Bruton's Tyrosine Kinase Inhibitors in Chronic Lymphocytic Leukemia and Lymphoma

Gaurav Varma, MSPH, Tyler P. Johnson, MD, and Ranjana H. Advani, MD

Mr Varma is a medical student at Drexel University College of Medicine in Philadelphia, Pennsylvania. Dr Johnson is a clinical fellow and Dr Advani is the Saul A. Rosenberg, MD, Professor of Lymphoma and a professor of medicine and oncology in the Division of Oncology of the Department of Medicine at Stanford University Medical Center in Stanford, California.

Corresponding author:
Ranjana H. Advani, MD
Saul A. Rosenberg, MD, Professor of Lymphoma
Stanford Cancer Institute
875 Blake Wilbur Drive
Stanford, CA 94305
Tel: (650) 725-6456
E-mail: radvani@stanford.edu

Abstract: The development of Bruton's tyrosine kinase (BTK) inhibitors and their introduction into clinical practice represent a major advance in the treatment of chronic lymphocytic leukemia (CLL) and other B-cell lymphomas. Although ibrutinib is the only BTK inhibitor that has been approved by the US Food and Drug Administration, several others are under investigation. Ibrutinib is currently approved for use in relapsed/refractory CLL, CLL with 17p deletion (del[17p]), relapsed or refractory mantle cell lymphoma, and Waldenström macroglobulinemia. Although it is clear that ibrutinib has altered treatment paradigms and outcomes in these diseases, several questions remain regarding (1) its role in frontline vs salvage therapy; (2) its use as a single agent vs in combination with biologic agents, other small molecules, or traditional chemoimmunotherapy; (3) the optimal duration of treatment; and (4) the treatment of patients who cannot tolerate or have disease resistant to ibrutinib. Because sparse clinical data are available on other BTK inhibitors, it is unclear at present whether their clinical efficacy and toxicity will differ from those of ibrutinib ***[corrected August 15, 2016].***

Keywords
Bruton's tyrosine kinase, B-cell receptor signaling, chronic lymphocytic leukemia, ibrutinib, non-Hodgkin lymphoma

Introduction

In 1952, Colonel Ogden Bruton identified a child who was unable to mount a humoral immune response and who experienced repeated serious infections.[1] The condition came to be known as X-linked agammaglobulinemia after the condition had been mapped to the X chromosome in 1986. In 1993, the molecular basis of X-linked agammaglobulinemia was identified as a defect in Bruton's tyrosine kinase (BTK), an integral mediator of B-cell receptor (BCR) signaling.[2]

BCR rearrangement is a critical step in B-cell development, and signaling through this pathway plays a central role in B-cell survival, function, and proliferation (Figure).[3] In normal B cells, the BCR displays tonic signaling as well as antigen-dependent signaling. Antigen-

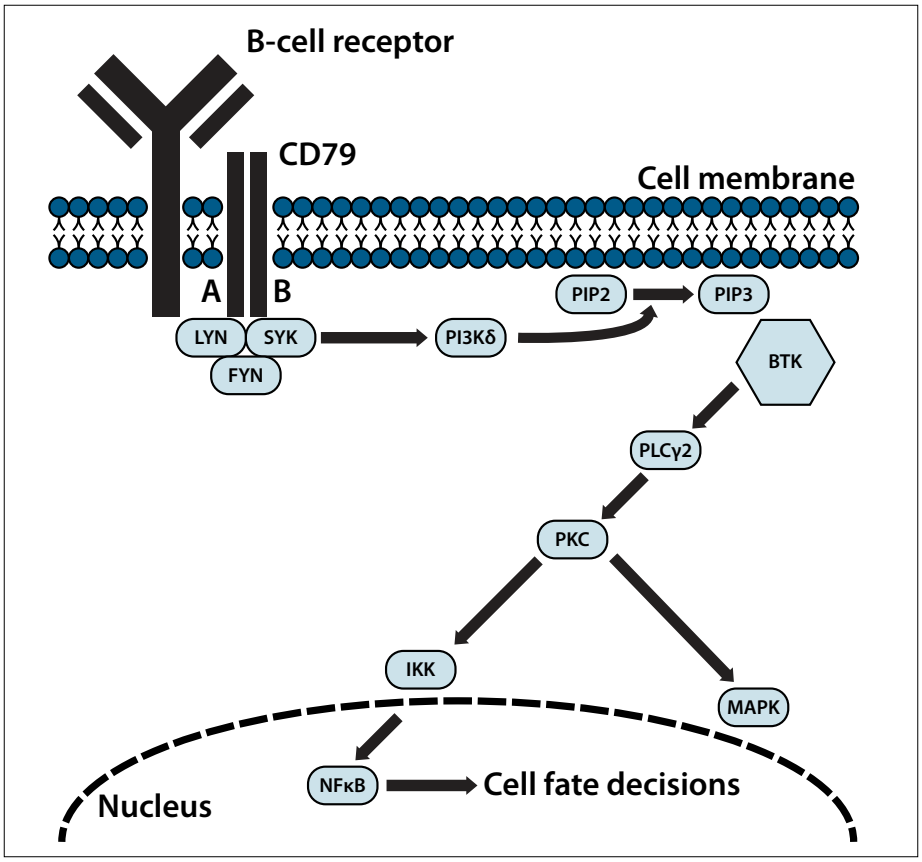

Figure. B-cell receptor signaling pathway. Antigen binding and cross-linking of B-cell receptors initiate a tyrosine kinase–mediated intracellular signaling cascade involving therapeutic targets spleen tyrosine kinase, phosphoinositide 3-kinase δ, and Bruton's tyrosine kinase. This ultimately leads to the upregulation of genes essential for cell activation, survival, and proliferation.

A, BCR adaptor protein CD79a; B, BCR adaptor protein CD79b; BTK, Bruton's tyrosine kinase; IKK, IκB kinase; LYN, Lck/Yes-related novel protein tyrosine kinase; MAPK, mitogen-activated kinase–like protein; NFκB; nuclear factor κB; PI3Kδ, phosphoinositide 3-kinase δ; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; PKC, protein kinase C; PLCγ2, phospholipase C γ2; SYK, spleen tyrosine kinase.

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induced cross-linking of adjacent BCRs results in the phosphorylation of immunotyrosine-based activation motifs on BCR-associated transmembrane proteins CD79a and CD79b by the tyrosine kinases FYN, Lck/Yes-related novel protein tyrosine kinase (LYN), and spleen tyrosine kinase (SYK). SYK further activates phosphoinositide 3-kinase δ (PI3Kδ), catalyzing the conversion of phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphate (PIP3) and recruiting BTK. BTK phosphorylates downstream targets, including phospholipase Cγ2 (PLCγ2). This leads to increased expression of nuclear factor κB (NFκB) and other pathways, promoting increased expression of genes essential to cell activation, proliferation, and survival.[3]

Antigen-independent, aberrant, or tonic signaling of the BCR has been implicated in the pathogenesis of many B-cell malignancies. Given the nonlethal nature of X-linked agammaglobulinemia and the central role of BTK in BCR signaling, several BTK-specific inhibitors have been evaluated in B-cell malignancies, including LFM-A13, ONO/GS-4059, CC-292, acalabrutinib, and ibrutinib (Imbruvica, Pharmacyclics/Janssen).[3] Of these, ibrutinib is the farthest along in development. Herein, we review the key clinical studies of BTK inhibitors in B-cell malignancies. Key phase 3 studies with ibrutinib are listed in Table 1, and results with other BTK inhibitors are listed in Table 2.

Ibrutinib

Ibrutinib is an oral, potent, small-molecule agent that binds to the cysteine 481 residue of BTK and selectively and irreversibly inhibits BTK.[4] In the first phase 1 study of ibrutinib in chronic lymphocytic leukemia (CLL) and other B-cell malignancies, full occupancy of the BTK binding site was seen at doses that were well tolerated and

Table 1. Phase 3 Trials With Ibrutinib

Disease	Regimen	Eligibility	Trial Name (ClinicalTrials.gov)	Status
CLL	IR vs FCR	Untreated, no del(17p)	E1912 (NCT02048813)	Ongoing
	BR vs IR vs ibrutinib	Untreated, ≥65 years, high-intermediate/high- risk	A041202 (NCT01886872)	Ongoing
	Ublituximab + ibrutinib vs ibrutinib	R/R, high-risk	UTX-IB-301 (NCT02301156)	Ongoing
	BR + ibrutinib vs BR + placebo	R/R, ≥1 prior therapy, no del(17p)	HELIOS (NCT01611090)	Active, not recruiting
	Ibrutinib vs ofatumumab	R/R, ≥1 prior therapy	RESONATE (NCT01578707)	Active, not recruiting
	Ibrutinib + obinutuzumab vs chlorambucil + obinutuzumab	Untreated	iLLUMINATE (NCT02264574)	Ongoing
	Ibrutinib vs chlorambucil	Untreated, ≥65 years, no del(17p)	RESONATE-2 (NCT01722487)	Completed
	Ibrutinib vs rituximab	R/R, ≥1 prior therapy, ineligible for purine analogue	BRILLIANCE (NCT01973387)	Ongoing
	Ibrutinib vs acalabrutinib	R/R, ≥1 prior therapy, high-risk	ACE-CL-006 (NCT02477696)	Ongoing
MCL	BR + ibrutinib vs BR + placebo	Untreated, ≥65 years	SHINE (NCT01776840)	Active, not recruiting
	Ibrutinib vs temsirolimus	R/R, ≥1 prior therapy	RAY (NCT01646021)	Active, not recruiting
WM	IR vs rituximab + placebo vs ibrutinib	All WM, no prior ibrutinib	iNNOVATE (NCT02165397)	Ongoing
DLBCL	R-CHOP + ibrutinib vs R-CHOP + placebo	Untreated, stage II-IV, R-IPI >1, non-GCB DLBCL	PHOENIX (NCT01855750)	Ongoing
	BEAMi or CBVi + ASCT + ibrutinib maintenance vs BEAM or CBV + placebo + ASCT + placebo maintenance	R/R, ABC DLBCL or B-cell lymphoma, unclassifiable with features intermediate between DLBCL and Burkitt lymphoma, <4 prior therapies	A051301 (NCT02443077)	Ongoing
FL	BR + ibrutinib vs BR + placebo	R/R, ≥1 prior therapy with anti-CD20	SELENE (NCT01974440)	Ongoing
MCL	BR + ibrutinib vs BR + placebo	R/R, ≥1 prior therapy with anti-CD20	SELENE (NCT01974440)	Ongoing

ABC, activated B cell-like; ASCT, autologous stem cell transplant; BEAM, carmustine, etoposide, cytarabine, and melphalan; BEAMi, carmustine, etoposide, cytarabine, and melphalan plus ibrutinib; BR, bendamustine and rituximab; CBV, cyclophosphamide, carmustine, and etoposide; CBVi, cyclophosphamide, carmustine, and etoposide plus ibrutinib; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; GCB, germinal center B cell-like; IR, ibrutinib and rituximab; MCL, mantle cell lymphoma; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; R-IPI, revised International Prognostic Index; R/R, relapsed/refractory; WM, Waldenström macroglobulinemia.

clinical activity was observed across histologic subtypes, with an overall response rate (ORR) of 60%. The maximum tolerated dose was not reached, and 560 mg daily was selected as the dosage for phase 2 studies.[5] Ibrutinib was subsequently evaluated extensively in multiple B-cell malignancies and has been approved by the US Food and Drug Administration (FDA) for relapsed/refractory CLL, CLL with 17p deletion (del[17p]), relapsed/refractory mantle cell lymphoma (MCL), and Waldenström macroglobulinemia (WM). The daily dose is 560 mg for MCL and 420 mg for the other indications.

Chronic Lymphocytic Leukemia

In vitro and in vivo studies demonstrate that ibrutinib acts on CLL cells through several mechanisms. Integrin-mediated adhesion to fibronectin and vascular cell adhesion molecule (VCAM) is inhibited, and chemokine-induced retention, migration, and homing of malignant cells to supportive microenvironments are blocked.[6] Ibrutinib reduces expression of NFκB, with decreased cell survival and tumor proliferation.[7] Consistent with this mechanism of action, clinical experiences with BCR-targeting agents demonstrate the development or worsening of lympho-

Table 2. Other Bruton's Tyrosine Kinase Inhibitors

Phase	Population	N	Response	Grade 3/4 Adverse Events
GS/ONO-4059 (irreversible inhibitor at Y223)				
Phase 1	R/R NHL/CLL	90	24/25 PR in CLL 5/12 CR in MCL 6/12 PR in MCL 3/31 PR in non-GCB DLBCL 8/31 PR in non-GCB DLBCL	Thrombocytopenia, anemia, neutropenia, rash, drug reaction, lower respiratory tract infection, pyrexia, petechiae[64]
CC-292 (irreversible inhibitor at C481)				
Phase 1b	R/R NHL/CLL	86	1/17 PR in NHL 17/50 PR in CLL	Thrombocytopenia, pneumonitis, altered mental status[66]
Phase 1	R/R CLL	84	39/83 PR 7/15 PR in del(17p)	Pneumonia, febrile neutropenia, diarrhea, neutropenia, thrombocytopenia, fever[67]
BGB-3111 (irreversible inhibitor at C481)				
Phase 1	R/R NHL/CLL	25	3/10 PR in NHL 1/10 CR in NHL 6/8 PR in CLL 5/6 PR in WM	Neutropenia[68]
Acalabrutinib (irreversible inhibitor at C481)				
Phase 1/2	R/R CLL	61	93% PR 100% PR in del(17p)	Anemia, pneumonia, hypertension[71]

C481, cysteine 481; CLL, chronic lymphocytic leukemia; CR, complete response; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B cell-like; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma; PR, partial remission; PR-L, PR with lymphocytosis; R/R, relapsed/refractory; WM, Waldenström macroglobulinemia; Y223, tyrosine 223.

cytosis during treatment with a concurrent decrease in lymphadenopathy, representing the redistribution of malignant cells from stroma to the blood.[8] The lymphocytosis is not associated with worsened outcome and has resulted in a new response category termed *partial response with lymphocytosis* (PR-L).[9,10]

Monotherapy Studies. Several early-phase studies demonstrate the efficacy of ibrutinib as monotherapy across CLL subtypes. Data from the phase 1 trial of ibrutinib reported an ORR of 69% in 16 patients with relapsed/refractory CLL. This led to a pivotal phase 1b/2 study evaluating 2 daily dose levels, 420 and 840 mg, in patients with relapsed/refractory CLL and in elderly patients with treatment-naive (TN) CLL. An initial report on 85 patients with relapsed/refractory disease demonstrated an ORR of 71% (PR-L, 15%-20%) overall and an ORR of 68% in patients with del(17p). Similar BTK occupancy and efficacy were seen at both dose levels, leading to the designation of 420 mg as the optimal dose for patients with CLL.[11] Long-term data after 3 years of follow-up showed an improvement in the ORR among the 101 patients with relapsed/refractory disease to 90% (complete response [CR], 7%) and estimated progression-free survival (PFS) to 69%: 48% in patients with del(17p), 74% in those with del(11q), and 87% in those with neither aberration. The depth of response also improved over time; one patient achieved a CR at 42.5 months, and 94% of the patients who initially achieved a PR-L achieved a deeper response.[12] In the 31 elderly patients with TN disease, the ORR at the median follow-up of 22.1 months was 71% (CR, 13%; PR-L, 13%), which improved to 84% (CR, 23%; PR-L, 6%) with a PFS of 96% at 3 years.[12,13] Another phase 2 study of patients with CLL and a *TP53* mutation—33 of whom had TN disease and 15 of whom had relapsed/refractory disease—reported similar results, with ORRs of 97% and 80%, respectively.[14]

Several phase 3 trials have evaluated ibrutinib vs standard treatments. RESONATE (A Phase 3 Study of Ibrutinib [PCI-32765] Versus Ofatumumab in Patients With Relapsed or Refractory Chronic Lymphocytic Leukemia) randomly assigned 391 patients with relapsed/refractory CLL to either ibrutinib 420 mg daily or ofatumumab (Arzerra, Novartis) for 20 weeks. At a median follow-up of 9.4 months, the median PFS had not been reached in the ibrutinib arm and was estimated at 8.1 months for the patients on ofatumumab (5.8 months in the subset with del[17p]). The ORRs with ibrutinib and ofatumumab were 43% (an additional 20% of patients achieved a PR-L) and 4%, respectively. Ibrutinib prolonged overall survival (OS), reducing the hazard of death at 1 year by 57%, and improved patient quality of life.[15,16] RESONATE-2 (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) randomly assigned 269 elderly patients (≥65 years) with TN, non-del(17p) CLL to ibrutinib 420 mg daily or chlorambucil 0.5 to 0.8 mg/kg on days 1 and 15 of 28-day cycles for up to 12 cycles. At a median follow-up of 18.4 months, significant improvements with ibrutinib vs chlorambucil were observed for ORR (86.0% vs 35.3%; CR, 4.4% vs 1.5%), median PFS (not reached vs 18.9 months), and 2-year OS (97.8% vs 85.3%). These differences were seen across all subgroups.[17]

Combination Studies. A phase 2 study of patients with high-risk or relapsed/refractory CLL treated with rituximab (Rituxan, Genentech/Biogen Idec) and ibrutinib reported a PFS of 78% (72% in patients with a *TP53* mutation) at 18 months.[18] Another phase 2 trial evaluated ibrutinib and ofatumumab in relapsed/refractory CLL and reported an ORR of 71% to 100%, depending on the dose administered. The estimated 12-month PFS was 75% to 89%, an improvement over that achieved with either agent alone.[15,19] A third trial of ibrutinib with ublituximab (an investigational anti-CD20 antibody) demonstrated an ORR of 94% in 18 patients with relapsed/refractory CLL.[20] The ongoing CLL2-BIG trial (Sequential Regimen of Bendamustine [B] Followed by GA101 and Ibrutinib [I] in CLL Patients; NCT02345863) is evaluating ibrutinib with obinutuzumab (Gazyva, Genentech) and optional bendamustine in 29 TN patients and 33 patients with relapsed/refractory disease.[21]

Ibrutinib has also demonstrated efficacy in combination with bendamustine and rituximab (BR) in relapsed/refractory CLL. In a phase 1b trial, the ORR was 93.3% (CR, 40%), with a 3-year PFS of 70%.[22] Early data from the phase 3 HELIOS trial (Randomized, Double-blind, Placebo-controlled Phase 3 Study of Ibrutinib, a Bruton's Tyrosine Kinase [BTK] Inhibitor, in Combination With Bendamustine and Rituximab (BR) in Subjects With Relapsed or Refractory Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma; NCT01611090) comparing ibrutinib plus BR vs BR alone reported improved ORR of 82.7% (CR, 10.4%) vs 67.8% (CR, 2.8%) and median PFS of not reached vs 13.3 months.[23]

Many studies involving combinations of ibrutinib with standard chemotherapy regimens (NCT02251548 and NCT02514083), anti-CD20 monoclonal antibodies, rituximab (NCT02007044 and NCT02232386), ofatumumab (NCT02388048), ublituximab (NCT02006485 and NCT02013128), and obinutuzumab (NCT02315768, NCT02537613, and NCT02611908) are ongoing. Studies are also ongoing to assess the efficacy of combining ibrutinib with other small molecules, including PI3K inhibitors (NCT02268851 and NCT02614508), thalidomide derivatives (NCT01886859, NCT02160015, NCT02200848, and NCT02406742), and other novel small molecules (NCT02352558).

Studies combining ibrutinib with checkpoint inhibitors (NCT02420912 and NCT02557516), the XPO1 inhibitor selinexor (NCT02303392), and the BCL2 inhibitor venetoclax (NCT02427451) are of particular interest, given the potential for synergistic action with these agents. Numerous phase 3 studies in CLL have also been initiated and are summarized in Table 1.

The prognosis of patients whose CLL fails to respond to ibrutinib therapy is poor. In a retrospective analysis of 127 patients enrolled in trials involving ibrutinib for CLL from 2010 to 2014, 33 patients had discontinued therapy at the time of reporting owing to progression to another malignancy (7), progressive disease (7), stem cell transplant (3), intolerable side effects (12), death on therapy (3), and patient choice (1). The majority of patients exhibited high-risk features, including poor-risk cytogenetics, with a median of 2 prior treatments. At the time of reporting, 25 of the 33 patients had died. Seven patients experienced disease transformation to diffuse large B-cell lymphoma (Richter's transformation). The median survival after discontinuation of ibrutinib was 3.1 months.[24] Another analysis demonstrated poor median survival in patients who discontinued ibrutinib owing to Richter's transformation (3.5 months) compared with those who discontinued owing to progression of CLL (17.6 months).[25] Although these data are grim, it is important to note that many patients enrolled in these trials had high-risk disease or had failed multiple prior therapies and likely were too sick to tolerate additional chemoimmunotherapy.

In summary, ibrutinib is highly efficacious across CLL subtypes both as monotherapy and in combination with anti-CD20 antibodies or chemoimmunotherapy. Its greatest impact is in patients with the *TP53* mutation and del(17p), for whom it is the now the treatment of choice; longer durations of response (DORs) are seen than with standard therapies. To date, however, therapy in this subset is not curative, and the role of allogeneic stem cell transplant in *TP53* mutation and del(17p) CLL remains an open question. Likewise, the role of ibrutinib in the up-front treatment of non-del(17p) CLL is unclear and is being addressed in an ongoing trial (NCT02048813). Moreover, further study is required to determine whether ibrutinib is best used as monotherapy or in combination with chemoimmunotherapy.

Mantle Cell Lymphoma

Immunogenetic studies suggest that MCL arises in an antigen-dependent manner, with BCR signaling as a potential driver toward MCL oncogenesis.[26] Additionally, gene expression studies have demonstrated that overexpression of SYK

and other proteins upstream of BTK in the BCR pathway may play a role in MCL growth and survival and suggest that inhibition of SYK or downstream targets, in particular BTK, may be important therapeutically in MCL.[27] Consequently, many studies have explored the role of ibrutinib in MCL.

Monotherapy Studies. The phase 1 trial of ibrutinib enrolled 9 patients with relapsed/refractory MCL; responses were seen in 7 patients (CR in 3 patients), leading to the initiation of a pivotal phase 2 trial in MCL.[5] In the latter trial, 115 patients (111 evaluable for response) with relapsed/refractory MCL were treated with ibrutinib 560 mg daily until unacceptable toxicity or disease progression. At a median follow-up of 15.3 months, the ORR was 68% (CR, 21%).[28] No significant differences were observed between the group exposed to bortezomib (Velcade, Millennium/Takeda Oncology) and the bortezomib-naive group.[28] Responses were durable, with a median DOR of 17.5 months—15.4 months for patients with a PR, and median not reached for patients with a CR. Long-term follow-up from this trial has reported 2-year PFS and OS of 31% and 47%, respectively.[29] Another phase 2 study evaluated the role of ibrutinib in 120 patients with MCL that was refractory to bortezomib. Results were similar to those in the pivotal trial; at a median follow-up of 14.9 months and a median treatment duration of 8 months, the ORR was 62.7% (CR, 20.9%). The median PFS was 10.5 months, with a median DOR of 14.9 months, and 61% of patients were alive at 18 months.[30]

Based on these results, ibrutinib received an FDA breakthrough therapy designation in February 2013 and FDA approval in November 2013 for patients with MCL that had relapsed or failed to respond to at least one prior line of treatment. The phase 3 RAY trial (Study of Ibrutinib Versus Temsirolimus in Patients With Relapsed or Refractory Mantle Cell Lymphoma Who Have Received at Least One Prior Therapy) randomly assigned 280 patients with relapsed/refractory disease to either ibrutinib or temsirolimus (Torisel, Pfizer). At a median follow-up of 20 months, the median PFS was 14.6 months for ibrutinib vs 6.2 months for temsirolimus (P<.0001), and the median OS was not reached vs 21.3 months (P=not significant). The lack of an OS advantage is likely confounded by the proportion of patients on temsirolimus who crossed over to ibrutinib (23%).[31]

Combination Studies. Several studies have explored combinations of ibrutinib with cytotoxic/immunomodulatory agents or chemoimmunotherapy for MCL. Ibrutinib in combination with BR has been evaluated in patients with MCL. A total of 17 patients (5 previously untreated) received either 280 or 560 mg of ibrutinib daily with standard BR for up to 6 cycles; responding patients could continue ibrutinib until unacceptable toxicity or disease progression. The ORR was 94% (CR, 76%); median DOR and PFS have not been reached.[32]

Early results from a phase 2 study of ibrutinib in combination with rituximab are also promising. In a single-institution study, 50 patients with relapsed/refractory MCL received ibrutinib 560 mg daily and rituximab for up to 2 years. Patients were classified as having an indolent (74%) or aggressive (26%) disease phenotype on the basis of their Ki-67 score (<50% vs ≥50%).[33] With a median follow-up of 16.5 months, the ORR was 88% (CR, 44%). Responses were impressive in the subset of patients with indolent disease, with an ORR of 100% (CR, 51.4%). The median DOR and PFS have not been reached.[33] A recently reported phase 2 trial examined ublituximab and ibrutinib in 15 patients with relapsed/refractory MCL. The ORR was 87% (CR, 33%), and improvement in the depth of response was seen with increased duration of treatment.[34]

Based on the promising response rates seen with the combination of BR and ibrutinib, which compare favorably with the historical experiences with single-agent ibrutinib and BR in MCL, the ongoing phase 3 SHINE trial (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; NCT01776840) is comparing BR with or without ibrutinib for patients older than 65 years with previously untreated MCL.

Several studies are evaluating combinations of ibrutinib with other approved agents for relapsed/refractory MCL. These include bortezomib (NCT02356458) and lenalidomide (Revlimid, Celgene) with rituximab (NCT02446236 and NCT02460276). Combinations of ibrutinib with agents that disrupt BCR signaling are also an area of active investigation in MCL; phase 1/1b and 2a trials of ibrutinib with novel PI3Kδ inhibitors (NCT02268851 and NCT02455297) are ongoing. Additional trials have combined ibrutinib with other investigational agents, including palbociclib (Ibrance, Pfizer; NCT02159755), venetoclax (NCT02419560 and NCT02471391), selinexor (NCT02303392), and carfilzomib (Kyprolis, Onyx; NCT02269085).

Additional trials incorporating ibrutinib into intensive, frontline therapy are ongoing; these include a phase 2 trial of ibrutinib with rituximab plus cyclophosphamide, vincristine, doxorubicin, and dexamethasone (Hyper-CVAD; NCT02427620) and a trial of ibrutinib maintenance following intensive frontline therapy (NCT02242097).

As observed in patients with CLL, those with MCL that fails to respond to treatment with ibrutinib have a poor prognosis. A retrospective study of 106 patients with MCL whose disease progressed on ibrutinib reported an OS following discontinuation of ibrutinib of 2.9 months,

with only 17.2% surviving for 1 year.[35] A lower Mantle Cell Lymphoma International Prognostic Index (MIPI) score before the initiation of ibrutinib, as well as longer duration of treatment with ibrutinib, predicted longer survival following failure to respond to ibrutinib.[35]

Cumulatively, these data suggest that ibrutinib will play an important part in the treatment of patients with MCL. Many of the issues in MCL are similar to those in CLL. Active studies of combinations are attempting to improve upon single-agent efficacy, and attempts to incorporate ibrutinib into initial therapy are ongoing. Questions remain regarding the role of intensive consolidation therapy for young, fit patients in first remission following ibrutinib and are under investigation in Europe. For patients not proceeding to intensive therapy, a major question is whether an up-front combination of ibrutinib with chemotherapy is required or whether ibrutinib should be kept in reserve until relapse. The optimal duration of therapy for patients who have achieved a CR remains unknown. Additionally, studies need to determine the impact of therapy on molecular remissions by assessing minimal residual disease (MRD) status.

Waldenström Macroglobulinemia

The substitution of leucine for proline at position 265 in *MYD88*, the $MYD88^{L265}$ mutation, has been identified in more than 90% of patients with WM.[36] The $MYD88^{L265}$ mutation is implicated in promoting WM tumor growth and survival through upregulation of NFκB via both BTK and interleukin 1 receptor–associated kinases (IRAK1 and IRAK4). Furthermore, in vivo studies have demonstrated that inhibition of BTK with ibrutinib induces apoptosis in WM cell lines.[37] Based on these data and the responses seen in the phase 1 trial (PR, 75%),[5] a phase 2 trial evaluated ibrutinib in patients with previously treated WM. A total of 63 patients received ibrutinib 420 mg daily until disease progression or unacceptable toxicity. The response to ibrutinib was rapid, with a median time to immunoglobulin M response and clinical responses of 4 weeks, along with improvement in the quality of response during prolonged therapy. The ORR was 90.5%, with a major response rate (MRR) of 73.0%, a 2-year PFS of 69.1%, and an OS of 95.2%.[38]

This study also explored the effect of somatic activating mutations in *CXCR4*, as reported in patients with warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) syndrome ($CXCR4^{WHIM}$) on response to ibrutinib. Previous reports have identified $CXCR4^{WHIM}$ mutations in 27.0% to 29.1% of patients with WM.[39,40] In this trial, $CXCR4^{WHIM}$ mutations were present in 34% of patients.[38] Responses to ibrutinib were examined in 3 genomic groups: $MYD88^{L265P}CXCR4^{WT}$, $MYD88^{L265}CXCR4^{WHIM}$, and $MYD88^{WT}CXCR4^{WT}$. ORR and MRR, respectively, were highest in the $MYD88^{L265P}$-$CXCR4^{WT}$ group (100.0% and 91.2%), followed by the $MYD88^{L265P}CXCR4^{WHIM}$ group (85.7% and 61.9%) and the $MYD88^{WT}CXCR4^{WT}$ group (71.4% and 28.6%).[38]

Based on the excellent response rate and acceptable toxicity profile in this trial, ibrutinib received its fourth FDA indication: treatment of WM both as frontline therapy and in relapsed/refractory disease. Currently, the iNNOVATE trial (Ibrutinib With Rituximab in Adults With Waldenström's Macroglobulinemia; NCT02165397) is comparing rituximab with and without ibrutinib in patients who have WM. Early data from the nonrandomized portion of this trial in 31 patients with rituximab-refractory disease receiving single-agent ibrutinib report a 7.7-month ORR of 84% and MRR of 65%.[41]

Overall, ibrutinib represents a significant advancement in the treatment of WM. Its impressive response rate and minimal toxicity are exciting; however, questions remain regarding the cost of continuous treatment with ibrutinib vs the limited and defined treatment periods with other therapeutic options.

Diffuse Large B-Cell Lymphoma

Gene expression profiling studies have identified 2 distinct molecular subtypes of diffuse large B-cell lymphoma (DLBCL): activated B cell-like (ABC) and germinal center B cell-like (GCB). These subtypes respond differently to standard treatment approaches, with inferior outcomes in patients who have the ABC subtype.[42] Studies also demonstrate that mutations in *CD79B* and *MYD88* lead to chronic BCR signaling and upregulation of NFκB, which are essential for survival in ABC cell lines. This suggests a potential role for BTK inhibition as a therapeutic strategy.[43,44]

Relapsed/Refractory Diffuse Large B-Cell Lymphoma. A phase 2 study of ibrutinib in 70 patients with relapsed or refractory DLBCL evaluated the hypothesis that ABC-type DLBCL might respond preferentially. An additional 10 patients from a prior pilot phase 1 expansion cohort were included in the final analysis. Patients received ibrutinib 560 mg daily until disease progression or unacceptable toxicity.[45] Gene expression profiles were evaluated in 58 patients (38 ABC and 20 GCB). Overall, 20 of 80 patients (25%) responded to treatment, including 8 with CRs. Consistent with the study hypothesis, responses were seen in 14 of 38 patients (37%) with the ABC subtype of DLBCL vs 1 of 20 patients (5%) with the GCB subtype.[45] Mutations in *CD79B* (23%) and *MYD88* also were evaluated, and the highest response rates were noted in patients with both *CD79B* and *MYD88* mutations (4/5, 80%) followed by patients with *CD79B* mutations (5/9, 55.5%). Importantly, the presence of these mutations was not necessary for a response to ibrutinib because 9 of 29 patients who had ABC DLBCL without mutations in *CD79B* also

responded, suggesting that mutations in BCR components themselves are not required for ABC DLBCL dependence on BCR signaling.[45]

Other, smaller studies have evaluated the safety and efficacy of ibrutinib combinations in patients with untreated or relapsed/refractory B-cell non-Hodgkin lymphoma (NHL). One phase 1/1b trial included 16 patients with relapsed/refractory DLBCL (4 GCB, 11 ABC, 1 unclassifiable) treated with BR plus ibrutinib 280 or 560 mg daily in 28-day cycles. Responses were seen in 6 of 16 patients (37%), with 5 patients (31%) achieving a CR.[32] Another phase 1 trial (NCT01955499) examined ibrutinib in combination with lenalidomide in patients with relapsed/refractory B-cell NHL and included 9 patients with DLBCL/transformed follicular lymphoma (FL). Patients received escalating doses of lenalidomide on days 1 to 21 and ibrutinib on days 1 to 28 of a 28-day cycle.[46] At the time of the most recent update, responses were seen in 7 of 18 evaluable patients, with disease control (stable disease, PR, or CR) in 12 of 18 patients.[46] Recruitment to this study is ongoing. Another phase 1b/2 trial studied the combination of ibrutinib (560 mg on days 1-7) and dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab (DA-EPOCH-R) with escalating doses of lenalidomide (days 1-7) in 15 patients who had relapsed/refractory DLBCL. In the 10 patients evaluable for response, the ORR was 50% (CR, 20%). Recruitment to the expansion cohort at the 25-mg dose of lenalidomide is ongoing.[47] Ibrutinib has also been incorporated into a novel regimen of temozolomide, etoposide, liposomal doxorubicin, dexamethasone, ibrutinib, and rituximab (DA-TEDDI-R) for patients with relapsed primary central nervous system lymphoma. Early results are encouraging, with 4 of 4 patients achieving a CR.[48]

Frontline Therapy of Diffuse Large B-Cell Lymphoma. Ibrutinib also has been evaluated in combination with standard chemoimmunotherapy in DLBCL. A phase 1b study examined the safety and efficacy of ibrutinib in combination with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) in patients who had untreated CD20+ B-cell NHL. In the dose escalation part of the study, 17 patients (49% with DLBCL) received escalating doses of ibrutinib (280-560 mg daily) in combination with standard R-CHOP. The combination was safe, and 560 mg of ibrutinib was recommended as the phase 2 dose in combination with R-CHOP. Subsequently, an additional 16 patients were enrolled.[49] Of the 23 patients with DLBCL, 22 received at least one dose of ibrutinib and 11 were evaluable for molecular subtype. A total of 5 of 7 patients with GCB disease and 4 of 4 patients with non-GCB disease achieved a CR. In the subset of 18 patients with DLBCL who received 560 mg, the ORR was 100% (CR, 15; PR, 3).[49]

Currently, several clinical trials are exploring the utility of ibrutinib in combination with chemoimmunotherapy. The phase 3 PHOENIX trial (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; NCT01855750) is randomly assigning patients with previously untreated non-GCB DLBCL to R-CHOP with or without ibrutinib. In the setting of relapsed disease, trials are exploring ibrutinib in combination with salvage chemoimmunotherapy with (NCT02142049) or without lenalidomide (NCT02219737), lenalidomide with or without rituximab (NCT02077166 and NCT01955499), and anti–programmed death ligand 1 (anti–PD-L1) immunotherapy (NCT02401048).

In conclusion, ibrutinib is a promising agent for patients with DLBCL. It has encouraging activity and a favorable toxicity profile as a single agent in patients with relapsed/refractory ABC DLBCL. The role of ibrutinib in combination with standard frontline chemoimmunotherapy in ABC DLBCL is being evaluated in the ongoing PHOENIX trial. If the results are positive, this regimen may represent an opportunity to improve outcomes for patients with ABC DLBCL.

Follicular Lymphoma

Antigenic stimulation of the BCR pathway in t(14;18)-expressing B cells has been proposed as a mechanism for the oncogenesis of FL and suggests a role for BTK inhibition in FL.[50] In 16 patients with relapsed/refractory FL included in the phase 1 trial, the ORR was 38% (CR, 19%).[5] Additionally, when responses were restricted to patients receiving at least 2.5 mg/kg per day, the ORR improved to 54.5% (CR, 27.3%), with a median DOR of 12.3 months and a median PFS of 13.4 months. In the subset receiving at least 5 mg/kg per day, there was a trend toward improved PFS at 19.6 months.[51] However, interim data from the phase 2 consortium trial of single-agent ibrutinib in 40 patients with relapsed/refractory grade 1/2/3a FL suggested only a moderate response. At a median follow-up of 6.5 months, the ORR was 30% (12/40 patients), with 1 CR and 11 PRs.[52,53]

The phase 1b combination study of ibrutinib with R-CHOP included patients with FL, but because of the small number of patients in this trial, it is difficult to make formal conclusions.[49,54] In a phase 1b trial of ibrutinib in combination with BR, a better ORR than with single-agent ibrutinib was noted: ORR of 90% (CR, 50%) in 12 patients with previously treated FL.[32] Another phase 1 study examined the triplet of rituximab, lenalidomide, and ibrutinib in 22 patients with TN FL. The ORR at all dose levels was 91% (CR, 63%), with a 12-month PFS of 86% at the highest dose level.[55]

Early results (median follow-up, 10.2 months) from a phase 2 study of rituximab and ibrutinib in 60 patients with TN FL are promising, with an ORR of 82% (CR, 27%). The median PFS, OS, and DOR have not yet been reached.[56]

Ongoing trials include a registration trial of single-agent ibrutinib (NCT01779791) and combination studies of ibrutinib with anti–PD-L1 therapy (NCT02401048), with rituximab and lenalidomide (NCT02532257), and with either BR or R-CHOP (NCT01974440).

Overall, the data on ibrutinib in FL are not as robust as those in the other histologic types previously described, and results of ongoing trials are required to determine the role of ibrutinib in FL.

Other Indolent Forms of Non-Hodgkin Lymphoma

Patients with other indolent forms of NHL have been included in many of the trials discussed earlier in this review, with variable responses to ibrutinib as a single agent or in combination with other agents and chemoimmunotherapy. A phase 2 trial of ibrutinib in patients with relapsed/refractory marginal zone lymphoma (NCT01980628) is ongoing.

Adverse Events With Ibrutinib

Across trials and histologic types, ibrutinib monotherapy is well tolerated, with the majority of adverse events being grade 1 and 2. The most common nonhematologic toxicities reported include diarrhea, fatigue, nausea, upper respiratory tract infection, peripheral edema, pyrexia, myalgia, constipation, bleeding, rash, and atrial fibrillation. Grade 3 and grade 4 toxicities were infrequent, occurring in approximately 5% to 15% of patients, and commonly were hematologic, including neutropenia, thrombocytopenia, and anemia. The most common grade 3 or worse nonhematologic adverse events were pneumonia (4%-6%) and atrial fibrillation (3%-12%). A rash developed in approximately 10% of patients taking ibrutinib, which typically occurred early during treatment and resolved with interruption of ibrutinib. Corticosteroids were occasionally required.[57] In CLL, toxicities occurred more frequently in patients with relapsed/refractory disease than in those with TN CLL. The most notable toxicities in this group were severe infections, which occurred in 51% of patients with relapsed/refractory disease vs 13% of those who were TN. Long-term follow-up at 3 years of patients with CLL and WM have not found additional safety signals.[12,58]

Between 40% and 61% of patients enrolled in the previously discussed trials experienced bleeding events of some grade, although severe bleeding (grade 3 or worse) is less common and often associated with the concomitant use of antiplatelet or anticoagulant agents. A similar pattern was seen in a retrospective analysis of 327 patients with CLL; major bleeding events occurred in 2% to 3% of patients and was associated with the use of antiplatelet or anticoagulant agents.[59] Given the increased risk of bleeding with ibrutinib, all trials were amended to prohibit the concomitant use of warfarin (alternative anticoagulants were allowed). Additionally, the package insert recommends withholding ibrutinib for at least 3 to 7 days before and after surgery.[57]

The incidence of atrial fibrillation also is increased with ibrutinib (3%-11%) and is higher than that observed in the general population (1%).[15,60] A retrospective analysis of 105 patients who had MCL treated with either ibrutinib alone or ibrutinib with rituximab found that coronary artery disease and hypertension were significant risk factors for atrial fibrillation. The presence of either of these comorbidities significantly elevated the odds for the development of atrial fibrillation compared with the odds in the general population: 4.54 vs 1.4 for coronary artery disease and 5.05 vs 1.35 for hypertension.[60]

A similar toxicity profile has been observed in trials combining ibrutinib with anti-CD20 antibodies, with the notable exception of an increased rate of neuropathy and stomatitis in patients who have CLL treated with ofatumumab and ibrutinib.[19] Combinations of ibrutinib with other new agents, in particular lenalidomide, may cause side effects that limit their tolerability. In the phase 1 study of ibrutinib with lenalidomide in relapsed/refractory B-cell NHL, 2 dose-limiting toxicities (one grade 2 ischemic stroke and one grade 3 rash) were observed at the initial dose levels, and de-escalation of the dose was required for both drugs; another dose-limiting toxicity of grade 3 rash was observed at the de-escalated dose levels. Additionally, 67% patients experienced grade 3 or grade 4 toxicities, including hematologic toxicity, rash, increased liver enzymes, pneumonia, hypokalemia, and syncope.[46] A similar toxicity profile was seen in patients who had TN FL treated with rituximab, lenalidomide, and ibrutinib, with 32% of patients experiencing grade 3 rash in addition to other common grade 3 or grade 4 toxicities of neutropenia, thrombocytopenia, anemia, atrial flutter, diarrhea, and febrile neutropenia.[55]

The toxicity of ibrutinib in combination with chemoimmunotherapy (eg, R-CHOP, BR, DA-EPOCH-R, and DA-TEDDI-R) was not significantly different from what is expected with these regimens alone. Neutropenia, thrombocytopenia, febrile neutropenia, and anemia were common, but there was no observed increase in risk for bleeding. Of note, a higher percentage of patients (25%) developed a grade 3 or grade 4 rash than traditionally has been seen in trials of ibrutinib used as a single agent.[32]

Overall, ibrutinib is well tolerated and has toxicities that are manageable in the majority of cases with dose interruption or reduction. Toxicities infrequently lead to therapy discontinuation, which occurs in approximately 4% to 12% of patients.

Ibrutinib Resistance

Although the majority of patients with CLL, MCL, or WM have prolonged responses to ibrutinib, relapse does occur in a sizeable proportion, and data regarding mechanisms of resistance are emerging. Despite a common mechanism of action in all B-cell malignancies (ie, disruption of BCR signaling), the observed mechanisms of resistance appear to vary based on the disease in question. In CLL, primary resistance appears to be rare, with most cases of acquired resistance due to either of 2 mechanisms. The first is a cysteine-to-serine mutation at position 481 in the BTK binding pocket, which renders ibrutinib unable to form a stable covalent bond with the BTK active site and so restores BCR signaling through BTK.[61] The second mechanism is based on gain-of-function mutations in *PLCγ2*, a signaling molecule downstream from BTK whose activation appears to decrease the reliance of CLL on BTK for proliferation.[62] In MCL, cases of primary resistance are more common and thought to be related to tonic sustained signaling through distal components of the BCR pathway (ie, sustained PI3K-AKT activation or reliance on the alternate NFκB pathway). Secondary resistance in MCL appears to arise through mechanisms similar to those in CLL. Finally, primary resistance in WM appears to be related to *CXCR4*^{WHIM} mutations that allow the sustained activity of downstream enzymes AKT and ERK and tumor escape from ibrutinib-induced apoptosis.[61] Future approaches to ibrutinib resistance may include combination treatment with additional agents that target the BCR pathway or the use of other BTK inhibitors under development.

Other Bruton's Tyrosine Kinase Inhibitors

In addition to ibrutinib, several other BTK inhibitors are at various stages of development. Some, such as LFM-A13, have not been evaluated in humans and are beyond the scope of this review. The ones that we discuss here are ONO/GS-4059, CC-292, BGB-3111, and acalabrutinib (Table 2).

ONO/GS-4059, formerly ONO-WG-307, is a potent, irreversible inhibitor that blocks autophosphorylation of BTK at tyrosine-223, and has demonstrated efficacy in FL, CLL, MCL and ABC DLBCL in vitro and in vivo.[63] A phase 1 trial of ONO/GS-4059 enrolled 90 patients with relapsed/refractory NHL and CLL with escalating doses either once or twice daily. Responses were seen across histologies. In the 28 patients with CLL (25 evaluable for response), rapid objective responses were seen in 24 (96%), including those with del(17p) and *TP53*. In 16 patients with MCL (12 evaluable for response) the ORR was 92% (CR, 41.6%). Finally, in 31 patients with non-GCB DLBCL, the ORR was 35% (CR, 9.6%), with a median DOR of 54 days. ONO/GS-4059 was well tolerated, with the majority of adverse events grade 1 or 2. Grade 3 and 4 events were less frequent and were primarily hematologic, including neutropenia (10%), anemia (13.3%), and thrombocytopenia (13.3%). 31.1% of patients received anticoagulation while on trial, with no observed increase in the risk of bleeding. Rash was observed in 3 patients with NHL, and 2 patients experienced a non-immune drug reaction.[64]

CC-292, formerly AVL-292, is a potent irreversible inhibitor of BTK that, in vitro, appears to have a mechanism of action and effect on CLL lines similar to those of ibrutinib.[65] Early clinical data suggest that CC-292 is effective in treating B-cell malignancies. A phase 1b study enrolled 23 patients with NHL, 6 patients with WM, and 57 patients with CLL, who received escalating doses of CC-292; the ORR was between 31% and 66.7% depending on the dose level. Most adverse events were grade 1 or grade 2, and 3 dose-limiting toxicities were observed.[66] A separate phase 1 study in 84 patients with CLL demonstrated a similar ORR range of between 31% and 67%, depending on the dose administered. Similarly, most adverse events were mild. Grade 3 and grade 4 adverse events that occurred in 5% or more of the patients included pneumonia (17%), febrile neutropenia (7%), diarrhea (7%), neutropenia (5%), thrombocytopenia (5%), and fever (5%).[67] Ongoing studies are evaluating CC-292 in DLBCL and CLL as monotherapy and in combination with other agents.

BGB-3111 is a novel, potent, irreversible inhibitor of BTK. In vitro, it has displayed selectivity for BTK greater than that of other tyrosine kinases, including ibrutinib. Early phase 1 data from 25 patients with advanced B-cell malignancies demonstrated an ORR between 0% and 83%, depending on histology. Three drug-related adverse events of grade 3 or worse were observed, all of which were self-limiting neutropenia in patients with CLL and two of which were in patients who had neutropenia at baseline. Ongoing studies are evaluating BGB-3111 as a single agent, as well as in combination with immunotherapeutic agents.[68]

Acalabrutinib, formerly ACP-196, is a novel, irreversible, second-generation BTK inhibitor that binds to cysteine 481. It has in vivo activity in mouse CLL xenograft and canine B-cell lymphoma models.[69] Additionally, acalabrutinib may have advantages over ibrutinib in regard to target specificity and drug-drug interactions.[70] A phase 1/2 study treated 61 patients who had relapsed/refractory CLL with escalating doses of acalabrutinib. Responses were rapid, with an ORR of 93% across CLL subtypes, including 100% in del(17p). The ORR in patients with prior exposure to idelalisib (Zydelig, Gilead) was 100%. The most common adverse events were

grade 1/2 and included headache (39%), diarrhea (33%), and upper respiratory infection (16%). Grade 3/4 adverse events included anemia (7%), pneumonia (7%), and hypertension (5%).[71] An extensive clinical development program has been initiated in CLL, MCL, WM, non-GCB DLBCL, and solid tumors that includes a phase 3 trial comparing acalabrutinib with ibrutinib in CLL (NCT02477696).

Overall, it is unclear how these novel BTK inhibitors will contribute to the treatment of B-cell malignancies, given the early stage of development and the high bar set by ibrutinib.

Conclusion

In summary, ibrutinib clearly has altered the treatment landscape for B-cell malignancies. In CLL, it offers unique therapeutic promise to patients with high-risk disease and another significant therapeutic option to patients with relapsed disease after conventional therapies; it may someday be established as a frontline option for most or all patients. Confirmatory trials are ongoing. In WM, ibrutinib is the only FDA-approved therapy and offers a relatively safe and well-tolerated therapeutic option. Similarly, in MCL, ibrutinib is an attractive alternative for monotherapy in relapsed/refractory disease and—as in CLL—eventually may become part of frontline therapy. The role of ibrutinib in the treatment of FL and GCB DLBCL is still unclear, given that its efficacy in these diseases is less robust. Ongoing trials will be informative. Although the efficacy of ibrutinib is remarkable, questions do remain regarding the optimal duration of therapy and the ratio of risk/cost to benefit, especially in CLL and WM, in which OS is generally long. Future studies should include discontinuation trials for patients in whom a minimal residual disease state has been achieved with re-treatment at the time of disease progression to determine if such a strategy is effective. Rational combinations (combining ibrutinib with pro-apoptotic agents such as venetoclax [ABT-199]) are being explored. Other BTK inhibitors are relatively early in development, and time will reveal if they offer any advantage over ibrutinib in terms of efficacy or safety.

Disclosures

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