Ther Adv Hematol
2016, Vol. 7(4) 179–186
DOI: 10.1177/2040620716654102

Ibrutinib in Waldenström macroglobulinemia: latest evidence and clinical experience

Jorge J. Castillo, M. Lia Palomba, Ranjana Advani and Steven P. Treon

Abstract: Ibrutinib is an oral Bruton's tyrosine kinase (BTK) inhibitor, which has recently gained approval by the United States (US) Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of patients with symptomatic Waldenström macroglobulinemia (WM). Herein, we review the role of BTK in the pathophysiology of WM, and present the results of the preclinical and clinical studies that led to the initial investigation and later approval of ibrutinib in WM. We also discuss aspects associated with ibrutinib therapy in WM patients, especially focusing on genomic profiling and the impact on response to ibrutinib, and the management of adverse events.

Keywords: Bruton's tyrosine kinase, ibrutinib, lymphoplasmacytic lymphoma, Waldenström macroglobulinemia

Introduction

In 1944, Dr Jan G. Waldenström first described a case series of patients who presented with anemia, hepatosplenomegaly, hyperviscosity, bleeding, a large serum protein ('macroglobulin') and a lymphoplasmacytic infiltrate in the bone marrow space [Waldenström, 1944]. Named after its discoverer, Waldenström macroglobulinemia (WM) is a rare lymphoproliferative disorder characterized by the malignant accumulation of lymphoplasmacytic lymphoma cells in the bone marrow and other organs, along with the presence of a monoclonal immunoglobulin (Ig)M paraprotein in the serum [Swerdlow *et al.* 2008].

WM is a heterogeneous disease and can present with cytopenias, lymphadenopathy, hepatosplenomegaly, hyperviscosity, neuropathy, cryoglobulinemia or amyloidosis [Treon, 2015]. Although patients with WM tend to survive for several years, even decades [Castillo *et al.* 2014, 2015], the condition is incurable and the disease course characterized, in the vast majority of patients, by symptomatic disease recurrences that affect the patient's quality of life and activities of daily living. The treatment of WM is not standardized, and the choice of therapy is highly personalized, dictated by the patient's age, symptoms, comorbidities or preferences.

In January 2015, the oral Bruton's tyrosine kinase (BTK) inhibitor ibrutinib (Imbruvica®, Pharmacyclics Inc, Sunnyvale, CA, USA) was approved by the United States (US) Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for its use in patients with symptomatic WM. The approval of ibrutinib in WM represents a historical landmark; the fruit of arduous basic, translational and clinical research. The objectives of this review are to describe the role of BTK in the pathophysiology of WM, and to discuss the preclinical and clinical data that supported the approval of ibrutinib as well as the experience in the clinical use of ibrutinib in WM.

The genomic landscape of Waldenström macroglobulinemia

The myeloid differentiation primary response gene 88 (MYD88) is an adapter protein used by Toll-like receptors (TLRs) to mediate innate immune responses [Beutler, 2009]. MYD88 is composed by a death domain (C terminus), an intermediate linker domain, and a Toll-interleukin (IL)-1 receptor domain (N terminus) [Warner and Nunez, 2013]. Under normal circumstances, the death domain is responsible for MYD88 dimerization and its interaction with IL-1 receptor-associated kinase 1 (IRAK1) and IRAK2.

Correspondence to:
Jorge J. Castillo, MD
Bing Center for Waldenström Macroglobulinemia, Dana-Farber Cancer Institute, Harvard Medical School, 450 Brookline Avenue, Boston, MA 02115, USA
Jorgej_castillo@dfci.harvard.edu

M. Lia Palomba, MD
Division of Hematology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Ranjana Advani, MD
Division of Oncology, Stanford University Medical Center, Stanford University, Stanford, CA, USA

Steven P. Treon, MD, PhD
Bing Center for Waldenström Macroglobulinemia, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

The intermediate domain also mediates the interaction between MYD88 and IRAK4, while the Toll receptor domain participates in intracellular signal transduction [Lin *et al.* 2010]. IRAK1 in turn activates the tumor necrosis factor receptor-associated factor 6 (TRAF6), which then phosphorylates the nuclear factor of kappa light polypeptide gene enhancers in B-cell inhibitor-alpha (IκBα), a regulator of the transcription factor nuclear factor-kappa B (NF-κB). Activation of IκBα induces ubiquitination and subsequent dissociation from NF-κB and degradation *via* the proteasome. The activated NF-κB then is transported into the nucleus where it is involved in DNA transcription and cell survival.

Whole genome sequencing (WGS) studies have shown that more than 90% of patients with WM carry a recurrent somatic point mutation in the MYD88 gene associated with a change from leucine to proline at position 265 [Treon *et al.* 2012]. In WM cells, the gain-of-function MYD88 L265P gene mutation triggers NF-κB through interaction with and activation of BTK [Yang *et al.* 2013].

In normal B-cells, the activation of BTK starts with its recruitment to the plasma membrane. Phosphatidylinositol-3,4,5-triphosphate (PIP_3) generated following B-cell receptor (BCR) activation, recruits BTK to the BCR complex. BTK is then phosphorylated by SRC-family kinases. Activated BTK in turn phosphorylates phospholipase C-γ2 (PLC-γ2), which activates protein kinase C β, and finally NF-κB. NF-κB induces further transcription of the BTK gene inducing, among other processes, inhibition of apoptosis. Co-immunoprecipitation studies showed that BTK could interact with proteins involved in TLR4 signal transduction such as MYD88, suggesting a role in lipopolysaccharide signal transduction [Jefferies *et al.* 2003]. Additionally, the interaction of MYD88 and BTK seems essential for the generation of appropriate IgM responses to infectious agents [Alugupalli *et al.* 2007].

BTK is constitutively activated in WM cells, and its activation seems to be dependent on a second, BCR-independent mechanism, involving complex formation with MYD88 L265P. Elegant preclinical experiments have shown that MYD88 knockdown was associated with increased WM cell death, while MYD88 L265P overexpression promoted survival of WM cells [Yang *et al.* 2013]. Therefore, MYD88 is a key regulator of BTK activity, and activation of BTK is mediated by MYD88 L265P overexpression in WM cells. Finally, in MYD88 L265P-expressing WM cells, the BTK inhibitor ibrutinib blocked activation of NF-κB, leading to tumor cell killing. These findings were crucial, as NF-κB plays an important role in the pathophysiology of WM [Leleu *et al.* 2008], and served as a platform for the clinical development of ibrutinib in WM.

More recent studies using WGS methodology have identified recurrent mutations in other genes associated with B-cell development [Hunter *et al.* 2014]. Of importance are mutations identified in the C-X-C chemokine receptor 4 (CXCR4) gene, which are found in 30–35% of patients with WM. These somatic mutations are similar to those found in the Warts, Hypogammaglobulinemia, Infection and Myelokathexis (WHIM) syndrome, which is a rare autosomal genetic disorder associated with frameshift or nonsense mutations in the c-tail of CXCR4 [Hernandez *et al.* 2003]. CXCR4 is a chemokine receptor that promotes WM cell survival, migration and adhesion to bone marrow stroma that is mediated by its only known ligand CXCL12 [Ngo *et al.* 2008]. The presence of the mutations impairs internalization of CXCR4 after binding to its ligand C-X-C motif chemokine 12 (CXCL12) thereby prolonging activation of the receptor [Lagane *et al.* 2008].

Ibrutinib

Ibrutinib is an irreversible inhibitor of BTK with an empirical formula of $C_{25}H_{24}N_6O_2$ and a molecular weight of 440.50. The chemical name is 1-[(3R)-3-[4-amin-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-y1]-1-piperidinyl]-2-propen-1-one. Ibrutinib is a small molecule that covalently binds to C481 within the phosphorylation site of BTK, leading to irreversible inactivation [Honigberg *et al.* 2010]. Ibrutinib is not an exclusive BTK inhibitor, and possesses a broad kinome, inducing irreversible inhibition of several other kinases with important roles in normal and malignant B-cell signaling [Hutchinson and Dyer, 2014; Yang *et al.* 2015]. Ibrutinib inhibits phosphorylation of BTK at very low concentrations (IC50, 0.5 nM) in B-cell lymphoma cell lines, as well as activation of B-cells after direct stimulation of the BCR [Burger and Buggy, 2013].

Ibrutinib is commercialized as capsules of 140 mg, and is rapidly absorbed and eliminated after oral administration. Mean peak plasma

concentrations are reached 1–2 hours after dosing. The mean half-life is 2–3 hours, and there is no accumulation of ibrutinib after repeated daily dosing [Advani *et al.* 2013]. Ibrutinib is metabolized in the liver by the cytochrome CYP3A. The elimination half-life is 4–6 hours. Dose adjustments are recommended for patients with hepatic impairment. Ibrutinib is not cleared significantly by the kidneys, and no dose adjustment is needed for renal dysfunction; however, patients should be monitored for toxicity. Systemic exposure to ibrutinib is increased when administered concomitantly with strong CYP3A inhibitors, and reduced with strong CYP3A inducers. Patients taking ibrutinib should avoid grapefruit, Seville oranges and starfruit.

Ibrutinib in Waldenström macroglobulinemia

In a phase I study, 56 patients with relapsed or refractory chronic lymphocytic leukemia (CLL) and B-cell non-Hodgkin's lymphoma were exposed to ibrutinib at increasing doses of 1.25–12.5 mg/kg per day, 28 days on, 7 days off [Advani *et al.* 2013]. Patients who achieved a response continued therapy until progression. Of 50 patients evaluable for response, 60% achieved a response. Overall, four patients had a diagnosis of WM, of whom three (75%) achieved a response to ibrutinib and continued to respond beyond 4 years [Furman *et al.* 2015]. During this study, a reversal of treatment-related lymphocytosis was seen in patients during the 7-day-off period supporting continued therapy.

In a recent phase II study, 63 symptomatic patients with relapsed or refractory WM were exposed to ibrutinib at a dose of 420 mg orally once daily until unacceptable toxicity or progression of disease [Treon *et al.* 2015a]. The median age was 63 years and 76% were men. The median number of previous treatments was two; 90% of patients had previously received monoclonal antibodies, 52% proteasome inhibitors, 51% alkylators and 24% nucleoside analogues. According to the International Prognostic Scoring System for WM (IPSSWM), 22%, 43% and 35% of patients had low, intermediate and high-risk disease; overall, 40% of patients had disease refractory to the most recent regimen. The MYD88 L265P mutation was detected in 89% of participants, and CXCR4 mutations in 34% of participants. In this study, MYD88 mutations were identified using the allele-specific polymerase chain reaction (AS-PCR) technique, and CXCR4 mutations were assessed using Sanger sequencing. The median time to at least minor response was 4 weeks. At the time of best response, the median IgM level decreased from 3520 mg/dl to 880 mg/dl, the median hemoglobin level increased from 10.5 g/dl to 13.8 g/dl, and the median bone marrow involvement decreased from 60% to 25%. Discordance between serum IgM levels and bone marrow involvement was observed at 6 months but by 12 and 24 months, a stronger correlation was evident. The overall response rate was 91% with 16% very good partial response, 57% partial response and 17% minor response rate. No complete responses were achieved. Response rates did not differ based on age, sex, performance status, IPSSWM, hemoglobin level, IgM level, bone marrow involvement, number of previous therapies or relapsed *versus* refractory disease. The overall and major response rates were higher in patients with the MYD88 L265P but CXCR4 wild type (100% and 91%, respectively), followed by MYD88 L265P and CXCR4 WHIM (86% and 62%, respectively), and MYD88 and CXCR4 wild type (71% and 29%, respectively). At 24 months, the estimated progression-free survival (PFS) and overall survival (OS) were 69% and 95%, respectively. A shorter PFS was observed in patients with high IPSSWM score, >3 lines of therapy and in MYD88 and CXCR4 wild type. The duration of treatment at the time of the study report was 19 months, which is relatively short for an indolent process such as WM. Overall, two of the seven patients who were MYD88 and CXCR4 wild type achieved a major response to ibrutinib. A recent study evaluated those two patients using Sanger sequencing, and showed that these patients carried non-L265P MYD88 mutations [Treon *et al.* 2015b]. Based on this new knowledge, the overall and major response rates for double wild type WM patients were 43% and 0%, respectively. From the approximately 30% of patients who stopped therapy, half did so due to lack of response, progression of disease or unacceptable 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).

Initial results of Arm C of the INNOVATE study were reported at the 2015 American Society of

Table 1. Response and survival outcomes of published selected regimens used to treat patients with Waldenström macroglobulinemia.

Agent	*N*	Overall response rate	Major response rate	Time to response	Progression-free survival	Overall survival
Rituximab [Gertz *et al.* 2004]	69	52%*	20% (previously treated)	Not reported	Not reported	2-year: ~70%
Extended rituximab [Treon *et al.* 2005]	29	66%*	48% (untreated and previously treated)	17 months	14 months	Not reported
Bortezomib twice weekly [Treon *et al.* 2007]	27	85%*	48% (previously treated)	1.4 months	8 months	Not reported
Cyclophosphamide, dexamethasone, rituximab (CDR) [Dimopoulos *et al.* 2007; Kastritis *et al.* 2015]	72	83%	74% (untreated)	4 months	35 months	2-year: 81%
Bortezomib twice weekly, dexamethasone, rituximab (BDR) [Treon *et al.* 2009]	23	96%	83% (untreated)	1.4 months	Not reached at 30 months	Not reported
Bortezomib weekly, dexamethasone and rituximab [Dimopoulos *et al.* 2013]	38	85%	68% (untreated)	Not reported	42 months	Not reported
Bendamustine and rituximab [Rummel *et al.* 2013]	22	Not reported	Not reported	Not reported	69 months	Not reported
Carfilzomib, dexamethasone, rituximab (CARD) [Treon *et al.* 2014]	31	87%	68% (untreated)	2.1 months	Not reached at 20 months	Not reported
Ibrutinib [Treon *et al.* 2015a]	64	91%*	73% (previously treated)	4 weeks	2-year: 68%	2-year: 95%

*No complete responses were observed.

Hematology Annual Meeting in Orlando, Florida, USA [Dimopoulos *et al.* 2015]. A total of 31 WM patients, who were refractory to rituximab, were started on ibrutinib 420 mg orally once daily until disease progression or unacceptable toxicity. Rituximab refractoriness was defined as either relapse within 12 months of rituximab therapy or failure to achieve at least a minor response to rituximab. The median age was 67 years, 42% had high-risk IPSSWM, and 68% had 3 or more lines of therapy. With a median follow up of 7.7 months, the overall and major response rates were 84% and 65%, respectively. Median IgM level was 3830 mg/dl, which declined by more than 50% by the end of cycle 1. Median hemoglobin increased from 10.3 g/dl to 11.4 g/dl. Grade 3 or 4 adverse events were seen in 52% of patients; the most common was neutropenia (13%), followed by anemia, diarrhea, hypertension and thrombocytopenia (6% each).

A comparison of response and survival outcomes between ibrutinib and other common regimens used in WM patients is shown in Table 1.

Clinical experience

Although the studies available were performed in WM patients with relapsed or refractory WM patients, the approval by the FDA supports the use of ibrutinib in previously-untreated symptomatic WM patients. On the other hand, the EMA approved ibrutinib in relapsed patients and supported ibrutinib therapy in untreated patients who are not candidates for chemoimmunotherapy. A study on single-agent ibrutinib as a frontline therapy in WM patients is ongoing in the US [ClinicalTrials.gov identifier: NCT02604511].

Patients who begin treatment with ibrutinib should get a baseline complete blood count (CBC), comprehensive metabolic panel (CMP), serum protein electrophoresis (SPEP) and 12-lead electrocardiogram. Patients should be seen monthly for the first 3 months with CBC, CMP and IgM levels, and every 3 months thereafter. Responses are observed earlier after initiation of ibrutinib therapy in MYD88-only mutated WM patients. Overall responses are seen in 91% of MYD88-only mutated patients within 3 cycles

of ibrutinib but in 76% of MYD88 and CXCR4-mutated patients by cycle 9. Major responses are expected in 74% of MYD88-only mutated patients within the first 3 cycles of therapy, but in 52% MYD88 and CXCR4-mutated patients within 9 months of therapy [Treon *et al.* 2015a]. Clinicians should be aware of this marked difference in time and depth of response based on genomic profile, and not to truncate potentially-effective ibrutinib therapy too early in MYD88 and CXCR4-mutated WM patients.

Approximately, 75% of patients with WM meet criteria for initiation of therapy on the basis of anemia. A quarter of the patients, however, meet criteria on the basis of hyperviscosity, lymphadenopathy, splenomegaly or neuropathy, among others. Based on the results of the phase II study, 68% of patients with lymphadenopathy, 57% of patients with splenomegaly, and 56% of patients with neuropathy experienced improvement of extramedullary symptoms after initiation of ibrutinib [Treon *et al.* 2015a]. Also, four patients who presented with hyperviscosity requiring plasmapheresis stopped plasmapheresis two cycles into ibrutinib therapy.

Bleeding has been reported as one of the adverse events associated with ibrutinib therapy, usually manifested as mucocutaneous or associated with invasive procedures [Byrd *et al.* 2013; Wang *et al.* 2013; Burger *et al.* 2015; Treon *et al.* 2015a]. An *in vitro* study showed that ibrutinib affects platelet aggregation induced by collagen and platelet adhesion onto von Willebrand factor (vWF). Restoration of platelet aggregation capabilities occurred within 60 hours of stopping ibrutinib exposure and also after addition of untreated platelets [Levade *et al.* 2014]. A separate *in vivo* study in 23 patients receiving ibrutinib confirmed the effect of ibrutinib on collagen-mediated platelet aggregation but showed that adenosine-mediated aggregation was not affected. The aggregation defects mediated by ibrutinib were fully reversible within 1 week of cessation and recurred within 1 week of reinitiation of ibrutinib therapy [Kamel *et al.* 2015]. A study on 85 patients with chronic lymphocytic leukemia showed in a multivariate model, that epinephrine closure prolongation as measured by platelet function analysis, low serum factor VIII (FVIII) levels and low vWF activity before initiation of ibrutinib therapy were associated with a higher risk of bleeding [Lipsky *et al.* 2015]. On the other hand, plasma clotting times do not seem to be affected by ibrutinib [Rigg *et al.* 2015]. Given the current evidence, the concurrent use of aspirin, other nonsteroidal anti-inflammatory drugs, other antiplatelet agents, anticoagulants and supplements associated with bleeding, such as fish oils, should be used with caution and only when strictly necessary in patients receiving ibrutinib. In patients who experience significant bleeding, stopping ibrutinib should be the first course of action with consideration of platelet transfusions depending on the severity of the bleeding.

The occurrence of atrial fibrillation has been described in patients receiving ibrutinib therapy with reported rates between 5–10% [Byrd *et al.* 2013; Wang *et al.* 2013; Burger *et al.* 2015; Treon *et al.* 2015a]. The mechanisms behind ibrutinib-induced atrial fibrillation are not well understood. BTK is expressed in cardiac tissue in patients with atrial fibrillation with higher BTK transcripts in atrial tissue in atrial fibrillation in comparison with sinus rhythm [McMullen *et al.* 2014]. BTK regulates the phosphoinositide 3-kinase (PI3K)-Akt pathway, a regulator of cardiac protection. A study has shown than mice with reduced PI3K-Akt activity had higher susceptibility to atrial fibrillation, and that surgical specimens from patients with atrial fibrillation showed lower PI3K-Akt activity than samples from patients in sinus rhythm [Pretorius *et al.* 2009]. In an *in vitro* study, rat myocytes were exposed to ibrutinib and were associated with reduced expression of PI3K, particularly the p110-alpha subunit, the dominant cardioprotective isoform of PI3K [Pretorius *et al.* 2009]. The management of atrial fibrillation in patients on ibrutinib has not been standardized but prompt initiation of anticoagulation and cardiology referral for rate or rhythm control should be sought. In patients who develop atrial fibrillation and are highly symptomatic from it or hard to control with standard cardiologic interventions, switching therapies should be considered.

In patients in whom withholding of ibrutinib is needed, increases in IgM levels have been seen, ensuing usually within 1 week of drug withholding. The increase in IgM does not represent abrupt progression of disease, and typically subsides once ibrutinib therapy is reintroduced. This is a similar phenomenon to the abrupt reversal of ibrutinib-related lymphocytosis seen in patients with CLL in whom ibrutinib therapy is temporarily withheld [Advani *et al.* 2013]. At the present time, there is no evidence that ibrutinib therapy

can be stopped at any time during the course of treatment in WM patients.

Future directions

At this time, single-agent ibrutinib should be considered the standard of care for patients with relapsed or refractory WM, specifically in patients carrying the MYD88 L265P gene mutation. The use of ibrutinib in the frontline setting is certainly reasonable in patients who are not candidates for chemoimmunotherapy, and should be further supported by the results of ongoing studies.

The clinical development of ibrutinib in WM continues. A logical next step is to combine ibrutinib with other active agents looking for biological synergy that could translate into deeper and longer responses. The INNOVATE study has just been closed to accrual [ClinicalTrials.gov identifier: NCT02165397]. In this phase III study, approximately 180 patients with relapsed or refractory WM were randomized to rituximab and ibrutinib (Arm A) or rituximab and placebo (Arm B). The study is undergoing analysis and results are eagerly awaited.

Preclinical studies support the combination of BTK inhibitors and proteasome inhibitors in lymphoma as well as myeloma cells [Dasmahapatra *et al.* 2013; Eda *et al.* 2014], supporting the clinical use of ibrutinib in combination with bortezomib, carfilzomib or ixazomib. Other agents that could be used in combination with ibrutinib in WM patients are the PI3K-inhibitor, idelalisib and the BCL2-antagonist, venetoclax, which have shown preliminary clinical efficacy in WM [Gopal *et al.* 2014; Gerecitano *et al.* 2015]. Single-agent studies on idelalisib [ClinicalTrials.gov identifier: NCT02439138] and venetoclax [ClinicalTrials.gov identifier: NCT02677324] in relapsed or refractory WM patients are undergoing accrual.

Other BTK inhibitors are currently undergoing clinical development. Acalabrutinib (ACP-196) was just recommended for orphan drug designation in Europe specifically for WM, and clinical studies in patients with WM are ongoing [ClinicalTrials.gov identifier: NCT02180724]. Acalabrutinib has shown to be well-tolerated and effective in patients with CLL [Byrd *et al.* 2016]. CC-292 and ONO-4959 are also under clinical development in CLL [ClinicalTrials.gov identifiers: NCT01744626, NCT01659255].

Conclusion

The oral BTK inhibitor ibrutinib is the only FDA-approved medication for patients with symptomatic WM. Ibrutinib has shown great efficacy at inducing deep and durable IgM responses, as well as improving hematologic parameters in patients with WM. As such, ibrutinib has changed how we manage patients with WM, although we all have to be mindful of its particular toxicity profile. Additional research is ongoing to investigate other molecularly-targeted agents in WM such as the PI3K-inhibitor, idelalisib and the BCL-2-antagonist, venetoclax, among others.

Funding

Research funding was provided by Pharmacyclics (USA) and AbbVie (USA).

Conflict of interest statement

The authors declare that there is no conflict of interest.

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