Review

Ibrutinib and Indolent B-Cell Lymphomas

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Abstract

Most patients with indolent B-cell lymphomas fail to achieve complete remission with current treatment approaches and invariably relapse. During the past decade, innovative immunochemotherapy strategies have substantially improved disease control rates but not survival, thus providing the rationale for development of novel agents targeting dysregulated pathways that are operable in these hematological malignancies. Ibrutinib, a novel first-in-human Bruton's tyrosine kinase (BTK) inhibitor, has progressed into phase III trials after early-phase clinical studies demonstrated effective target inhibition, increased tumor response rates, and significant improvement in survival, particularly in patients with indolent B-cell lymphomas. Recently, the compound was designated a "breakthrough therapy" by the United States Food and Drug Administration for the treatment of patients with relapsed or refractory mantle cell lymphoma and Waldenström macroglobulinemia. This review summarizes recent achievements of ibrutinib, with a focus on its emerging role in the treatment of patients with indolent B-cell lymphoid malignancies.

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Introduction

Indolent B-cell lymphomas comprise a heterogeneous group of lymphoproliferative disorders, and encompass low-grade and some categories of intermediate-grade non-Hodgkin lymphomas (NHLs) in the Working Formulation Classification.[1] These B-cell disorders include grade I to IIIA follicular lymphoma (FL), marginal zone lymphoma (MZL), mantle cell lymphoma (MCL), lymphoplasmacytic lymphoma (Waldenström macroglobulinemia [WM]) and small lymphocytic lymphoma/chronic lymphocytic leukemia [SLL/CLL]).[2] Overall, they account for approximately 40% of all NHLs and are more common with advanced age.[3,4] Indolent B-cell lymphomas, a result of clonal mature B-cell overproliferation, are typically characterized by slow-growth, advanced-stage disease at presentation and a propensity to transform to a more aggressive histological subtype in the terminal phase. However, each disease is unique with a distinct morphology, genetic profile, and clinical behavior.[3]

Though indolent B-cell lymphomas remain incurable, there have been modest gains in the response rates of patients over the past several decades with immunochemotherapy strategies. These disorders demonstrate a relentless progressive-relapsing course, and the vast majority of patients ultimately succumb to the disease.[5] At present, rates of complete remission (CR) are still low, responses are not durable, and survival is not substantially prolonged with conventional therapies. These challenges have encouraged the search for novel therapeutic alternatives targeting the numerous dysregulated pathways (Lyn, Syk, and phosphatidylinositide 3-kinase [PI3K]) that are operable in these hematological malignancies. Preclinical and clinical data indicated that this approach could represent a very promising therapeutic strategy and new treatment option for patients with indolent lymphoproliferative disorders.[6-8]

Recently, Bruton's tyrosine kinase (BTK), a terminal kinase enzyme in the B-cell antigen receptor (BCR) signaling, has emerged as a potential therapeutic target for inhibition.[9] As a critical effector molecule that governs normal B-cell development, differentiation, and functioning, BTK activity is dysregulated frequently in human B-cell malignancies and exploited by tumor cells for increased proliferative potential, evasion of apoptosis, enhanced survival, and tumor progression.[10]

Ibrutinib, a novel first-in-human BTK inhibitor, has demonstrated substantial antitumor activities across a wide variety of B-cell lymphoproliferative disorders in preclinical models. Early-phase clinical studies have shown effective target inhibition, increased tumor responses, and significant prolongation of survival, particularly in patients with indolent B-cell lymphomas. Overall, the compound showed a favorable safety and tolerability profile across all patients. On the basis of these promising data, ibrutinib was granted a 'breakthrough therapy' designation by the United States Food and Drug Administration (FDA) in February 2013, and has

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also progressed into phase III registration trials. Here, we review our current understanding of the BTK signaling pathway and its role in health and disease, as well as discuss the preclinical models and most current clinical experiences with ibrutinib in the treatment of indolent B-cell lymphoma disorders.

The BTK Signaling Pathway and Its Role in Health and Disease

BTK, a nonreceptor tyrosine kinase, belongs to the tyrosine-protein kinase (TEC) superfamily of protein kinases that share structural homology within their catalytic and noncatalytic domains, and have similar mechanism of activation. BTK consists of a conserved domain structure: amino terminal pleckstrin homology (PH) and Tec homology (TH) domains, a central Src homology (SH) 3 and SH2 domains, and a carboxyl-terminal regulatory and catalytic region that contains the tyrosine kinase (TK) or SH1 domains.[11]

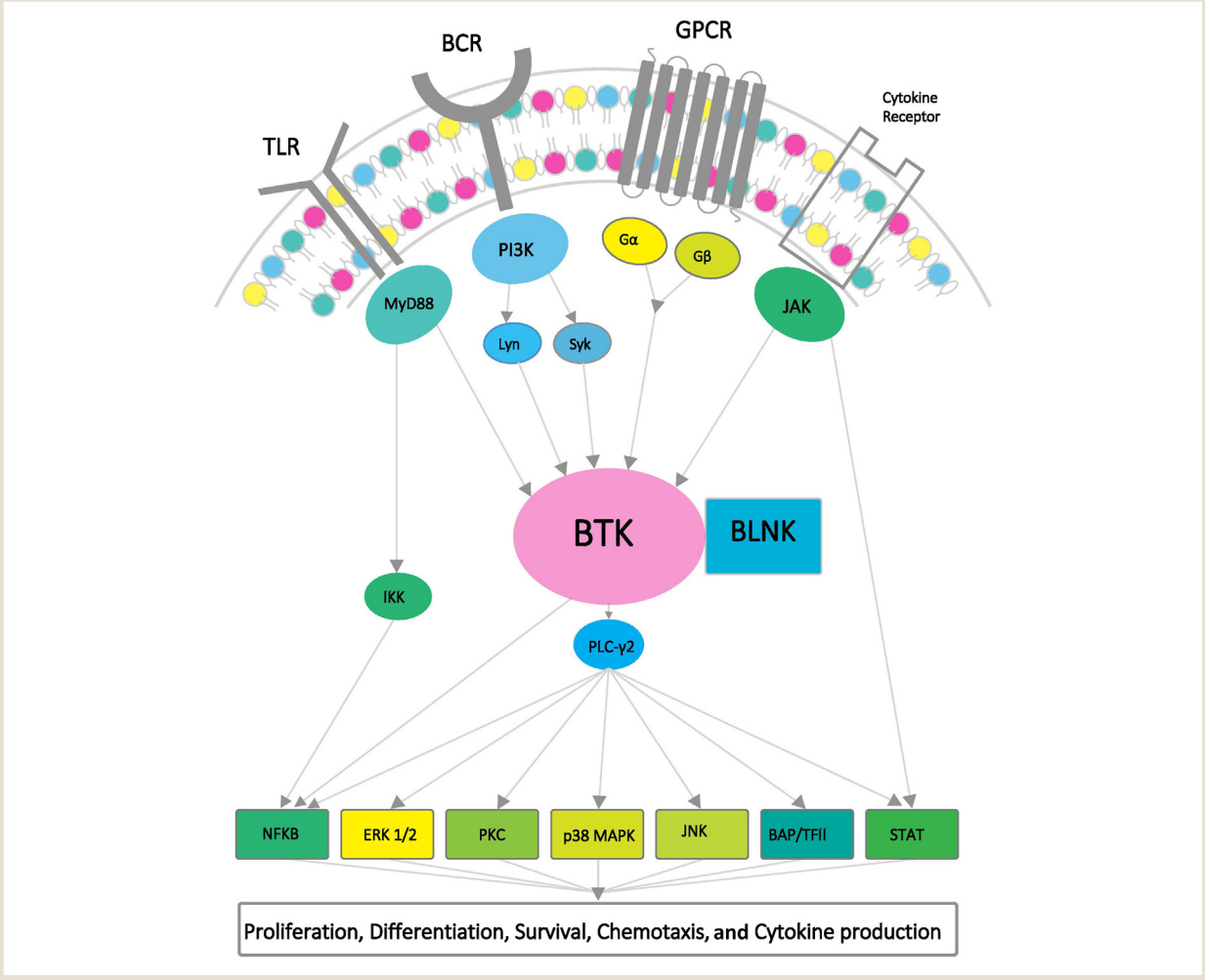
The N-terminally located PH domain of BTK consisting of approximately 50 amino acid residues interacts with membrane lipid products such as phosphatidylinositol (3,4,5) triphosphate (PIP3) produced by PI3K.[12] Biochemical analysis also demonstrated that the PH domain binds transcription factor Bruton's tyrosine kinase-associated Protein 135/transcription factor II-I (BAP-135/TFII-I)[13] and harbors the inhibitory sites for down-regulators such as peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (PIN1), and inhibitor of BTK (IBTK).[14] Following the PH domain is an amino-terminal extension of about 80 residues designated as the TH domain, which shares a high degree of similarity with other TEC kinases such as interleukin 2−inducible kinase (ITK), TEC, bone marrow tyrosine kinase in chromosome X (BMX), and tyrosine-protein kinase TXK/receptor-like kinase (TXK/RLK).[15] It contains conserved regions known as BTK motif (zinc cofactor binding site) and proline-rich stretch[15] and serves as major determinant binding site for protein kinase C-beta (PKC-β).[16] The SH3 and SH2 domains that are located in the central region of the molecule contain the major autophosphorylation sites,[17,18] nuclear localization signals (NLS), and the nuclear export sequence (NES) required for nucleocytoplasmic shuttling of BTK.[19] For all members of the TEC family of kinases, autophosphorylation of residues in these Src domains is necessary for full activation of the kinase. The carboxyl-terminal of BTK possesses the kinase catalytic apparatus, nucleotide (adenosine triphosphate [ATP]) binding site, and the allosteric inhibitory segments.[20]

BTK is predominantly expressed by B cells at various stages of their development (except in terminally differentiated plasma cells) and less commonly in myeloid and erythroid progenitor cells.[21] It functions downstream of multiple receptors and integrates inputs from at least 7 major extracellular and intracellular cues—growth factors, cytokines, B-cell antigen, pathogen-associated molecular patterns, stress, reactive oxygen intermediates, and chemokines—to control diverse major processes, including proliferation, survival, differentiation, motility, angiogenesis, antigen presentation, and protein and lipid synthesis.[22-25] A schematic diagram of the BTK signaling pathway is illustrated in Figure 1. BTK activation is a complex process, and a crucial step in this cascade requires the translocation of BTK to the plasma membrane.[12] PI3K, which is activated by tyrosine kinase and G-protein−coupled receptors, is a key upstream regulator of BTK, and it functions by generating second messenger phosphatidylinositol 3,4,5-trisphosphates (PIP3)[12]. Through high-affinity binding to the PH domain of BTK, PIP3 recruits BTK to the plasma membrane and alters its conformation to allow subsequent transphosphorylation at Tyr-551 residue by Lyn and Syk kinases.[26] Although transphosphorylation at Tyr-551 partially activates BTK, full activation requires autophosphorylation at a second site (Tyr-223) located within the SH3 domain.[18] Many of the upstream signals transmitted by BTK impinge on phospholipase C (PLC)-γ2, which in turn triggers a cascade of events that culminates in sustained intracellular calcium influx and indirect activation of multiple effector kinase pathways, including extracellular signal-regulated kinases (ERK)1/2, p38 mitogen-activated protein kinase (MAPK), nuclear factor of activated T cells (NFAT), nuclear factor κB (NFκB), and stress-activated protein kinase/Jun N-terminal kinase (JNK/SAPK) pathways to regulate proliferation, differentiation and survival.[27-30] BTK also signals to NFκB via myeloid differentiation primary response gene 88 (MyD88)/MyD88-adaptor-like/Toll/interleukin-1 receptor domain−containing adapter-inducing interferon-β (MyD88/MAL/TRIF)−dependent pathways to promote the production of inflammatory cytokines.[24,31] Phosphorylated BTK has direct effects on the apoptosis pathway as well, for example, it can target and downregulate the antiapoptotic activity of signal transducer and activator of transcription 3 (STAT3) transcription factor, resulting in induction of programmed cell death.[32] On BCR engagement, BTK has also been shown to mediate BAP-135/TFII-I -induced expression of some critical genes, such as immunoglobulin heavy-chain gene.[13] Another recently identified substrate of BTK is transcription factor Bright (B-cell regulator of immunoglobulin H [IgH] transcription)/ AT-rich interactive domain (ARID)−3a/Dril1[13], which cooperates with TFII-I to augment IgH gene transcription 3- to 6-fold. In recent studies, several proteins that bind to and inhibit the activity of BTK have been identified.[14,16,33,34] For instance, PKC-β can directly phosphorylate BTK at Ser-180, resulting in its retranslocation to the cytosolic compartment.[16]

Orderly B-cell development is by far the best-characterized process controlled by BTK.[11,23] Otherwise known as agammaglobulinemia tyrosine kinase (ATK) or B-cell progenitor kinase (BPK), BTK was initially identified as the defective protein in the human congenital immunodeficiency state known as X-linked agammaglobulinemia (XLA). This disorder is characterized by defective B-cell maturation, lack of immunoglobulin synthesis, and increased patient susceptibility to recurrent bacterial and viral infections.[35,36] Furthermore, to a large extent, BTK regulate B-cell survival through transcription factors that control the expression of numerous pro- and antiapoptotic genes.[32,37,38]

The BTK signaling pathway is now recognized as one of the most dysregulated pathways in B-cell lymphoproliferative disorders, and it induces several processes required for cancer cell growth, survival, and proliferation.[10,39-42] Oncogenic activation of BTK signaling represents an absolute prerequisite for CLL leukemogenesis and progression in IgH.ETmu CLL mouse models.[43] There is also emerging evidence for a role of aberrant BTK signaling in solid tumor development.[44] The pathway is also found to be abnormally activated in autoimmune disorders, including rheumatoid arthritis and systemic lupus erythematosus.[45]

Figure 1 **Mechanism of Bruton Tyrosine Kinase Signaling. In This Figure, Bruton Tyrosine Kinase (BTK) Integrates Extracellular Inputs Through Intermediacy of 4 Major Integral Membrane Receptors —Toll-Like Receptor (TLR), B-Cell Antigen Receptor (BCR), G-protein Coupled Receptor (GPCR) and Chemokine Receptor—to Control Diverse Cellular Processes Such as Proliferation, Survival, Differentiation, Motility, Angiogenesis, and Cytokine Synthesis. The Multiple Effector Kinase Pathways Activated by BTK are Shown in the Figure**



Abbreviations: BAP = Bruton's tyrosine kinase-associated protein 135; BLNK = B-cell linker protein; Ca^{2+} = calcium; ERK 1/2 = extracellular-signal-regulated kinases 1/2; Gα = G-protein's α subunit; Gβ = G-protein's β subunit; IKK = inhibitor of κ B kinase; JAK = janus kinase; JNK = c-Jun N-terminal kinases; Lyn = v-yes-1 Yamaguchi sarcoma viral related oncogene homolog; MyD88 = myeloid differentiation primary response gene 88; NFκB = nuclear factor κ-light-chain-enhancer of activated B cells; p38 MAPK = p38 mitogen-activated protein kinase; PKC = protein kinase C; PLC-γ2 = phospholipase C γ2; PI3K = phosphatidylinositide 3-kinase; SAPK = stress-activated protein kinase; STAT = signal transducers and activators of transcription; Syk = spleen tyrosine kinase; TFII-I = transcription factor II-I.

Recently, BTK inhibitors have emerged as a promising new class of potential therapeutics for the treatment of B-cell hematological malignancies and autoimmune disorders.[45-47] Ibrutinib, a first-in-class BTK inhibitor has progressed through advanced preclinical development to phase III clinical trials and is currently being tested as a single agent and in combination with cytotoxic chemotherapeutic agents and other targeted agents in the treatment of patients with indolent B-cell lymphomas.

Ibrutinib Activity in Preclinical Models of Indolent B-Cell Lymphomas

Ibrutinib (formerly PCI-32765) is an orally bioavailable, first-generation, multitargeted allosteric inhibitor with potent activity against BTK at subnanomolar concentration (half maximal inhibitory concentration [IC_{50}] = 0.5 nM) in biochemical assays.[48] Ibrutinib exerts its BTK-inhibitory property by covalently interacting with both Cys-481 (TK domain) and Tyr-223 (SH3 domain) residues within BTK and irreversibly inhibits its enzymatic kinase activity and autophosphorylation respectively.[49,50] Preclinical studies evaluating ibrutinib in human cancer cell lines in vitro and human tumor xenografts in vivo have shown its potent antitumor activity. The compound exhibits strong antiproliferative activity against a panel of MCL tumor cell lines as a single agent[51] and, in combination with ACY1215, a selective histone deacetylase 6 (HDAC6) inhibitor, it augments apoptosis by 3-fold, indicating a synergistic effect of BTK and HDAC6 inhibition in MCL.[52]

Table I Trials of Ibrutinib in Indolent B-cell Lymphomas

Study	Phase	Disease Characteristics	Treatment	ORR %	Survival
Advani et al.[58] (n = 56)	I	R/R CLL/SLL, MCL, WM, FL, DLBCL, MZL	Ibrutinib alone	Overall: 60 CLL/SLL: 79 MCL: 78	mPFS: 13.6 mo
Byrd et al.[61] (n = 85)	IB/II	R/R CLL/SLL	Ibrutinib alone	71	26 mo PFS: 75% OS: 83%
Byrd et al.[63] (n = 31)	IB/II	TN CLL/SLL	Ibrutinib alone	71	22 mo PFS: 96%
Burger et al.[66] (n = 40)	I	R/R CLL/SLL	Ibrutinib + rituximab	85	NR
Brown et al.[69] (n = 30)	I	R/R CLL/SLL	Ibrutinib + BR	93	NR
Jaglowski et al.[70] (n = 27)	I	R/R CLL/SLL, PLL	Ibrutinib + ofatumumab	100	9.8 mo PFS: 100%
Fowler et al.[71] (n = 16)	I	R/R FL	Ibrutinib alone	55	mPFS: 13.4 mo
Wang et al.[73] (n = 111)	II	R/R MCL	Ibrutinib alone	68	mPFS: 13.9 mo
Blum et al.[76] (n = 11)	I	R/R B-cell NHL including TN MCL	Ibrutinib + BR	Overall: 38 MCL: 100	NR
Younes et al.[78] (n = 17)	I	TN B-cell NHL including MCL, FL	Ibrutinib + R-CHOP	100	NR
RESONATE 1 (n = 350)	III	R/R CLL	Ibrutinib versus ofatumumab	NA	NA
RESONATE 2 (n = 272)	III	TN CLL	Ibrutinib versus chlorambucil	NA	NA
RESONATE-17 (n = 111)	II	R/R CLL with 17p$^-$	Ibrutinib alone	NA	NA
HELIOS (n = 580)	III	R/R CLL/SLL	Ibrutinib + BR versus Placebo + BR	NA	NA
DAWN (n = 110)	II	R/R FL	Ibrutinib alone	NA	NA
RAY (MCL3001) (n = 280)	III	R/R MCL	Ibrutinib versus Temsirolimus	NA	NA
SPARK (MCL2001) (n = 110)	II	R/R MCL	Ibrutinib alone	NA	NA
SHINE (n = 520)	III	TN MCL	Ibrutinib + BR versus Placebo + BR	NA	NA

Abbreviations: BR = bendamustine and rituximab; mPFS = median progression free survival; NA = not available; NR = not reported; ORR = objective response rate; OS = overall survival; PFS = progression free survival; R-CHOP = rituximab, cyclophosphamide, adriamycin, vincristine, prednisone; R/R = relapsed/refractory; TN = treatment-naïve.

Ibrutinib has also displayed excellent antitumor activities against CLL tumor cell lines in concentration- and time-dependent manners by inducing the proapoptotic caspase pathway and abrogating the growth-promoting Toll-like receptor signaling.[9,53,54] The small-molecule inhibitor also inhibits cysteine X cysteine chemokine ligand (CXCL) 12-induced, CXCL13-induced, and C-C motif chemokine ligand (CCL) 19-induced signaling, adhesion, and migration in a set of primary CLL cells.[55] In addition, data on trophoblast cell line 1 (TCL1) CLL xenografts demonstrated that treatment with ibrutinib (25 mg/kg/d) profoundly delays disease progression.[53]

In addition to blocking BTK in B-cells as previously reported, recent ex vivo and in vivo studies by Dubovsky and colleagues indicated that ibrutinib also exhibits potent and irreversible inhibitory action against ITK, a crucial molecule that drives proximal T-cell receptor (TCR) signaling, by abrogating autophosphorylation of ITK at Tyr-180 residue.[56] Notably, this inhibition results in robust Th1 and CD8 T-cell responses (because of compensatory RLK activity) with concomitant suppression of survival signals provided by Th2-polarized CD4 T-cells in the CLL cells microenvironment.[56,57] This potential immunomodulatory property of ibrutinib may contribute to its antitumor effects against indolent B-cell lymphomas.

Clinical Trials of Ibrutinib in Indolent B-Cell Lymphomas

Given the activity of ibrutinib in earlier preclinical studies, several early-phase clinical trials have evaluated the pharmacokinetics, pharmacodynamics, and the maximum tolerated dose (MTD) of ibrutinib, either alone or in combination with cytotoxic chemotherapeutic agents and other targeted agents, in patients with advanced indolent B-cell tumors (Table 1).

The first-in-human phase I study with a dose-escalation design evaluated intermittent versus continuous dosing of ibrutinib in 56 patients with relapsed or refractory FL, SLL/CLL, MCL, MZL, diffuse large B-cell lymphoma (DLBCL), and WM.[58] Ibrutinib was found to be generally well tolerated at doses up to 12.5 mg/kg/d without reaching MTD. Reversible nonhematologic adverse effects were the most common, including arthalgia, myalgia, rash, fatigue, and gastrointestinal (GI) toxicities such as nausea and diarrhea. Pharmacodynamics data confirmed that the mode of action of ibrutinib was irreversible, as BTK occupancy was maintained for $\geq$ 24 hours after rapid absorption and elimination of the drug. In this trial, 60% of the evaluable 50 patients across all histological subtypes achieved at least a partial response (PR). Among 9 patients with MCL, the objective response rate (ORR) was 78%, including 3 complete responses (CRs), whereas 11 of the 16 patients with CLL responded, including 3 CRs, suggesting that ibrutinib was more effective in these subgroups. In addition, the study also clearly demonstrated that the activity of single-agent ibrutinib in relapsed or refractory CLL and MCL is superior to current standard therapies.[59,60] Characteristically, the rapid resolution of lymph nodes induced by ibrutinib in all CLL patients was accompanied by a surge in lymphocytosis resulting from redistribution from the lymphatic tissues into the bloodstream.[58] At the time of data cutoff, responses were noted to be durable, with a median progression-free survival (mPFS) of 13.6 months.[58] Given the possibility of

reversed biologic effect with intermittent dosing indicated by transient reversal of treatment-related lymphocytosis during the drug off-period, the study recommended a continuous fixed dosing schedule for subsequent trials.

More recently, through work by a number of different groups, the long-term safety, tolerability, and effectiveness of ibrutinib in the treatment of indolent B-cell lymphoproliferative disorders have also been examined. The phase IB/II study conducted by Byrd and colleagues investigated the safety of 2 fixed-dose regimens of ibrutinib (420 mg/d vs. 840 mg/d) in patients with CLL/SLL until onset of disease progression or unacceptable toxicity[61]. Among the 85 patients included in the study, unfavorable risk factors according to advanced-stage disease, 17p13.1 deletion, bulky lymph node ($\geq$ 5 cm in diameter), and 11q22.3 deletion were present in 65%, 33%, 52%, and 36% respectively. At final analysis, the median follow-up was 20.9 months, and 7 patients (8%) had discontinued treatment because of serious adverse effects that included pneumonia, sepsis, staphylococcal bacteremia, and GI bleed, indicating that long-term treatment with ibrutinib was associated with modest toxicity. The incidence of drug discontinuation was greater in the 840-mg/d cohort (12%) compared with that in the 420-mg/d cohort (4%). The clinical benefit rates, which included CR and PR, were high, and the 2 dosing regimens did not differ in the magnitude of ORR (71%). Remarkably, there were no significant differences in response rates between patients with high-risk prognostic features and those with favorable-risk disease. A substantial proportion of patients had sustained improvement in anemia (82%), neutropenia (77%), and thrombocytopenia (78%). To further characterize the benefit of ibrutinib in the relapsed/refractory CLL disease setting, a randomized phase III trial (RESONATE 1) is underway comparing single-agent ibrutinib versus ofatumumab with a planned accrual of 350 patients across 9 countries[62]. Similarly, a phase II trial (RESONATE 17) of ibrutinib in patients with relapsed/refractory CLL/SLL with 17p deletion is underway.

Interestingly, a subset analysis of 31 treatment-naive CLL patients aged 65 years or older initially enrolled in the previously reported phase IB/II study showed an ORR of 71%, favoring ibrutinib as an effective upfront therapy in this cohort.[63] To confirm these data, RESONATE 2, an international, double-blind, randomized, phase III trial designed to assess the efficacy of ibrutinib as a frontline therapy in comparison with chlorambucil in patients 65 years or older with treatment-naive CLL/SLL, has been initiated.[64]

Although 11 out of 85 patients (13%) developed progressive disease with ibrutinib as reported by Byrd and colleagues, response to ibrutinib was still found to be durable with a substantially high estimated 26-month PFS and overall survival (OS) of 75% and 83% respectively.[61] As such, resistance to single-agent ibrutinib appears not to be a major problem at present in patients with CLL/SLL. Nonetheless, molecular sequencing analyses performed by Chang and associates have identified distinct missense mutations in BTK (C481S) and PLC-γ2 (R665W) as potential mechanisms of acquired resistance to ibrutinib in 3 patients who experienced disease progression with ibrutinib therapy.[65] Although the occurrence of these mutations appears to be low, screening of patients with these genomic assays may be useful for identifying patients who would substantially benefit from ibrutinib therapy.

Amid the enthusiasm that has developed for ibrutinib monotherapy in advanced CLL/SLL, there has been significant development of this novel agent in combination regimens, particularly for patients with high-risk disease. A preliminary analysis of the safety and efficacy of ibrutinib plus rituximab was conducted in patients with high-risk features that included del17p, *TP53* mutation, PFS $<$ 36 months after frontline immunochemotherapy, and del11q.[66] Data were gathered for 40 patients, who received ibrutinib 420 mg daily, in combination with weekly rituximab (375 mg/m^2) for weeks 1 to 4 (cycle 1), then daily ibrutinib plus monthly rituximab until cycle 6, followed by daily single-agent ibrutinib afterward. Of the 40 patients, 13 experienced transient, but largely treatment-unrelated, serious adverse events, such as neutropenia, fatigue, pneumonia, insomnia, and bone aches. At a median follow-up of 4 months, the ORR was 85%, indicating profound activity of this combination regimen in patients with unfavorable-risk features. The study was small, evaluated only 20 patients for response, but the responses with ibrutinib plus rituximab appear superior to those achieved with BR (bendamustine plus rituximab) in previous studies of patients with relapsed or refractory CLL.[67,68] Another phase I trial combining ibrutinib with rituximab and bendamustine was associated with a highly favorable safety and tolerability profile, including low incidence of myelosuppression and a superior clinical benefit rate (ORR, 93%) after 8.5 months of median follow-up.[69] Given these favorable results, HELIOS (also known as CLL3001), a phase III randomized trial, has been initiated to assess the combination of IBR (ibrutinib plus bendamustine and rituximab) versus placebo plus BR in refractory or relapsed CLL/SLL. The study primary endpoint is progression-free survival (NCT01611090).

Jaglowski et al. have also addressed whether ibrutinib can be used with ofatumumab in patients with heavily pretreated relapsed or refractory CLL/SLL.[70] Interim data regarding safety showed that the combination was tolerable, with no occurrence of serious adverse events. Again, the response was higher (ORR, 100%) and faster, giving support to the rationale for further study of this combination.

The clear clinical benefit of ibrutinib therapy in CLL/SLL has paved the way for the development of this novel agent in patients with advanced FL. A phase I study by Fowler and colleagues enrolled 16 patients with relapsed/refractory FL who were treated with ibrutinib on a 28-day on, 7-day off (intermittent) or a once daily dose (continuous) schedules.[71] Dosing was initiated at 1.25 mg/kg in the intermittent cohort, with dose escalation guided by the emergence of protocol-defined, dose-limiting toxicities (DLTs) or until 3 dose levels above attainment of full BTK occupancy. The drug was administered at 8.3 mg/kg and 560 mg fixed dose in the continuous dosing schedule. No DLT was observed at the highest dose of 12.5 mg/kg, and the MTD was not reached. Serious adverse events noted included neutropenia, anemia, anxiety, hypersensitivity reaction, hypokalemia, hypophosphatemia, pneumonia, and vomiting. Of 11 patients who received ibrutinib at $\geq$ 2.5 mg/kg and were evaluated for response, the ORR was 54.5%, including 3 CRs. Though the overall median PFS was 13.4 months, there was a slight trend toward improved PFS (19.6 mo) favoring patients who received $\geq$ 5 mg/kg of ibrutinib. As such, a dose of 5 mg/kg or higher was recommended for phase II studies.[71] On the basis of these data and considerations, the DAWN (also known as

FLR2002) study has been launched to establish the efficacy and safety of a daily dose of 560 mg ibrutinib in 110 patients with immunochemotherapy -resistant FL. The primary objective of the study is to evaluate the ORR.[72]

Currently, much of the emphasis in targeted treatment of MCL is focused on ibrutinib, given the encouraging results initially reported by Advani and colleagues.[58] The most compelling data, however, demonstrating the benefit of ibrutinib in advanced MCL came from a recently published phase II study.[73] A total of 111 patients with relapsed or refractory MCL classified as either having received treatment with bortezomib ($\geq$ 2 cycles) or not having received such treatment ($<$ 2 complete cycles or no prior bortezomib therapy) were treated with ibrutinib 560 mg daily in a continuous 28-day cycle. Patients were heavily pretreated, with a median of 3 prior chemotherapy regimens. The primary endpoint of the trial was met. The response rate was substantially high (68%) and uncommon for standard single-agent therapies in this setting.[74,75] Responses were durable with the median duration of response and median PFS being 17.5 months and 13.9 months, respectively.[73] The median OS was not reached. The most frequent grade 3/4 adverse events were neutropenia, thrombocytopenia, diarrhea, fatigue, and abdominal pain. On the basis of these collective data, ibrutinib was granted a "breakthrough therapy" status by the FDA in February 2013 for the treatment of relapsed or refractory MCL. However, further research is needed to confirm these data, and that has led to the initiation of a phase III trial (RAY) comparing single-agent ibrutinib versus temsirolimus in the same patient population. Likewise, a phase II single-arm study (SPARK/MCL 2001) using ibrutinib in patients with MCL that progressed after bortezomib therapy and who have received at least 1 prior rituximab-containing chemotherapy regimen has just completed enrolment for the planned 110 patients.

Although most conventional immunochemotherapy regimens generally achieve high initial response rates, most patients with MCL eventually relapse and die from this disease. To optimize high rates of durable remissions in this patient population, therapeutic strategies of combining ibrutinib with immunochemotherapy are being explored in the clinic. Blum and associates recently reported the interim results from an open-label, single-arm, phase I trial of 11 patients with advanced NHL, including previously untreated MCL deemed ineligible for autologous stem cell transplantation.[76] The median number of prior therapies was 3 (range, 0–10). Ibrutinib was administered at escalating doses of 280 mg or 560 mg on days 1 to 28 every 28 days, combined with rituximab 375 mg/m^2 day 1, and bendamustine 90 mg/m2 days 1 and 2, for 6 cycles. Overall, the combination had a highly favorable safety profile without unexpected toxicity. No DLT was reported. In a subgroup analysis, 3 patients with MCL included in the study achieved 2 CRs and 1 PR with the regimen.[76] These promising results led to the design of the SHINE study, in which elderly patients with newly diagnosed MCL are being randomized to treatment with either IBR or placebo plus BR. The primary study endpoint is PFS.[77]

From the safety data we have so far, the addition of ibrutinib does not appear to increase the toxicities of current standard immunochemotherapy strategies for the treatment of indolent NHL. In another phase IB combination study of 17 patients with previously untreated NHL, including mantle cell lymphoma (29%) and follicular lymphoma (24%), patients who received ibrutinib plus standard doses of R-CHOP (rituximab–cyclophosphamide, doxorubicin, vincristine, and prednisone) (IR-CHOP) achieved a response rate of 100%, including 7 CRs in 10 evaluable patients.[78] No new safety signal emerged from this trial, suggesting that the combination of IR-CHOP has an acceptable safety profile. Safety analysis indicated no overlap of key toxicities. The majority of adverse events were neutropenia, thrombocytopenia, vomiting, anemia, and nausea consistent with previously reported toxicities in earlier studies. It is hard to say whether this combination with ibrutinib is considerably better or not, owing to the fact the study was small, follow-up was short, and disease-specific details were lacking. These observations provide fertile areas for additional research of this regimen before any robust conclusions can be drawn.

Conclusion and Future Direction

Overall, ibrutinib, a novel first-in-class selective oral inhibitor of BTK, looked impressive both a single agent and when given in combination with standard therapies across multiple histological subtypes of indolent B-cell lymphomas. Ibrutinib is likely to become a key element in the treatment of CLL and mantle cell lymphoma, and the durable remissions obtained thus far suggest that many patients may be treated successfully with ibrutinib monotherapy. Nonetheless, ibrutinib combined with chemotherapy or immunotherapy might prove to be an effective approach. Future directions should focus on identifying subgroups or develop tools to predict which patients will derive the greatest benefit from targeted BTK inhibition strategy.

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Disclosure

The authors have stated that they have no conflicts of interest.

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