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REVIEW



Novel approaches to targeting MYD88 in Waldenström macroglobulinemia

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ABSTRACT

Introduction: Waldenström macroglobulinemia (WM) is an incurable lymphoma characterized by the accumulation of IgM-producing lymphoplasmacytic cells in the bone marrow and other organs. Although WM patients can experience prolonged remissions, the disease invariably recurs advocating for the need of novel treatments in order to achieve higher response and survival rates. The discovery of a recurrent mutation in the MYD88 gene and an increased understanding behind the biology of MYD88 signaling have provided the opportunity to developing novel agents targeting the MYD88 pathway.

Areas covered: The present review focuses on potential therapies that could change the landscape of treatment of patients with WM, specifically focusing on inhibitors of the Bruton tyrosine kinase (BTK), phosphatidylinositol-3 kinase, hematopoietic cell kinase, interleukin-1 receptor associated kinase and MYD88 assembly.

Expert commentary: Novel agents such as the BTK inhibitor ibrutinib has shown to be safe and highly effective in the treatment of WM. Ibrutinib has been approved in Europe and the United States for its use in patients with symptomatic WM. Prospective studies are ongoing and/or planned to study many other novel agents alone and in combination with aims at improving response, survival and quality of life in patients with WM.

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1. Introduction

Waldenström macroglobulinemia (WM) is a rare lymphoma, characterized by the accumulation of malignant IgM-secreting lymphocytes, lymphoplasmacytoid cells, and plasma cells in the bone marrow and other organs such as lymph nodes and spleen [1]. The median overall survival of patients with WM has improved in recent years and is now approximating a decade, as shown in recent population-based studies [2,3]. The more prolonged survival of patients with WM is likely due to improved treatments and supportive therapies as well as a higher involvement of patients and families in the care of the patient. However, WM remains incurable, despite intensity of therapy, and agents with novel mechanisms of action are needed.

In 2012, a recurrent mutation in the MYD88 gene (MYD88 L265P) was identified in over 90% of patients with WM [4], finding that has been independently validated by several other research centers around the world [5–8]. Interestingly, other MYD88 non-L265P gene mutations have also been identified, although they are rare [9]. With a deeper understanding of the biology of MYD88 signaling, it is likely that the treatment of WM can be improved by targeting MYD88-driven pathways. This review will focus on potential targets associated with MYD88 signaling including the Bruton tyrosine kinase (BTK), phosphatidylinositol-3 kinase (PI3K), toll-like receptors (TLRs), hematopoietic cell kinase (HCK), and interleukin-1 receptor-associated kinase (IRAK) pathways, as well as targeting the MYD88 molecule itself.

2. The MYD88 pathway

MYD88 is an adaptor molecule in all TLRs, except TLR3 and interleukin-1 receptor (IL-1R) as well as interleukin-18 receptor (IL-18R) signaling. After stimulation of the TLRs or IL-1R or IL-18R, MYD88 is recruited to the activated receptor complex as a homodimer and forms complexes with IRAK4, the so-called ‘Myddosome,’ leading to activation of IRAK1 and IRAK2. Tumor necrosis factor receptor-associated factor 6 (TRAF6) is then activated by IRAK1, leading to phosphorylation of I κ B- α and activation of NF- κ B. A depiction of the MYD88 activation pathway is shown in Figure 1.

3. BTK inhibition

In WM cell lines, more robust MYD88 co-immunoprecipitation was observed with phospho-BTK in MYD88 L265P-expressing cells versus MYD88 wild-type cells [10]. In MYD88 L265P-expressing cells, exposure to the BTK inhibitor ibrutinib reduced binding of BTK and MYD88. Furthermore, phospho-BTK expression was also significantly decreased when WM cells were treated with an inhibitor of MYD88 signaling. Conversely, MYD88 L265P overexpression stimulated BTK activation in WM cells. Preclinically, the downstream effects of ibrutinib included inhibition of I κ B- α phosphorylation, with resulting blockade of NF- κ B signaling. Ibrutinib also induced higher levels of killing in MYD88 L265P-expressing cells than in wild-type cells. In all, current evidence supports BTK as a downstream target of MYD88 L265P signaling.

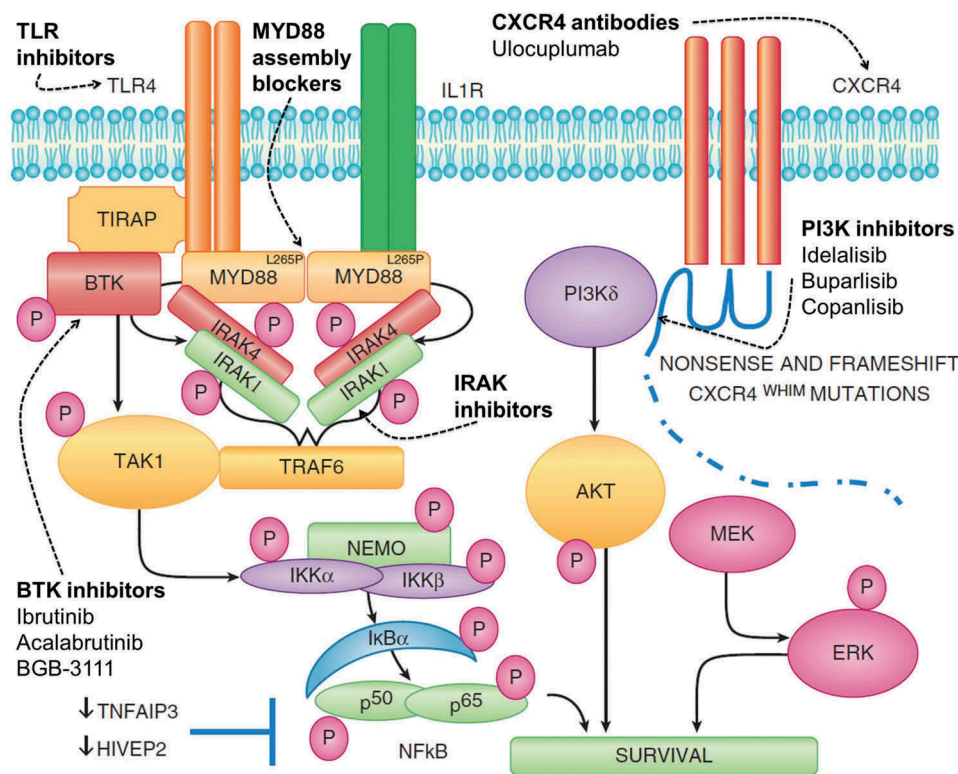


Figure 1. The MYD88 activation pathway. Dashed lines indicate inhibition.

A single-arm, investigator-initiated phase II study evaluated ibrutinib at 420 mg PO daily in 63 patients with previously treated symptomatic WM [11]. High response rates were observed with an overall response rate (ORR) of 91%, a major response of 73%, and a median time to response of 4 weeks. The response rates associated with the genomic profile of WM patients. The major response rate in patients with MYD88 but no CXCR4 mutations was 92% versus 62% in MYD88- and CXCR4-mutated patients. Among MYD88 wild-type patients ($n = 5$), no major responses were observed. Grade 3 or 4 adverse events included neutropenia, thrombocytopenia, anemia, atrial fibrillation, pneumonia, herpes zoster, endocarditis, subcutaneous abscess, urinary tract infection, hematoma, and syncope. Based on these results, ibrutinib was approved by the US FDA and the European Medicines Agency to treat symptomatic WM. A study evaluating ibrutinib in previously untreated symptomatic WM patients is completing accrual (NCT02604511).

In the Arm C of the INNOVATE study, a total of 31 WM patients who were refractory to rituximab were started on ibrutinib at 420 mg PO once daily until disease progression or unacceptable toxicity. With a median follow-up time of 8 months, the overall and major response rates were 84% and 65%, respectively. Grade 3 or 4 adverse events were seen in 52% of patients and included neutropenia, anemia, diarrhea, hypertension, and thrombocytopenia [12].

Acalabrutinib (ACP-196) is a second-generation BTK inhibitor that appears to have greater selectivity to BTK than ibrutinib [13]. Acalabrutinib does not inhibit HCK [14]. HCK is regulated by MYD88 signaling, seems to mediate WM cell survival, and appears relevant for the effect of ibrutinib [15]. It is not clear if

this biological difference between ibrutinib and acalabrutinib has any actual clinical implication. *In vivo* studies have shown that thrombus formation is unaffected in mice exposed to acalabrutinib, while thrombus formation was inhibited with ibrutinib. Clinically, acalabrutinib has shown an ORR of 95% in previously treated chronic lymphocytic leukemia (CLL) patients. The ORR was 100% in patients with 17p deletion [14]. No bleeding or atrial fibrillation was observed although follow-up can be considered short. A phase Ib/II study in previously treated patients with WM is ongoing (NCT02180724).

Other BTK inhibitors undergoing clinical development in hematologic malignancies are BGB-3111, CC-292 (AVL-292), and ONO-4059 (GS-4059). BGB-3111 has demonstrated nanomolar BTK inhibition with a more restricted off-target activity than ibrutinib [16]. Specifically, BGB-3111 did not inhibit rituximab-induced NK cell interferon secretion, unlike ibrutinib, consistent with weak interleukin-2-inducible T cell kinase inhibition. The survival of mouse models was longer on BGB-3111 than on ibrutinib. BGB-3111 has shown to be safe in a phase I study in patients with relapsed/refractory B-cell malignancies, including observed responses in five of six WM patients [17]. A recent abstract reported an experience on 31 WM patients receiving oral doses of BGB-3111 between 40 and 320 mg daily [18]. After a median follow-up of 8 months, the ORR was 92% with a major response rate of 83%. The median serum IgM level decreased from 2990 to 300 mg/dl, and the median hemoglobin increased from 10.1 to 13.5 g/dl. The median time to response was 29 days. Lymphadenopathy was present in eight patients at baseline and improved with therapy in all patients. CC-292 has shown anti-BTK activity in kinase assays and anti-B-cell receptor

(BCR) signaling in lymphoma and myeloma cell lines [19]. ONO-4059 has also shown activity against BTK and BCR signaling [20]. A phase I dose-escalation study evaluated ONO-4059 in patients with B-cell lymphomas including three patients with WM and showed efficacy with a favorable toxicity profile [21].

An important issue is the resistance to BTK inhibition already emerging in CLL and mantle cell lymphoma (MCL), typically associated with mutations in BTK C481S and PLC γ 2 [22]. Recent studies evaluated mechanisms of resistance in patients with WM [23]. Not surprisingly, BTK and PLC γ 2 mutations were associated with ibrutinib resistance in WM. However, CARD11 mutations were also identified. Most of these mutations were subclonal to MYD88. Interestingly, the development of BTK mutations was identified only in WM patients carrying CXCR4 mutations, supporting genomic instability as the basis of clonal evolution.

4. PI3K inhibition

Preclinically, Ingenuity Pathway Analysis showed significant enrichment for BCR, PI3K/AKT, and ERK/MAPK signaling following MYD88 activation in WM cell lines. Western blot analysis confirmed the activation of AKT as a downstream effect of PI3K activation. Furthermore, exposure to a MYD88 inhibitor significantly decreased phosphorylation of AKT-S473 and AKT-T308. Finally, exposure to WM cells to the PI3K-delta inhibitor idelalisib mediated robust cell killing with EC₅₀ of 30–50 nM [24].

In a phase II study in patients with relapsed and/or refractory B-cell lymphomas, 10 patients with WM were exposed to idelalisib 150 mg PO twice daily (BID) [25]. From these, eight patients (80%) experienced at least a 25% decrease in lymphadenopathy. It is unclear if there were any responses based on serum IgM levels, which is the standard marker of response in patients with WM. Also, no data were provided on the toxicity profile of idelalisib specifically in WM patients in this study. Given these encouraging results, a single-arm phase II study was conducted evaluating idelalisib at 150 mg PO BID for 6 months followed by 150 mg PO QD until disease progression or unacceptable toxicity in patients with previously treated WM [26]. The study was aimed at accruing 30 patients but was terminated early due to liver toxicity. Patient 1 died of disease progression. Patients 2–4 were exposed to idelalisib experienced an increase in liver enzymes that was considered grade 3 or higher. Idelalisib was held and dose-reduced according to protocol with recurrence of toxicity. In March 2016, Gilead Sciences stopped six prospective studies with idelalisib combinations in patients with hematologic malignancies due to an increased mortality rate associated with cytomegalovirus (CMV) reactivation and *Pneumocystis jiroveci* pneumonia. Patients who were still receiving active therapy decided to stop idelalisib. All surviving patients were tested for CMV viral load without evidence of active infection. Patient 5 had experienced a decrease in serum IgM level of 16% (stable disease) without liver toxicity at day 28, when the study was terminated.

Buparlisib (NVP-BKM120) is an oral pan-class I PI3K inhibitor that has shown efficacy in lymphomas. Specifically, exposure to buparlisib decreased cell proliferation and adhesion and increased apoptotic death via upregulation of the proapoptotic PUMA and BIM and downregulation of the antiapoptotic BCL-XL and MCL-1 in diffuse large B-cell lymphoma (DLBCL)

and WM cells [27,28]. A study evaluating buparlisib and rituximab in patients with indolent B-cell lymphomas, including WM, is ongoing (NCT02049541). Other PI3K inhibitors of interest in WM are the dual gamma/delta inhibitor duvelisib (IPI-145), which has shown efficacy in CLL, and the pan-inhibitor copanlisib (BAY80-6946), which has shown efficacy in follicular lymphoma and DLBCL [29,30].

5. IRAK inhibition

MYD88 L265P activates multiple downstream signaling pathways including BTK, PI3K, and IRAK1/IRAK4 that support malignant cell growth and survival [10,31]. Ibrutinib targets BTK and shows high overall and major clinical response rates, though no complete responses are observed, suggesting the presence of alternative survival signaling. Phospho-flow analysis of lymphoplasmacytic cells taken from the bone marrow of WM patients after 6 months of ibrutinib therapy demonstrated highly active IRAK1 and IRAK4, but not BTK. These findings prompted further investigation of the impact of IRAK1 and IRAK4 in supporting WM cell survival [32]. Using lentiviral transduction, shRNAs were identified, which produced reduction of protein levels for both IRAK1 and IRAK4. Compared to scrambled control vector, knockdown of IRAK1 or IRAK4 produced decreased tumor cell survival in MYD88-mutated cells. Treatment with ibrutinib and IRAK4/IRAK1 inhibitor of primary WM cells from untreated patients and of cells of WM patients on ibrutinib therapy and MYD88-mutated WM cells lines resulted in more robust reductions in NF- κ B signaling, suggesting a synergistic effect even in cells previously exposed to ibrutinib. These findings would support pursuing clinical trials using concurrent inhibition of BTK and IRAK1/IRAK4. The IRAK4 inhibitor PF-06650833 is being evaluated in healthy subjects (NCT02485769).

6. HCK inhibition

HCK is a member of the SRC family of tyrosine kinases, and it is one of the most aberrantly upregulated genes in WM cells [33]. PCR and Western blot techniques have shown that HCK transcription was increased in WM cell lines expressing MYD88 L265P and MYD88 S222R, but HCK expression was virtually absent in MYD88 WT cells. Additionally, HCK knockdown by lentiviral transduction resulted in sustained reduction in WM cell viability when compared with cells transduced with a control vector. Transduction of HCK in WM cells lines triggered PI3K/AKT, MAPK, and BTK, while knockdown of HCK induced a reciprocally contrasted pattern of decreased PI3K/AKT, MAPK, and BTK signaling. Interestingly, ibrutinib seems to bind to HCK and affect HCK Tyr⁴¹¹ phosphorylation in MYD88-expressing WM cells. These findings suggest that HCK is a downstream target of MYD88, which promotes WM cell survival signaling via activation of PI3K/AKT, MAPK, and BTK. IRAK activation was not impacted by HCK overexpression or knockdown, suggesting that IRAK activation might be independent of HCK signaling in WM cells.

7. Inhibition of Myddosome assembly

MYD88 dimerization seems to be necessary for assembly of the Myddosome, a structure composed of MYD88 dimers that recruit

and activate IRAK1/2/4. The MYD88:IRAK4:IRAK1/2 complex can then trigger NF- κ B activation mediating cell growth and survival signaling [10,31,34]. The Myddosome can also activate BTK, which can promote NF- κ B signaling in mutated MYD88 cells [10]. The MYD88 protein is composed of an N-terminal death domain, an intermediate linker domain, and a C-terminal Toll/IL1 receptor (TIR) domain. In normal circumstances, upon ligand binding to TLR/IL1R, the TIR domain facilitates binding to the receptor complex, promoting MYD88 dimerization. In contrast, mutated MYD88 protein can assemble without external stimuli and promote constitutive NF- κ B activation [4,10,31]. Previous studies have shown that mutations on Glu¹⁹⁶ in the TIR domain interfered with TIR-mediated MYD88 dimerization and IRAK recruitment and reduced NF- κ B activation [35]. Recent preclinical studies have evaluated vector-mediated transduction of mini-peptides targeting Glu¹⁹⁶ into WM cells [36]. Expression of MYD88^{181–202} blocked growth of MYD88-mutated WM cells, but did not impact the growth of MYD88 WT cells. Other mini-peptides targeting Ser²⁵⁷ and Arg³⁰¹ coding for MYD88^{256–292} and MYD88^{295–302} showed weak induction of apoptosis and reduction of pro-survival signaling without growth-inhibitory effect. Interestingly, the transduction of DD domain MYD88^{40–85} minipeptides was associated with increased apoptosis, sustained cell growth inhibition as well as reduced NF- κ B activation. Based on these findings, efforts are underway to develop stapled peptides and peptidomimetics to block MYD88 assembly.

8. Expert commentary

Mutations in the MYD88 gene are recurrent in WM and found in over 90% of WM patients. Not surprisingly, this discovery has focused research interests on targeting MYD88 through a series of novel approaches, such as BTK, PI3K, IRAK, and HCK, as members of the Myddosome, and finally MYD88 assembly itself. Table 1 highlights data from selected agents undergoing development in WM. Of these, BTK inhibition is the one more extensively developed. Ibrutinib is already approved by the FDA and the EMA for the treatment of symptomatic patients with WM. Acalabrutinib and BGB-311 appear very effective as well and might have a different, and desirable, safety profile. BeiGene, the manufacturer of BGB-3111, has announced the initiation of a phase III randomized trial to determine whether

BGB-3111 is superior to ibrutinib in patients with symptomatic WM, regardless of prior therapy. BGB-3111 will be administered at 160 mg PO twice daily and ibrutinib at 420 mg PO once daily. The outcome of interest is deep responses, measured by the combination of complete and very good partial responses [37]. BTK inhibition, however, is not curative in WM, and the next logical step is to use BTK inhibitors in combination with other agents. The randomized INNOVATE study evaluating rituximab $\pm$ ibrutinib has completed accrual, and the results are eagerly awaited (NCT02165397). The INNOVATE study enrolled 181 symptomatic WM patients regardless of prior therapy in almost 50 study locations, with a primary outcome of progression-free survival.

PI3K inhibition has been less successful in WM. A phase II study evaluating idelalisib in relapsed/refractory WM patients had to be stopped early due to hepatic toxicity. However, other PI3K inhibitors with distinct safety profiles might be of value, and prospective clinical trials are encouraged, as preclinical data on PI3K inhibition have shown glimpses of efficacy. IRAK and HCK inhibition has shown preclinical promise, but no clinical data are available at this time. Once IRAK and HCK inhibitors are clinically available, well-designed prospective studies will be warranted.

Although targeting MYD88 has the potential to revolutionize the treatment of patients with WM, we have to acknowledge that other targets might be of interest, especially other targets that could potentially potentiate the activity of MYD88-acting agents. Among these, CXCR4-directed therapy is of great interest. CXCR4 mutations have been detected in over 40% of patients with WM [38]. Over 40 mutations have been identified, and in some cases, more than one CXCR4 mutation has been identified in the same patient. CXCR4 mutations can be divided in frameshift and non-sense and appear to have distinct clinical features, as well as associated with distinct response properties to ibrutinib [34]. CXCR4 can be potentially targeted with monoclonal antibodies. Ulocuplumab is a fully human IgG4 monoclonal antibody that has shown preclinical efficacy in CLL cell lines, inducing cell death at a nanomolar concentration in the absence or presence of stromal support [39]. Similarly, in myeloma cell lines, ulocuplumab regulates extramedullary dissemination by modulating epithelial-to-mesenchymal transition transcriptional patterns [40]. Anti-CXCR4 therapy in combination with BTK inhibition would be highly active in CXCR4-mutated WM patients.

Table 1. Selected drugs in current clinical development for the treatment of Waldenström macroglobulinemia.

Agent	Target	Study phase	Setting	ORR	PFS	OS
Ibrutinib	BTK inhibitor	II	Rel/Ref	91%	2 years: 69%	2 years: 95%
Ibrutinib	BTK inhibitor	II	Rel/Ref to R	90%	18 months: 86%	18 months: 97%
Ibrutinib	BTK inhibitor	II	Untreated	–	–	–
Ibrutinib + R vs. R alone	BTK inhibitor	III	Untreated, Rel/Ref	–	–	–
Acalabrutinib	BTK inhibitor	II	Rel/Ref	–	–	–
BGB-3111	BTK inhibitor	I/II	Rel/Ref	92%	–	–
BGB-3111 vs. ibrutinib	BTK inhibitor	III	Untreated, Rel/Ref	–	–	–
Idelalisib	PI3K inhibitor	II	Rel/Ref	Study stopped early due to liver toxicity		
Buparlisib	PI3K inhibitor	I/II	Rel/Ref	–	–	–
Ulocuplumab	CXCR4 antibody	I/II	Rel/Ref	–	–	–
Venetoclax	BCL2 antagonist	II	Rel/Ref	–	–	–
Daratumumab	CD38 antibody	II	Rel/Ref	–	–	–

ORR: overall response rate; PFS: progression-free survival; OS: overall survival; Rel/Ref: relapsed/refractory; R: rituximab; BTK: Bruton tyrosine kinase; PI3K: phosphatidylinositol-3 kinase; CXCR4: C-X-C motif chemokine receptor 4; BCL2: B-cell lymphoma 2; CD38: cluster of differentiation 38 (a plasma cell marker).

Other novel targets of interest include BCL2 and CD38. The BCL2 antagonist venetoclax has shown to be safe and effective in CLL patients. The FDA granted approval to venetoclax for the treatment of patients with del17p CLL. BCL2 is highly overexpressed in WM cells [41]. Recent transcriptome analysis has confirmed these findings in WM cells, regardless of their MYD88 or CXCR4 mutational status [42]. A phase II prospective study evaluating venetoclax in relapsed and/or refractory patients with WM is underway (NCT02677324). Preclinical data have shown synergism in cell killing induction with concurrent BTK and BCL2 inhibition, making it an interesting clinical question for the near future. Finally, CD38 is highly expressed by the lymphoplasmacytic as well as the plasma cell components in WM patients [43,44]. The anti-CD38 monoclonal antibody daratumumab, given its safety and efficacy profiles, has recently been approved for its use in previously treated patients with multiple myeloma. Combinations including agents directed against CD20, BTK, CD38, BCL2 and/or CXCR4 could have the potential of positively transforming the treatment of patients with WM by increasing efficacy.

9. Five-year view

The field of WM therapy will move toward genomically driven personalization. The identification of the MYD88 L265P mutation as well as the CXCR4 mutations has lead us to profile patients and recommend therapies based on such profiles. For example, patients with MYD88 mutation and without CXCR4 mutation will greatly benefit from ibrutinib therapy when compared to patients without MYD88 mutation. In patients who carry both MYD88 and CXCR4 mutations and prompt control of the disease is desirable, then using bendamustine and rituximab might be a better option than ibrutinib, as responses can be delayed in double-mutant WM patients. Finally, in patients without MYD88 or CXCR4 mutations, the use of bendamustine and rituximab or bortezomib, dexamethasone, and rituximab might be better options, as these patients do not derive significant clinical benefit from ibrutinib therapy.

Using next-generation sequencing as well as transcriptome analysis, other recurrent alterations have been identified in patients with WM, which include mutations in ARID1A (15–20%), CD79B (10–15%), and TP53 (5%), among others [42,45]. The clinical implications of the presence of these abnormalities is at this time unknown, but it could help dictate therapeutic decisions in the near future.

Key issues

- The MYD88 L265P gene mutation is identified in 90–95% of patients with WM, and can help define the disease.
- CXCR4 mutations can be seen in 30–40% of patients with WM, and can confer resistance to ibrutinib manifested as slower, more superficial and shorter responses.
- The BTK inhibitor ibrutinib is safe, highly effective, and approved by the FDA for the treatment of patients with symptomatic WM.

- Other targeted agents of interest in WM include venetoclax (BCL2 antagonist), ulocuplumb (anti-CXCR4 monoclonal antibody) and daratumumab (anti-CD38 monoclonal antibody).

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References

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.

1. Swerdlow SH, Berger F, Pileri SA, et al. Lymphoplasmacytic lymphoma. In: Swerdlow SH, Campo E, Harris NL, editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon, France: IARC; 2008. p. 194–195.
2. Castillo JJ, Olszewski AJ, Cronin AM, et al. Survival trends in Waldenstrom macroglobulinemia: an analysis of the surveillance, epidemiology and end results database. *Blood*. 2014;123:3999–4000.
3. Castillo JJ, Olszewski AJ, Kanan S, et al. Overall survival and competing risks of death in patients with Waldenstrom macroglobulinemia: an analysis of the surveillance, epidemiology and end results database. *Br J Haematol*. 2015;169:81–89.
4. Treon SP, Xu L, Yang G, et al. MYD88 L265P somatic mutation in Waldenstrom's macroglobulinemia. *N Engl J Med*. 2012;367:826–833.
- This is the first report on the presence of MYD88 L265P gene mutations in WM.
5. Jimenez C, Sebastian E, Chillon MC, et al. MYD88 L265P is a marker highly characteristic of, but not restricted to, Waldenstrom's macroglobulinemia. *Leukemia*. 2013;27:1722–1728.
6. Mori N, Ohwashi M, Yoshinaga K, et al. L265P mutation of the MYD88 gene is frequent in Waldenstrom's macroglobulinemia and its absence in myeloma. *Plos One*. 2013;8:e80088.
7. Poulain S, Roumier C, Decambre A, et al. MYD88 L265P mutation in Waldenstrom macroglobulinemia. *Blood*. 2013;121:4504–4511.
8. Varettoni M, Arcaini L, Zibellini S, et al. Prevalence and clinical significance of the MYD88 (L265P) somatic mutation in Waldenstrom's macroglobulinemia and related lymphoid neoplasms. *Blood*. 2013;121:2522–2528.
9. Treon SP, Xu L, Hunter Z. MYD88 mutations and response to ibrutinib in Waldenstrom's macroglobulinemia. *N Engl J Med*. 2015;373:584–586.
10. Yang G, Zhou Y, Liu X, et al. A mutation in MYD88 (L265P) supports the survival of lymphoplasmacytic cells by activation of Bruton tyrosine kinase in Waldenstrom macroglobulinemia. *Blood*. 2013;122:1222–1232.
11. Treon SP, Tripsas CK, Meid K, et al. Ibrutinib in previously treated Waldenstrom's macroglobulinemia. *N Engl J Med*. 2015;372:1430–1440.
- This is the prospective study that supported the FDA approval of ibrutinib in WM.
12. Dimopoulos MA, Trotman J, Tedeschi A, et al. Ibrutinib for patients with rituximab-refractory Waldenstrom's macroglobulinemia

- (INNOVATE): an open-label substudy of an international, multicentre, phase 3 trial. *Lancet Oncol.* **2017**;18:241–250.
13. Covey T, Barf T, Gulrajani M, et al. Abstract 2596: ACP-196: a novel covalent Bruton's tyrosine kinase (Btk) inhibitor with improved selectivity and *in vivo* target coverage in chronic lymphocytic leukemia (CLL) patients. *Cancer Res.* **2015**;75:2596.
 14. Byrd JC, Harrington B, O'Brien S, et al. Acalabrutinib (ACP-196) in relapsed chronic lymphocytic leukemia. *N Engl J Med.* **2016**;374:323–332.
 15. Yang G, Buhrlage SJ, Tan L, et al. HCK is a survival determinant transactivated by mutated MYD88, and a direct target of ibrutinib. *Blood.* **2016**;127:3237–3252.
 16. Li N, Sun Z, Liu Y, et al. Abstract 2597: BGB-3111 is a novel and highly selective Bruton's tyrosine kinase (BTK) inhibitor. *Cancer Res.* **2015**;75:2597.
 17. Tam C, Grigg AP, Opat S, et al. The BTK inhibitor, Bgb-3111, is safe, tolerable, and highly active in patients with relapsed/refractory B-cell malignancies: initial report of a phase 1 first-in-human trial. *Blood.* **2015**;126:832–832.
 18. Tam CS, Trotman J, Opat S, et al. High major response rate, including very good partial responses (VGPR), in patients (pts) with Waldenstrom macroglobulinemia (WM) treated with the highly specific BTK inhibitor Bgb-3111: expansion phase results from an ongoing phase I study. *Blood.* **2016**;128:1216–1216.
 19. Evans EK, Tester R, Aslanian S, et al. Inhibition of Btk with CC-292 provides early pharmacodynamic assessment of activity in mice and humans. *J Pharmacol Exp Ther.* **2013**;346:219–228.
 20. Yasuhiro T, Yoshizawa T, Daub H, et al. Abstract 2021: ONO-WG-307, a novel, potent and selective inhibitor of Bruton's tyrosine kinase (Btk), results in sustained inhibition of the ERK, AKT and PKD signaling pathways. *Cancer Res.* **2012**;72:2021.
 21. Walter HS, Rule SA, Dyer MJ, et al. A phase 1 clinical trial of the selective BTK inhibitor ONO/GS-4059 in relapsed and refractory mature B-cell malignancies. *Blood.* **2016**;127:411–419.
 22. Woyach JA. Patterns of resistance to B cell-receptor pathway antagonists in chronic lymphocytic leukemia and strategies for management. *Hematology Am Soc Hematol Educ Program.* **2015**;2015:355–360.
 23. Xu L, Tsakmaklis N, Yang G, et al. Acquired mutations associated with ibrutinib resistance in Waldenstrom macroglobulinemia. *Blood.* **2017**;129:2519–2525.
 24. Yang G, Liu X, Zhou Y, et al. PI3K/AKT pathway is activated by MYD88 L265P and use of PI3K-delta inhibitors induces robust tumor cell killing in Waldenstrom's macroglobulinemia. *Blood.* **2013**;122:4255.
 25. Gopal AK, Kahl BS, De Vos S, et al. PI3Kdelta inhibition by idelalisib in patients with relapsed indolent lymphoma. *N Engl J Med.* **2014**;370:1008–1018.
 26. Castillo JJ, Gustine JN, Meid K, et al. Idelalisib in Waldenstrom macroglobulinemia: high incidence of hepatotoxicity. *Leuk Lymphoma.* **2017**;58:1002–1004.
 27. Sahin I, Azab F, Mishima Y, et al. Targeting survival and cell trafficking in multiple myeloma and Waldenstrom macroglobulinemia using pan-class I PI3K inhibitor, buparlisib. *Am J Hematol.* **2014**;89:1030–1036.
 28. Zang C, Eucker J, Liu H, et al. Inhibition of pan-class I phosphatidylinositol-3-kinase by NVP-BKM120 effectively blocks proliferation and induces cell death in diffuse large B-cell lymphoma. *Leuk Lymphoma.* **2014**;55:425–434.
 29. Balakrishnan K, Peluso M, Fu M, et al. The phosphoinositide-3-kinase (PI3K)-delta and gamma inhibitor, IPI-145 (Duvelisib), overcomes signals from the PI3K/AKT/S6 pathway and promotes apoptosis in CLL. *Leukemia.* **2015**;29:1811–1822.
 30. Patnaik A, Appleman LJ, Tolcher AW, et al. First-in-human phase I study of copanlisib (BAY 80-6946), an intravenous pan-class I phosphatidylinositol 3-kinase inhibitor, in patients with advanced solid tumors and non-Hodgkin's lymphomas. *Ann Oncol.* **2016**;27:1928–1940.
 31. Ngo VN, Young RM, Schmitz R, et al. Oncogenically active MYD88 mutations in human lymphoma. *Nature.* **2011**;470:115–119.
 32. Yang G, Liu X, Chen J, et al. Targeting IRAK1/IRAK4 signaling in Waldenstrom's macroglobulinemia. *Blood.* **2015**;126:4004–4004.
 33. Gutierrez NC, Ocio EM, De Las Rivas J, et al. Gene expression profiling of B lymphocytes and plasma cells from Waldenstrom's macroglobulinemia: comparison with expression patterns of the same cell counterparts from chronic lymphocytic leukemia, multiple myeloma and normal individuals. *Leukemia.* **2007**;21:541–549.
 34. Xu L, Hunter ZR, Tsakmaklis N, et al. Clonal architecture of CXCR4 WHIM-like mutations in Waldenstrom macroglobulinaemia. *Br J Haematol.* **2016**;172:735–744.
 35. Loiarro M, Volpe E, Ruggiero V, et al. Mutational analysis identifies residues crucial for homodimerization of myeloid differentiation factor 88 (MyD88) and for its function in immune cells. *J Biol Chem.* **2013**;288:30210–30222.
 36. Liu X, Hunter ZR, Xu L, et al. Targeting Myddosome assembly in Waldenstrom macroglobulinaemia. *Br J Haematol.* **2017**;177:808–813.
 37. GlobeNewswire. BeiGene Initiates Global Phase III Trial of the BTK Inhibitor BGB-3111 in Waldenström's Macroglobulinemia. **2017** [cited February 10]. Available from: <https://globenewswire.com/news-release/2017/01/25/910721/0/en/BeiGene-Initiates-Global-Phase-III-Trial-of-the-BTK-Inhibitor-BGB-3111-in-Waldenstr%C3%B6m-s-Macroglobulinemia.html>
 38. Hunter ZR, Xu L, Yang G, et al. The genomic landscape of Waldenstrom macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell lymphomagenesis. *Blood.* **2014**;123:1637–1646.
 - **This is the first study reporting on the identification of CXCR4 mutations in WM.**
 39. Kashyap MK, Kumar D, Jones H, et al. Ulocuplumab (BMS-936564 /MDX1338): a fully human anti-CXCR4 antibody induces cell death in chronic lymphocytic leukemia mediated through a reactive oxygen species-dependent pathway. *Oncotarget.* **2016**;7:2809–2822.
 40. Roccaro AM, Mishima Y, Sacco A, et al. CXCR4 regulates extramedullary myeloma through epithelial-mesenchymal-transition-like transcriptional activation. *Cell Rep.* **2015**;12:622–635.
 41. San Miguel JF, Vidrales MB, Ocio E, et al. Immunophenotypic analysis of Waldenstrom's macroglobulinemia. *Semin Oncol.* **2003**;30:187–195.
 42. Hunter ZR, Xu L, Yang G, et al. Transcriptome sequencing reveals a profile that corresponds to genomic variants in Waldenstrom macroglobulinemia. *Blood.* **2016**;128:827–838.
 43. Konoplev S, Medeiros LJ, Bueso-Ramos CE, et al. Immunophenotypic profile of lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia. *Am J Clin Pathol.* **2005**;124:414–420.
 44. Morice WG, Chen D, Kurtin PJ, et al. Novel immunophenotypic features of marrow lymphoplasmacytic lymphoma and correlation with Waldenstrom's macroglobulinemia. *Mod Pathol.* **2009**;22:807–816.
 45. Hunter ZR, Yang G, Xu L, et al. Genomics, signaling, and treatment of Waldenstrom macroglobulinemia. *J Clin Oncol.* **2017**. [Epub ahead of print].