Ave, Boston, MA 02215 (e-mail: jorgej_castillo@dfci.harvard.edu).

ISSN: 1528-9117

THE BTK PATHWAY

Bruton tyrosine kinase is a cytoplasmic protein that belongs to the TEC family of kinases and is predominantly expressed on B cells, marrow-derived stem cells, and developing myeloid cells.[7] Bruton tyrosine kinase, however, does not seem to be expressed in plasma cells or T cells.[8] Loss of function of BTK translates into maturation inhibition of B cells with subsequent lack of production of immunoglobulins. Individuals with X-linked agammaglobulinemia, the first primary immunodeficiency state ever described, lack BTK function, which manifests as panhypogammaglobulinemia and an increased risk of developing viral, bacterial, and parasitic infections.

As a cytoplasmic protein, the activation of BTK starts at the level of the plasma membrane. Following BCR activation, PI3K is phosphorylated and generates PIP3, which in turn will recruit BTK. Bruton tyrosine kinase then is phosphorylated at the site Y551 by LYN, FYN, and BLK, leading to autophosphorylation at the site Y223. Bruton tyrosine kinase then phosphorylates phospholipase C γ2 (PLC-γ2), which activates protein kinase Cβ, and finally NF-κB. Nuclear factor κB would then induce transcription of the BKT gene and inhibit apoptosis. Bruton tyrosine kinase, however, plays also a dual function as it can mediate apoptosis through p38 mitogen-activated protein kinase. A detailed review on the BCR activation pathway has been published.[9] A schematic representation of the BTK pathway is shown in Figure 1.

BTK IN NHL

Mantle Cell Lymphoma

The BCR repertoire appears biased in mantle cell lymphoma (MCL), which strongly suggests a role for chronic antigenic stimulation in MCL pathogenesis.[10] Further evidence of the important role of BCR signaling in MCL is suggested by the constitutive activation of upstream components of the pathway such as LYN, SYK, and PKC-β.[11] In addition, MCL cells show activation of NF-κB and AKT. Specifically, BTK is strongly expressed in MCL cells. Exposure of primary MCL cells or cell lines to ibrutinib has resulted in decreased adhesion and migration.[12] In a phase I clinical trial, approximately 80% of patients with MCL experienced a response to ibrutinib.[13]

These findings prompted a phase II clinical trial of ibrutinib in patients with relapsed and/or refractory MCL.[14] A total of 111 patients were exposed to ibrutinib 560 mg orally (PO) daily. The median age at initiation of therapy was 68 years, and 86% of patients had intermediate- or high-risk disease. The overall response rate was 68%; 21% obtained complete response, and 47% partial response. The median time to response was 2 months. Similar response rates occurred regardless of age, sex, race, performance status, tumor bulk, number of prior therapies, prognostic score, or previous exposure to lenalidomide or bortezomib. Approximately one-third of patients in this study experienced transient lymphocytosis (>50% increase in absolute lymphocyte count and >5000 lymphocytes/mm^3) with a peak at 4 weeks with return to baseline during cycle 4 or 5. With a median follow-up of 15 months, the median progression-free survival (PFS) was

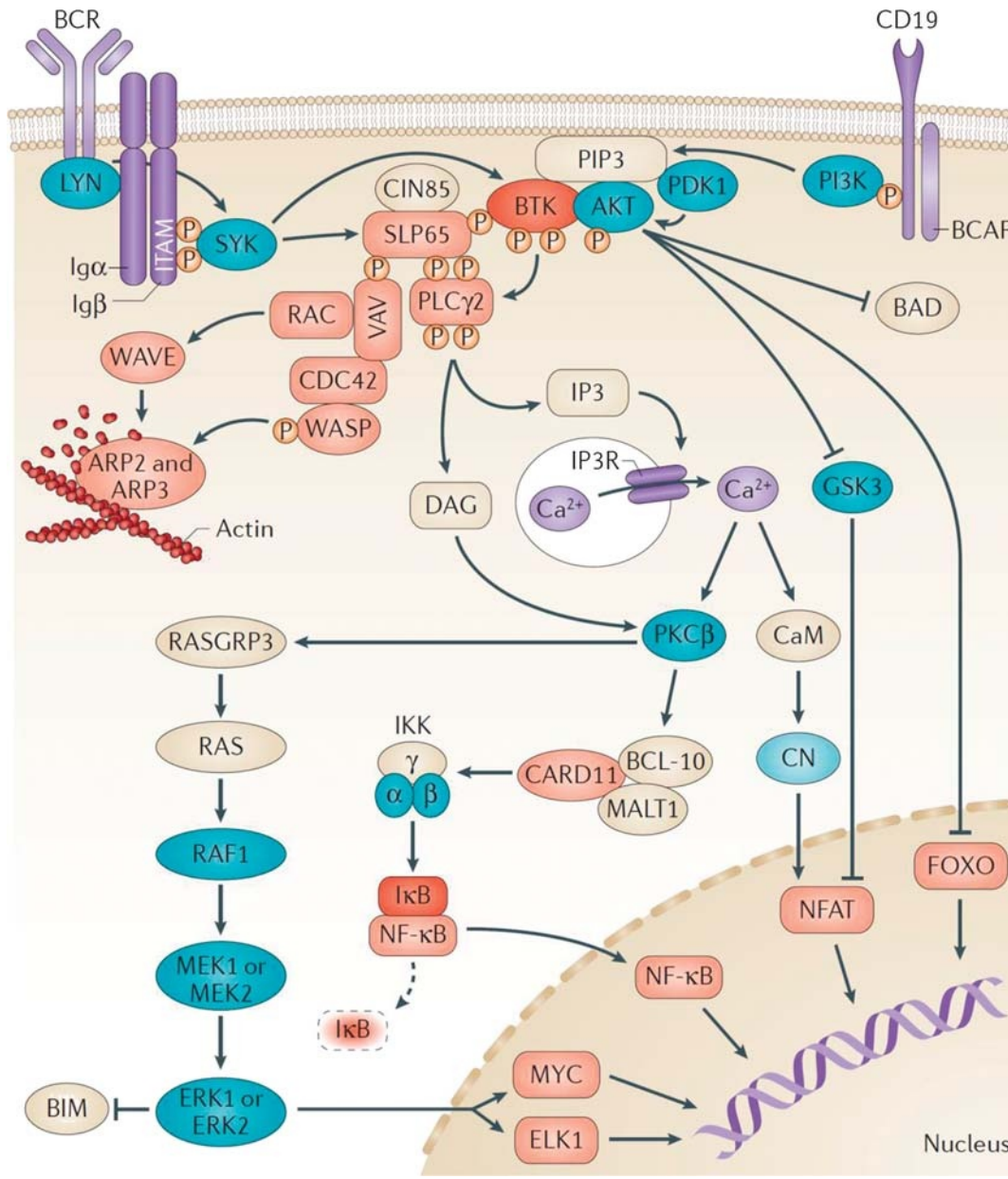

FIGURE 1. Involvement of BTK in B cell receptor signaling.

14 months, and the overall survival (OS) rate was 58% at 18 months. Grades 3 and 4 adverse events included neutropenia (16%), thrombocytopenia (11%), diarrhea (6%), fatigue (5%), and abdominal pain (5%).

Based on these results, on November 13, 2013, the US Food and Drug Administration (FDA) approved ibrutinib in patients with relapsed and/or refractory MCL who had received at least 1 prior line of therapy. Selected ongoing studies evaluating ibrutinib in patients with MCL are shown in Table 1.

Diffuse Large B-Cell Lymphoma

Activated B-cell–type (ABC) diffuse large B-cell lymphoma (DLBCL) cells are heavily dependent on NF-κB signaling for their survival and proliferation.[15] Activated B-cell–type DLBCL cells not only express high levels of NF-κB but also have demonstrated an increased sensitivity of NF-κB inhibition, which can be explained by chronic BCR signaling. CARD11 and MYD88 are mediators of NF-κB signaling, and mutations in these genes can be seen in approximately 50% and 30% of patients with ABC-DLBCL, respectively.[16,17]

In a phase I study, 2 of 6 patients with DLBCL experienced partial response to single-agent ibrutinib.[13] In a phase IB clinical trial, 33 patients with $CD20^+$ NHL were treated with ibrutinib at a dose of 560 mg PO daily in combination with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP).[18] Of the 18 patients with DLBCL treated with the combination, all exhibited a response to therapy. Specifically, 70% and 100% of patients with GC-DLBCL and ABC-DLBCL had complete response. There seemed to be no pharmacokinetic interaction between ibrutinib and R-CHOP, with greater than 90% BTK occupancy at the recommended dose of 560 mg. Another phase

TABLE 1. Selected Ongoing Studies Evaluating Ibrutinib in Patients With B-Cell NHL

Identifier	Indication	Phase	Approach
NCT01880567	MCL	II	Ibrutinib and rituximab
NCT02242097	MCL	II	Ibrutinib maintenance after intensive induction therapy
NCT02269085	MCL	II	Ibrutinib and carfilzomib
NCT02460276	MCL	II	Ibrutinib, lenalidomide, and rituximab (PHILEMON)
NCT02471391	MCL	II	Ibrutinib and ABT-199 (BCL-2 inhibitor) (AIM)
NCT02558816	MCL	II	Ibrutinib and obinutuzumab (OASIS)
NCT02356458	MCL	I/II	Ibrutinib and bortezomib followed by ibrutinib maintenance
NCT01974440	iNHL	III	Bendamustine/rituximab or R-CHOP with and without ibrutinib
NCT01955499	iNHL	II	Ibrutinib and lenalidomide
NCT02451111	FL	II	Rituximab with and without ibrutinib
NCT01855750	ABC-DLBCL	III	R-CHOP with and without ibrutinib
NCT02142049	DLBCL	I/II	Ibrutinib, lenalidomide, and DA-R-EPOCH
NCT02219737	DLBCL	I	Ibrutinib and ICE
NCT02165397	WM	III	Rituximab with and without ibrutinib (INNOVATE)

ABC indicates activated B cell; DA-R-EPOCH, dose-adjusted rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin; FL, follicular lymphoma; ICE, ifosfamide, carboplatin, and etoposide; iNHL, indolent NHL; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone.

II study evaluated the combination of ibrutinib, bendamustine, and rituximab in 48 patients with B-cell NHL.[19] The complete response rate in the 16 patients with DLBCL was 31%. Selected ongoing clinical trials evaluating ibrutinib in DLBCL are shown in Table 1. Ibrutinib is not approved for use in patients with DLBCL.

BTK IN WM

Through whole-genome sequencing studies, a recurrent mutation in the MYD88 gene was identified in more than 90% of patients meeting clinicopathological criteria for WM.[20] Since then, several research groups have confirmed the presence of the MYD88 L265P gene mutation in patients with WM, with prevalence rates ranging from 80% to 100%.[21–23] Approximately a third of patients with WM also carry activating mutations in the C-terminal domain of the CXCR4 gene.[24] Patients with nonsense (truncating mutations in the C-terminal domain) present with a higher burden of bone marrow disease and higher levels of serum immunoglobulin M (IgM), including symptomatic hyperviscosity.[25] Preclinical studies have shown that the MYD88 L265P gene mutation triggers BTK activation that leads to NF-κB–dependent growth and survival in WM cells,[26] whereas CXCR4 mutations confer drug resistance, including the BTK inhibitor ibrutinib.[27] A schematic representation of BTK involvement in Toll-like receptor (MYD88) activation is shown in Figure 2.

These findings prompted the design and execution of a phase II clinical trial on ibrutinib in patients with relapsed and/or refractory WM.[28] In that study, 63 patients with symptomatic WM who had received at least 1 previous treatment received ibrutinib 420 mg PO daily until disease progression or unacceptable toxicity. At the time of best response, 10 patients achieved a very good partial response (IgM decrease >90% with residual M-spike in serum protein electrophoresis), 36 achieved partial response (IgM decrease >50% but <90%), and 11 minor response (IgM decrease between 25% and 50%). The median time to at least a minor response was 4 weeks. The overall response rates were similar regardless of age, performance status, International Prognostic Scale System for Waldenstrom macroglobulinemia score, β2-microglobulin level, hemoglobin level, IgM level, bone marrow involvement, or previous number of regimens. Response rates to ibrutinib, however, differed based on genomic profile. Updated response data showed that patients who carried the MYD88 mutation alone experienced a major response rate (at least partial response) of 92% compared with 62% in patients with MYD88 and CXCR4 mutations (double mutant) and in none of the patients who were wild type for both MYD88 and CXCR4.[29]

After a median duration of treatment of 19 months, approximately 70% of patients continue on active therapy. Half of the patients stopped therapy because of nonresponse, progression, or toxicity. The most common grade 3 or 4 adverse events were neutropenia (15%) and thrombocytopenia (13%). Other grade 3 adverse events included anemia, atrial fibrillation, pneumonia, herpes zoster, endocarditis, subcutaneous abscess, urinary tract infection, hematoma, and syncope (2% each).

Based on these data, on January 29, 2015, the US FDA approved ibrutinib for the treatment of patients with symptomatic WM. On July 10, 2015, the European Commission granted marketing authorization for ibrutinib in all 28 members of the European Union for WM patients who had received at least 1 prior line of treatment or in untreated WM patients otherwise not suitable for chemoimmunotherapy. A randomized study evaluating rituximab +/− ibrutinib in untreated and relapsed patients with WM is ongoing (Table 1).

BTK IN CLL

Bruton tyrosine kinase is fundamental to the pathophysiology of CLL cells because it serves as one of the main nodes in the BCR pathway, which is stimulated by the stromal microenvironment to promote CLL cell survival. Lymph node–derived CLL cells have a gene expression profile similar to activated B cells that leads to increased proliferation compared with matched peripheral blood samples from the same patients.[30] In addition to its prosurvival role, BCR signaling also facilitates CLL cell trafficking within the microenvironment.[31] Tonic signaling through the BCR has been described, which may further contribute to its functional importance in the CLL cell.[32] Interestingly, approximately one-third of CLL patients have a stereotyped BCR, which is associated with distinct clinical characteristics and may respond differently to antigen than nonstereotyped BCRs.[33]

An important early observation when BTK and other BCR kinase inhibitors were first given to CLL patients was that the

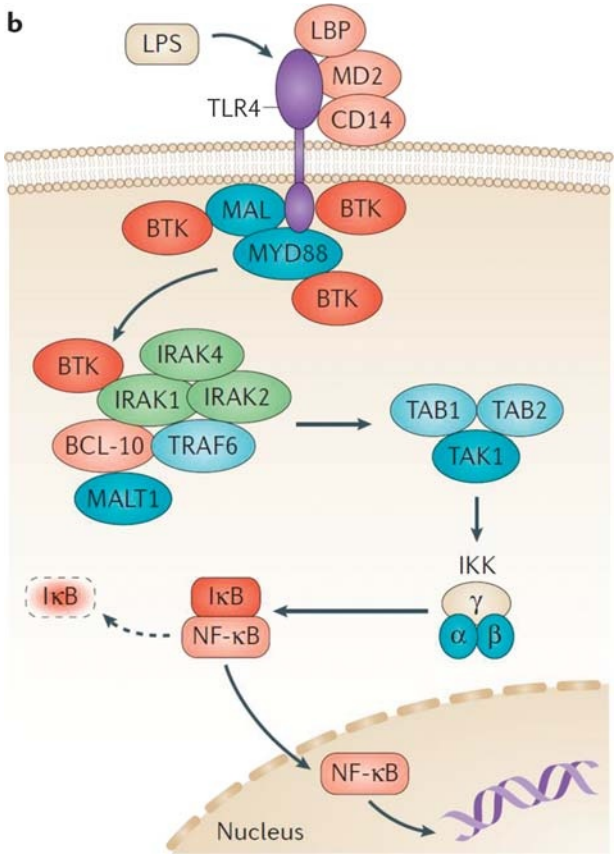

FIGURE 2. Involvement of BTK in Toll-like receptor signaling.

majority experienced an initial rise in their lymphocyte count in the setting of lymph node shrinkage. This "lymphocyte redistribution" phenomenon is thought to result from the release of CLL cells into the peripheral blood from the microenvironmental niches such as the bone marrow and lymph nodes. Lymphocyte redistribution typically occurs within days of initial dosing of ibrutinib and resolves for most patients within a few months, although some patients have persistent lymphocytosis for a year or more.[34] Because patients with lymphocytosis on ibrutinib usually have clear signs of disease response and clinical benefit, these patients are best classified as having achieved a nodal response with lymphocytosis.[35] It appears that these patients with lymphocytosis have equally good outcomes as do those who do not experience it.[36]

The clinical benefit of ibrutinib in CLL was first observed as part of the phase I study in lymphoid malignancies and prompted a CLL/small lymphocytic lymphoma-specific phase IB/2A trial that enrolled both patients with relapsed refractory disease as well as a small cohort of older patients with previously untreated disease. In the relapsed/refractory cohort, 85 patients with CLL/SLL were enrolled and received either 420 mg (n = 51) or 840 mg (n = 34) daily on a continuous schedule until time of progression or unacceptable toxicity.[37] The patient population was enriched for high-risk prognostic markers, and most patients had several lines of prior therapy. The overall response rate based on standard International Workshop on-CLL criteria was 71% (including 2 complete responses). When including the additional patients with nodal response with lymphocytosis, approximately 88% of patients achieved clinical benefit. The response rate was equivalent in patients with high-risk del(17p) and unmutated Immunoglobulin heavy chain variable region gene CLL. A recent update of this data set was published and suggested that these responses were durable for most patients, with an estimated PFS of 69% at 30 months of follow-up.[34] The only subgroup thus far with clearly suboptimal PFS is the del(17p) population, where the median PFS is only 28 months.

Several of the del(17p) patients who progressed were subsequently found to have C481S mutations in the BTK gene, and 2 patients had mutations in PLC-γ2 that were thought to directly lead to treatment resistance.[38] Based largely on the data from this study, the FDA granted accelerated approval for ibrutinib for relapsed/refractory CLL on February 12, 2014.

The benefits of ibrutinib in the relapsed/refractory CLL population were subsequently confirmed in the RESONATE trial, a randomized phase III study of ibrutinib versus the anti-CD20 monoclonal antibody ofatumumab.[39] In addition to a dramatic improvement in PFS for ibrutinib versus ofatumumab (not reached vs 8.1 months), ibrutinib also strikingly showed an OS benefit, with a 12-month OS of 90% versus 81%, despite the fact that crossover from the ofatumumab to the ibrutinib arm was allowed. The data from this study led to the full FDA approval of ibrutinib for CLL on July 28, 2014. Based on a high response rate in del (17p) and the paucity of effective treatment options for this

TABLE 2. Selected Ongoing Studies Evaluating Ibrutinib in Patients With CLL

Identifier	Indication	Phase	Approach
NCT01886872	Untreated	III	Bendamustine and rituximab vs rituximab and ibrutinib vs ibrutinib alone
NCT02048813	Untreated	III	FCR vs ibrutinib and rituximab
NCT02301156	R/R, high-risk	III	Ibrutinib and ublituximab (novel anti-CD20 monoclonal antibody) vs ibrutinib alone
NCT02427451	Untreated and R/R	II	Ibrutinib, obinutuzumab and ABT-199 (BCL-2 inhibitor)
NCT02420912	Untreated, high-risk	II	Ibrutinib and nivolumab (anti-PD-1 monoclonal antibody)
NCT02537613	R/R	II	Ibrutinib and obinutuzumab
NCT02268851	R/R	II	Ibrutinib and TGR-1202 (PI3K inhibitor)
NCT02160015	R/R	II	Ibrutinib, lenalidomide, and rituximab
NCT02007004	R/R	II	Ibrutinib and rituximab vs ibrutinib alone
NCT01886859	R/R	II	Ibrutinib and lenalidomide

PI3K indicates phosphatidylinositol-3 kinase; R/R, relapsed and/or refractory.

subgroup, approval was also given for frontline therapy of del (17p) CLL patients.

The 31 patients with previously untreated elderly CLL/SLL who received ibrutinib as initial therapy were recently reported.[40] Twenty-two (71%) of 31 patients achieved an objective response, including 4 patients (13%) with a CR and 1 patient (3%) with a nodular PR. An additional 4 patients (13%) achieved a partial response with lymphocytosis. The promising efficacy and safety of ibrutinib in this population have raised the question of whether this should be the preferred option for initial therapy for most elderly patients with CLL. This question will be addressed by the recently completed large, randomized phase III RESONATE-2 study of ibrutinib versus chlorambucil (NCT01722487), which if positive may establish ibrutinib as a new standard of care for frontline CLL therapy in the elderly. Other key ongoing randomized studies with ibrutinib in the frontline setting include a phase III Alliance randomized study of BR versus ibrutinib versus ibrutinib-R (NCT01886872) and an Eastern Cooperative Oncology Group randomized study of FCR (fludarabine, cyclophosphamide, rituximab) versus ibrutinib-R (NCT02048813).

Although highly effective as monotherapy, ibrutinib alone rarely leads to CR in CLL. Therefore, numerous combination strategies are currently being explored. Given the large number of these combination studies, a detailed description of each is beyond the scope of this review. However, broadly speaking, combinations are being explored in 3 general categories: ibrutinib with chemotherapy, ibrutinib with antibodies, and ibrutinib with other novel agents. In the ibrutinib with chemotherapy category, a recently published phase IB study found that ibrutinib plus bendamustine/rituximab was safe and had promising efficacy in relapsed/refractory CLL.[41] An ongoing study is exploring the combination of ibrutinib with FCR for the upfront treatment of younger, fit patients with CLL (NCT02251548). In the ibrutinib plus antibody category, data have recently been published on ibrutinib + rituximab[42] and ibrutinib + ofatumumab,[43] with another trial recently opening of ibrutinib + obinutuzumab (NCT02537613). Ongoing novel-agent-only combination studies include a trial exploring dual BCR blockade with ibrutinib + the delta-isoform PI3K inhibitor TGR-1202 (NCT02268851) and a triple combination strategy of ibrutinib + obinutuzumab + venetoclax (NCT02427451). The large number of ongoing ibrutinib combination studies will provide crucial data to help inform our approach to the management of individual CLL patients with ibrutinib-based regimens in the future (Table 2).

FUTURE DIRECTIONS

Modulation of the BCR through BTK inhibition has the potential of becoming paramount for the treatment of B-cell disorders. Ibrutinib has shown to be safe and effective in several prospective studies in patients with CLL, MCL, WM, and others. Several aspects of ibrutinib therapy make it a highly desirable option for patients with B-cell lymphoproliferative disorders (LPDs). These include oral administration of the drug, favorable safety profile, and not only single-agent activity but also ease of combination with other chemotherapeutic and/or targeted agents.

The use of ibrutinib as a single agent, however, has not translated into a cure. Specifically in WM, not 1 patient had obtained a CR while undergoing therapy with ibrutinib. Therefore, combination regimens are warranted to deepen responses and also prolong PFS and/or OS. A number of studies combining ibrutinib with chemotherapeutic agents are ongoing and are already showing early evidence of efficacy without concerning safety issues. The design of such studies, however, would need to define the role of BTK inhibition. Concurrent versus sequential regimens are currently being evaluated in clinical trials. Perhaps the most interesting approach would be to combine BTK inhibitors with other orally administered molecularly targeted agents such as PI3K or BCL2 inhibitors, among others. Again, preclinical data are encouraging, but prospective clinical trials are necessary to further evaluate the safety of such combinations.

Besides improving the outcome of patients with B-cell LPDs, combinations containing BTK inhibitors could potentially consider stopping therapy in the future. As it stands now, BTK inhibition is indefinite, until progression of disease or unacceptable toxicity, based on the design of the studies that supported the approval of ibrutinib. Stopping BTK inhibition is not only appealing from the perspective of patients not taking pills indefinitely, but also to decrease the financial burden in our already overwhelmed medical system. At a cost of approximately $100,000 per year of therapy, ibrutinib is among the most expensive medications currently marketed in the United States and Europe. Given the costs associated with ibrutinib therapy, its use would have to be rational and thoughtful. The use of a genomic-based treatment approach such as the use of MYD88 and CXCR4 mutation status in WM could help delineate more appropriate candidates for ibrutinib therapy. This is paramount in the context of a number of novel oral agents coming up to the market at high prices.

Further research is needed to understand the biological basis for the adverse events associated with ibrutinib therapy such as bleeding and the increased risk of atrial fibrillation. In addition, resistance mechanisms would have to be elucidated. Resistance to ibrutinib appears heterogeneous among different B-cell processes and ranges from BTK and PLC-γ2 mutations in CLL to CDK4 overexpression in MCL to CXCR4 mutations in WM. Understanding these mechanisms would help not only improving our selection of patients for ibrutinib therapy but also designing studies focused on those patients who are less likely to benefit from BTK inhibition.

Newer oral BTK inhibitors have entered clinical trials. CC-292 (Celgene) has reported PR rates ranging from 31% to 67% in patients with relapsed and/or refractory CLL/SLL (Brown ASH 2014). ONO-4059 (Ono Pharma) has shown overall response rate (ORR) of 84% in relapsed/refractory CLL with 89% ORR in patients with del17p (Salles ASH 2013). ORR of 42% was reported in patients with iNHL with ORR of 52% in MCL (Rule ASH 2013). ACP-196 (Acerta Pharma) has also entered clinical trials for CLL and indolent NHL. Notably, a randomized study of ACP-196 versus ibrutinib in previously treated patients with high-risk CLL is underway (NCT02477696).

Overall, BTK inhibition is here to stay as it has already shown unprecedented efficacy with a manageable safety profile. The careful design of prospective clinical trials would further define the role of BTK inhibition in patients with B-cell LPDs.

REFERENCES

1. Young RM, Staudt LM. Targeting pathological B cell receptor signalling in lymphoid malignancies. *Nat Rev Drug Discov*. 2013;12: 229–243.
2. Niemann CU, Wiestner A. B-cell receptor signaling as a driver of lymphoma development and evolution. *Semin Cancer Biol*. 2013;23: 410–421.
3. Reth M. Antigen receptor tail clue. *Nature*. 1989;338: 383–384.
4. Liu W, Meckel T, Tolar P, et al. Antigen affinity discrimination is an intrinsic function of the B cell receptor. *J Exp Med*. 2010;207: 1095–1111.
5. Castillo JJ, Furman M, Winer ES. CAL-101: a phosphatidylinositol-3-kinase p110-delta inhibitor for the treatment of lymphoid malignancies. *Expert Opin Investig Drugs*. 2012;21: 15–22.
6. Winer ES, Ingham RR, Castillo JJ. PCI-32765: a novel Bruton's tyrosine kinase inhibitor for the treatment of lymphoid malignancies. *Expert Opin Investig Drugs*. 2012;21: 355–361.

7. Mohamed AJ, Yu L, Bäckesjö CM, et al. Bruton's tyrosine kinase (BTK): function, regulation, and transformation with special emphasis on the PH domain. *Immunol Rev*. 2009;228: 58–73.
8. Smith CI, Baskin B, Humire-Greiff P, et al. Expression of Bruton's agammaglobulinemia tyrosine kinase gene, BTK, is selectively down-regulated in T lymphocytes and plasma cells. *J Immunol*. 1994;152: 557–565.
9. Hendriks RW, Yuvaraj S, Kil LP. Targeting Bruton's tyrosine kinase in B cell malignancies. *Nat Rev Cancer*. 2014;14: 219–232.
10. Hadzidimitriou A, Agathangelidis A, Darzentas N, et al. Is there a role for antigen selection in mantle cell lymphoma? Immunogenetic support from a series of 807 cases. *Blood*. 2011;118: 3088–3095.
11. Rinaldi A, Kwee I, Taborelli M, et al. Genomic and expression profiling identifies the B-cell associated tyrosine kinase Syk as a possible therapeutic target in mantle cell lymphoma. *Br J Haematol*. 2006;132: 303–316.
12. Chang BY, Francesco M, Steggerda SM, et al. Ibrutinib inhibits malignant cell adhesion and migration and reduces tumor burden in lymph node and bone marrow in a murine model of mantle cell dissemination and progression. *Cancer Res*. 2013;73:Asbtract 923.
13. Advani RH, Buggy JJ, Sharman JP, et al. Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. *J Clin Oncol*. 2013;31: 88–94.
14. Wang ML, Rule S, Martin P, et al. Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. *N Engl J Med*. 2013;369: 507–516.
15. Davis RE, Brown KD, Siebenlist U, et al. Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. *J Exp Med*. 2001;194: 1861–1874.
16. Lenz G, Davis RE, Ngo VN, et al. Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. *Science*. 2008;319: 1676–1679.
17. Ngo VN, Young RM, Schmitz R, et al. Oncogenically active MYD88 mutations in human lymphoma. *Nature*. 2011;470: 115–119.
18. Younes A, Thieblemont C, Morschhauser F, et al. Combination of ibrutinib with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) for treatment-naive patients with CD20-positive B-cell non-Hodgkin lymphoma: a non-randomised, phase 1B study. *Lancet Oncol*. 2014;15: 1019–1026.
19. Maddocks K, Christian B, Jaglowski S, et al. A phase 1/1B study of rituximab, bendamustine, and ibrutinib in patients with untreated and relapsed/refractory non-Hodgkin lymphoma. *Blood*. 2015;125: 242–248.
20. Treon SP, Xu L, Yang G, et al. MYD88 L265P somatic mutation in Waldenström's macroglobulinemia. *N Engl J Med*. 2012;367: 826–833.
21. Jiménez C, Sebastián E, Chillón MC, et al. MYD88 L265P is a marker highly characteristic of, but not restricted to, Waldenström's macroglobulinemia. *Leukemia*. 2013;27: 1722–1728.
22. Poulain S, Roumier C, Decambron A, et al. MYD88 L265P mutation in Waldenstrom macroglobulinemia. *Blood*. 2013;121: 4504–4511.
23. Varettoni M, Arcaini L, Zibellini S, et al. Prevalence and clinical significance of the MYD88 (L265P) somatic mutation in Waldenstrom's macroglobulinemia and related lymphoid neoplasms. *Blood*. 2013;121: 2522–2528.
24. Hunter ZR, Xu L, Yang G, et al. The genomic landscape of Waldenstrom macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell lymphomagenesis. *Blood*. 2014;123: 1637–1646.
25. Treon SP, Cao Y, Xu L, et al. Somatic mutations in MYD88 and CXCR4 are determinants of clinical presentation and overall survival in Waldenstrom macroglobulinemia. *Blood*. 2014;123: 2791–2796.
26. Yang G, Zhou Y, Liu X, et al. A mutation in MYD88 (L265P) supports the survival of lymphoplasmacytic cells by activation of Bruton tyrosine kinase in Waldenström macroglobulinemia. *Blood*. 2013;122: 1222–1232.
27. Cao Y, Hunter ZR, Liu X, et al. The WHIM-like CXCR4(S338X) somatic mutation activates AKT and ERK, and promotes resistance to ibrutinib and other agents used in the treatment of Waldenstrom's macroglobulinemia. *Leukemia*. 2015;29: 169–176.
28. Treon SP, Tripsas CK, Meid K, et al. Ibrutinib in previously treated Waldenström's macroglobulinemia. *N Engl J Med*. 2015;372: 1430–1440.
29. Treon SP, Xu L, Hunter Z. MYD88 mutations and response to ibrutinib in Waldenström's macroglobulinemia. *N Engl J Med*. 2015;373: 584–586.
30. Herishanu Y, Pérez-Galán P, Liu D, et al. The lymph node microenvironment promotes B-cell receptor signaling, NF-kappaB activation, and tumor proliferation in chronic lymphocytic leukemia. *Blood*. 2011;117: 563–574.
31. Davids MS, Burger JA. Cell trafficking in chronic lymphocytic leukemia. *Open J Hematol*. 2012;3: ppi–3.
32. Monroe JG. ITAM-mediated tonic signalling through pre-BCR and BCR complexes. *Nat Rev Immunol*. 2006;6: 283–294.
33. Agathangelidis A, Darzentas N, Hadzidimitriou A, et al. Stereotyped B-cell receptors in one-third of chronic lymphocytic leukemia: a molecular classification with implications for targeted therapies. *Blood*. 2012; 119: 4467–4475.
34. Byrd JC, Furman RR, Coutre SE, et al. Three-year follow-up of treatment-naïve and previously treated patients with CLL and SLL receiving single-agent ibrutinib. *Blood*. 2015;125: 2497–2506.
35. Cheson BD, Byrd JC, Rai KR, et al. Novel targeted agents and the need to refine clinical end points in chronic lymphocytic leukemia. *J Clin Oncol*. 2012;30: 2820–2822.
36. Woyach JA, Smucker K, Smith LL, et al. Prolonged lymphocytosis during ibrutinib therapy is associated with distinct molecular characteristics and does not indicate a suboptimal response to therapy. *Blood*. 2014;123: 1810–1817.
37. Byrd JC, O'Brien S, James DF. Ibrutinib in relapsed chronic lymphocytic leukemia. *N Engl J Med*. 2013;369: 1278–1279.
38. Woyach JA, Furman RR, Liu TM, et al. Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. *N Engl J Med*. 2014;370: 2286–2294.
39. Byrd JC, Brown JR, O'Brien S, et al. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. *N Engl J Med*. 2014;371: 213–223.
40. O'Brien S, Furman RR, Coutre SE, et al. Ibrutinib as initial therapy for elderly patients with chronic lymphocytic leukaemia or small lymphocytic lymphoma: an open-label, multicentre, phase 1B/2 trial. *Lancet Oncol*. 2014;15: 48–58.
41. Brown JR, Barrientos JC, Barr PM, et al. The Bruton tyrosine kinase inhibitor ibrutinib with chemoimmunotherapy in patients with chronic lymphocytic leukemia. *Blood*. 2015;125: 2915–2922.
42. Burger JA, Keating MJ, Wierda WG, et al. Safety and activity of ibrutinib plus rituximab for patients with high-risk chronic lymphocytic leukaemia: a single-arm, phase 2 study. *Lancet Oncol*. 2014;15: 1090–1099.
43. Jaglowski SM, Jones JA, Nagar V, et al. Safety and activity of BTK inhibitor ibrutinib combined with ofatumumab in chronic lymphocytic leukemia: a phase 1B/2 study. *Blood*. 2015;126: 842–850.