Waldenström macroglobulinemia: From biology to treatment

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Abstract

Waldenström macroglobulinemia (WM) is distinct B-cell lymphoproliferative disorder primarily characterized by bone marrow infiltration of lymphoplasmacytic cells along with production of a serum monoclonal immunoglobulin M (IgM). In this review, we describe the biology of WM, the diagnostic evaluation for WM with a discussion of other conditions that are in the differential diagnosis and clinical manifestations of the disease as well as current treatment options. Within the novel agents discussed are everolimus, perifosine, enzastaurin, panobinostat, bortezomib and carfilzomib, pomalidomide, and ibrutinib. Many of the novel agents have shown good responses and have a better toxicity profile compared to traditional chemotherapeutic agents, which makes them good candidates to be used as primary therapies for WM in the future.
Introduction

Waldenström macroglobulinemia (WM) is a B-cell lymphoproliferative disorder characterized by bone marrow infiltration of lymphoplasmacytic cells along with production of a serum monoclonal immunoglobulin M (IgM)\cite{1,2}. WM first described by Jan Gosta Waldenström in 1944 by reporting two patients presented with oronasal bleeding, anemia, high serum viscosity, hepatosplenomegaly and infiltration of the BM by lymphoid cells. Unlike multiple myeloma, the patients had lymphocytic bone marrow infiltration rather than plasmacytic and no bone lesions or symptoms \cite{3,4}.

WM is classified as a lymphoplasmacytic lymphoma (LPL) according to World Health Organization (WHO) and the Revised European American Lymphoma (REAL) classificiations \cite{4,5}. WM is a rare cancer with an incidence rate of about 3 cases per million people per year in the United States.1,500 new cases are diagnosed with WM each year in the United States \cite{6,7}. The median age at diagnosis varies between 63 and 68 years \cite{1}. Men are twice more commonly affected compared to women and, incidence rates for white population are 2-3 fold higher than in black population \cite{8}.

Biology of WM

The cell of origin

WM is thought to originate from an arrested B cell that has undergone somatic hypermutation in germinal center without differentiating to plasma cells \cite{9}. It has been shown that WM may originate from an IgM+ and/or IgM+IgD+ memory B cell without initiating switching events. WM cells are characterized by a specific immunophenotype (positive for surface IgM, CD19, CD20, CD22, CD25, CD27, FMC7; negative for CD103, and variable CD5, 10, 23, CD138 expression), which help in differentiating from other NHL and multiple myeloma that appear morphologically similar.

Cytogenetic findings and copy number variations
The most common cytogenetic abnormality in WM patients is the deletion of the long arm of chromosome 6, which was reported in about 40-50% in recent studies [10-12]. Other studies demonstrated the presence of other cytogenetic abnormalities, including trisomy 4, trisomy 5, monosomy 8, and deletion of the long arm of chromosome 20 [10].

It has been reported that 83% of patients with WM have various chromosomal abnormalities [13]. Gain of 6p was the second most common abnormality (17%), which was always concomitant with 6q loss. B lymphocyte-induced maturation protein-1 (BLIMP-1) and tumor necrosis factor α-induced protein 3 (TNFAIP3 or A20) are the two candidate tumor suppressor genes mapping two distinct minimal deleted regions on the long arm of chromosome 6 [14,15]. BLIMP-1 is a transcription factor acting mainly as a repressor, which plays a crucial role in orchestrating the differentiation of B cells into plasma cells [16]. TNFAIP3 is an ubiquitin-modifying enzyme that acts as a negative regulator of TNF and toll-like receptor (TLR) mediated responses. Inactivating mutations were observed in TNFAIP3 and TNF receptor-associated factor 3 (TRAF3) resulting in activation of the nuclear factor kappa B (NF-kB) signaling pathways. Moreover, a minimal deleted region, including MIRN15A and MIRN16-1, was demonstrated on 13q14 in about 10-13% of patients [13,17].

**Whole genome sequencing**

Treon et al has recently reported the presence of MYD88 L265P somatic mutation (in chromosome 3p22.2) in over 90% of WM patients via whole genome sequencing [18]. High frequency of this mutation in WM patients (70-100%) was also confirmed by other studies via Sanger sequencing, polymerase chain reaction (PCR) or allele specific polymerase chain reaction (AS-PCR) [19-23]. MYD88 L265P was also detected in non-IgM LPL patients, suggesting that this mutation can be seen in LPL patients independent of isotype [18,24,25]. Recent studies also showed that this mutation might be seen in up to 80% of patients with IgM monoclonal gammopathy of undetermined significance (MGUS) [18,20,22,26]. In order to assess the impact of this mutation on the risk of
progression from IgM-MGUS to WM, Varettoni et al performed a case-control approach in IgM-MGUS patients and found that the MYD88 L265P mutation was associated with a significantly higher risk of progression to WM or to other lymphoproliferative disorders [20]. Together, these findings suggest a key role of MYD88 L265P somatic mutation for the development of WM. This nonsynonymous mutation causes a leucine (L) to proline (P) substitution in codon 265 (L265P) of MYD88, which is thought to be a crucial player in the activation of the canonical NF-κB pathway, downstream of TLR and interleukin-1 receptor (IL-1R) signaling [18,27-29]. When combined with immunoglobulin heavy chain variable gene (IGHV) mutation status, MYD88 L265P mutation appears to be a unique genetic signature as a diagnostic tool that distinguishes WM from other similar B cell malignancies [21]. The second most common somatic gene mutation in WM are C-terminal mutations, which are similar to those found in the germline of patients with WHIM syndrome [30]. Up to 30% of WM patients harbor CXCR4 mutations which enhances tumor dissemination and survival of WM cells [31,32].

**Epigenetics**

Epigenetic regulation of tumor suppressors and oncogenes play an important role in the development of many tumor types [33,34]. The main epigenetic changes include DNA methylation and histone modification [35]. Other regulators include non-coding RNAs such as miRNAs.

Upregulation of miRs including 155, 184, 206, 363, 494 and 542-3p, and downregulation of miR-9 are identified in WM cells. Among these, miRNA-155 was found to be a critical regulator of tumor growth and proliferation of WM cells both in vitro and in vivo [36]. Increased expression of miRNA-206 and reduced expression of miRNA-9 increased histone acetylation leading to enhanced gene transcription [37]. In addition, MIRN15A and MIRN16-1 are believed to act as tumor suppressors by preventing the transcription of mRNAs encoding for proteins involved in proliferation, cell cycle and anti-apoptosis, such as BCL-2 [38].
Gene Expression Profiling (GEP)

A study of GEP in Waldenström macroglobulinemia demonstrated that WM has a homogenous expression profile similar to that of patients with chronic lymphocytic leukemia (CLL) [39]. Another study showed that the genes including ATXN1, FMOD, LEF1 (WNT/b-catenin pathway), MARCKS discriminated WM cells from CLL cells [14]. Furthermore, a recent study performed GEP on WM BM CD19 + versus IgM MGUS BM CD19 + cells showed that 151 genes were differently expressed. PI3K/Akt/mTOR, JAK/STAT and MAPK signaling pathways were found to be the most important signaling pathways in these two disorders [40].

Proteomic analysis

Hatjiharissi et al employed protein expression profiling of untreated WM and normal bone marrow controls using antibody-based protein microarrays to demonstrate proteins that are dysregulated in WM. The study showed that Ras family proteins such as Rab-4 and p62DOK, as well as Rho family proteins such as CDC42GAP and ROK α were up-regulated by >2-fold in WM. Other proteins including cyclin-dependent kinases, apoptosis regulators, and histone deacetylases were up-regulated by >1.3-fold [41].

Deregulated molecular pathways

MYD88/IRAK

Recent findings of the existence of a mutation in MYD88 in most of WM patients have indicated that MYD88/IRAK signaling pathway might have a crucial role in pathogenesis of WM. In this pathway, upon TLR or IL-1R stimulation, interleukin-1 receptor-associated kinase 4 (IRAK4) recruited to the receptor by the adaptor MyD88, which then activates IRAK1 that ultimately resulting in activation of NF-κB [42]. However, the signaling cascade(s) by which MYD88 L265P induce NF-κB activation in WM remains unclear. Studies showed that targeting MYD88/IRAK signaling attenuated NF-κB signaling and survival of MYD88
L265P–expressing WM cells \[18,23]\). Moreover, Yang et al demonstrated increased phosphorylated Bruton tyrosine kinase (BTK) in WM cells transduced to overexpress L265P compared to wild-type MYD88 \[43\]. Taken together, these findings would suggest that MYD88 L265P triggers NF-κB via dual, which signal through BTK and/or IRAK1/IRAK4 \[44\]. Therefore, combined suppression of both BTK and IRAK signaling may provide synergistic inhibition of NF-κB signaling.

**PI3K/Akt/mTOR**

PI3K/AKT signaling inhibits apoptosis and regulates cell growth, survival, and proliferation \[45\]. Although there is no evidence that there are activating mutations in the PI3K/Akt/mTOR pathway in WM, it has been shown that this pathway is activated in WM \[46,47\]. Recently, Yang et al showed that MYD88 L265P can activate PI3K delta \[48\]. External stimulation through the bone marrow microenvironment with cytokines such as insulin-like growth factor (IGF-1) or stromal-derived factor (SDF-1) may also contribute to activation of this pathway \[46\]. Gene and protein expression of PTEN, a negative regulator of PI3K/Akt pathways, were found to be decreased which lead to consistent activation of the PI3K/Akt pathway in WM \[47\]. Additionally, inhibition of Akt by perifosine, mTOR by RAD001, or dual PI3K/ mTOR inhibitor (NVP-BEZ235) display activity against WM even in the presence of bone marrow stromal cells \[46,49\].

**JAK/STAT**

It was shown that expression of both Jak1 and Stat3 proteins is significantly higher in the WM patients as compared to healthy controls \[41\]. Although no mutations associated with this constitutive Jak/Stat signaling have been described in WM to date, cytokine-mediated stimulation of this pathway may be involved in the dysregulation of IgM \[50\]. One important cytokine believed to play pivotal role is IL-6. It is widely accepted that IL-6 activates MAPK/ERK pathway and the transcription factor Stat3 via the tyrosine kinase receptor signaling of JAK1 and 2 \[51\].
**The nuclear factor kappa B pathway**

NF-κB comprises a family of transcription factors that serve as important regulators of the transcription of many genes involved in inflammation, innate immunity, cell growth, and apoptosis [52]. Inhibition of this pathway by proteasome inhibitors such as bortezomib, NPI-0052 and Onyx0912 can inhibit WM cells and overcome resistance induced by bone marrow stromal cells [53,54]. Studies using bortezomib alone or in combination with rituximab in newly diagnosed or even in relapsed WM patients have shown significant activity with over 80% response rate [55-57].

**Risk factors for developing WM**

The underlying causes of WM are poorly understood. The main risk factor for the development of WM is pre-existing IgM-MGUS. Other risk factors include familial history of WM or other B-cell malignancies and immunological factors.

*IgM-MGUS*. IgM-MGUS patients are up to 46 times more likely to develop WM than the general population [58]. IgM-MGUS progresses mostly to WM; Kyle et al demonstrated the cumulative incidence of progression was 10% at 5 years, 18% at 10 years, and 24% at 15 years, respectively [58]. Abnormal serum free light chain (FLC) ratio, the serum monoclonal protein and serum albumin concentrations were shown to be risk factors for progression [58,59]. In addition, recent work by Varettoni et al has shown that MYD88 L265P mutation is also an independent predictor of progression [60]. IgM-MGUS patients are also at increased risk of developing lymphoproliferative disorder, lymphoma, primary amyloidosis and chronic lymphocytic leukemia [61]. We have previously demonstrated that increased serum FLC (with a cut off of 60 mg/L) is a new marker in WM disease and reflective of tumor burden. Therefore, it can be used to differentiate IgM-MGUS from symptomatic WM [62]. Similarly, 6q deletion is not seen in IgM-MGUS patients, whereas it is observed in about 30%-50% of WM patients [63].
Familial WM. Although WM represents a sporadic disease in most cases; various reports reported strong familial predisposition. A study with clinicopathological analysis from 257 unrelated WM patients showed that 48 (18.7%) patients had at least one first-degree relative with WM or another B-cell disorder, including non-Hodgkin lymphoma (NHL), Hodgkin lymphoma, MGUS, MM, chronic lymphocytic leukemia (CLL) and acute lymphoblastic leukemia [64]. A large study with 2144 LPL/WM patients has demonstrated first-degree relatives of LPL/WM patients to have 20-fold, 3.0-fold, 3.4-fold, and 5.0-fold increased risks of developing LPL/WM, NHL, CLL, and MGUS, respectively [65]. The excess risks among parents, siblings, and offspring were similar.

Immunological Risk Factors. It has been shown that history of certain autoimmune and infectious diseases was associated with an increased risk of LPL/WM [65].

Diagnosis

WM is primarily characterized by bone marrow infiltration of lymphoplasmacytic cells which produce excessive amounts of a serum monoclonal IgM [1,2]. Of note, an IgM paraprotein of any level is not sufficient for the diagnosis of WM, since there are other lymphoproliferative disorders that produce IgM [66]. In clinical practice, characteristic immunophenotype of high levels of surface CD19, CD20, and Ig light chain expression in association with nonparatrabecular pattern of BM infiltration is diagnostic for WM [1,4,5,67]. The intratrabecular pattern of BM infiltration is mostly found in WM, whereas an exclusively paratrabecular pattern is rare and should raise suspicion of follicular lymphoma. Dutcher bodies-containing plasma cells and increased number of mast cells close to lymphoid aggregates are commonly found in WM.

Immunophenotypic studies showed the following profile for WM cells: positive for surface IgM, CD19, CD20, CD22, CD25, CD27, FMC7; negative for CD103, and variable CD5, 10, 23, CD138 expression [29,68].

Clinical features
The majority of WM patients present with signs and symptoms related to elevated IgM levels and/or to infiltration of bone marrow and extramedullary sites by malignant B cells. Cytopenia, which is associated with tumor cells replacement in the BM, is the frequent clinical presentation of the disease. Hyperviscosity resulting from high levels of proteins can lead to impairment of microcirculation in the central nervous system. This may result in headache, blurry vision, visual loss and epistaxis in WM patients. Some patients may experience hepatosplenomegaly and lymphadenopathy, and some patients may present with B symptoms including night sweats, fever, and weight loss. Moreover, some patients with WM may present with other common manifestations including neuropathy, cryoglobulinemia, skin rash (Schnitzler syndrome), cold-agglutinin hemolytic anemia, and amyloidosis [69].

**Progression in WM: IgM-MGUS, asymptomatic WM, and symptomatic WM**

Normally, WM is preceded by IgM-MGUS or smoldering WM [70]. IgM-MGUS is described as an IgM<3 g/dL and <10% lymphoplasmacytic cells in the BM, whereas smoldering WM is described as an IgM>3 g/dL or >10% LPL cells in the BM in the absence of any symptoms attributable to WM. A recent study showed that the cumulative probability of progression of smoldering WM to symptomatic WM, amyloidosis, or lymphoma is 6% at 1 year, 39% at 3 years, and 59% at 5 years, respectively [71]. The percentage of lymphoplasmacytic cells in the bone marrow, size of the serum M-spike, and the hemoglobin value were the major risk factors for the progression. Although abnormal serum FLC ratio was shown as a risk factor for progression in MGUS, it was not a predictive factor of progression in patients with smoldering WM [71]. Since the criteria used for IgM-MGUS and smoldering WM are not widely adopted in the WM literature, another classification based on the consensus recommendation is more frequently used, According to this classification, patients present with Ig protein but no LPL cells in the BM should be diagnosed with Ig-MGUS, and patients with any level of IgM monoclonal protein and presence of lymphoplasmacytic cells in the BM should
be diagnosed with WM (asymptomatic or symptomatic) [4]. Patients diagnosed with asymptomatic WM should be recognized and not treated because of the stability of the disease for many years. In an analysis of 31 asymptomatic patients, the median time to disease progression was 6.9 years [5].

**Prognosis**

In a study of 337 symptomatic WM patients, the median survival of patients was 6.4 years, and the median disease-specific survival was 11.2 years [72]. Despite indolent nature of the disease, WM prognosis shows wide variation, which makes important to define prognostic factors. Cytopenias, high β2-microglobulin, low albumin, serum IgM monoclonal protein, organomegaly and advanced age have shown to be poor prognostic factors for WM patients [72-76]. International Prognostic Scoring System (ISS-WM) is the most commonly used prognostic system, which is based on 5 risk factors and the survival of patients at 5 years [77]. Based on this system, high-risk WM patients were shown to have a 5-year survival of 36%, whereas low-risk patients had a 5-year survival of 87%.

**Diagnostic workup and differential diagnosis**

Important points of workup for a patient with WM are summarized in Table 1. Diseases that need to be considered for differential diagnosis are IgM-MM, mantle cell lymphoma, marginal zone lymphoma (MZL), and other IgM-secreting lymphomas.

Plasmacytic cell infiltration of BM and the presence of osteolytic lesions differentiate IgM-MM from WM [1,5,69]. Renal insufficiency can be seen in IgM-MM, whereas it is rare in WM. Some cytogenetic characteristics such as 13q deletion, 11:14, or 4:14 translocations may also help to differentiate MM from WM. In addition, MYD88 L265P mutation presents in over 90% of WM patients but not MM patients [18-20]. This may also help to differentiate WM from not only from MM but also from other B cell malignancies.
Infiltration of BM by monomorphous, small medium lymphoid cells with irregular nuclei may help to differentiate mantle cell lymphoma from WM. In addition to the BM, involvement of extranodal sites and lymph nodes may be observed in mantle cell lymphoma. Moreover, almost all cases present with t(11;14) (q13;q32) [1,5,69].

Distinguishing WM from MZL can be difficult, particularly from splenic MZL (SMZL). SMZL mostly involves the spleen and only rarely presents with lymphadenopathy. It virtually always infiltrates the bone marrow with a characteristic intertrabecular and intrasinusoidal pattern, which is not typical of NMZL [78]. In immunophenotyping, some markers such as CD22 and CD11c are overexpressed in patients with SMZL compared to patients with WM, whereas CD25 is mostly seen in WM (88% vs 44%). Therefore, combination of CD25 and CD22 may help to differentiate between WM and SMZL [69]. The CD103 Ag is absent in WM patients, whereas it is present in 40% of SMZL patients. The most common cytogenetic abnormality in WM patients is 6q deletion (30%-50% in WM), whereas the most common abnormalities in SMZL are loss of 7q (19%) along with +3q (19%) and +5q (10%). The use of MYD88 L265P may be particularly helpful, as few if any patients with MZL demonstrate this mutation whereas >90% of WM patients express this mutation [18].

Follicular lymphoma can be differentiated from WM with BM infiltration of small, cleaved cells that are usually paratrabecular. In addition, rearrangement of Bcl-2 is the molecular hallmark in follicular lymphoma and seen in 70%-90% of the cases [1,5,69].

Lastly, IgM myeloma may also share similar morphological features with WM, including expression of CD20. Translocation involving chromosome 11 (t 11;14) are common in IgM myeloma, but absent in WM [79].

**Primary treatment options for WM**

Despite the advances in the biology and treatment of WM, there is no standard of care for the treatment of WM. Treatment decisions are based on the presence of
symptoms, patient factors including age and functional status and disease factors including presence of cytopenias, rate of disease progression, the level of IgM protein, the presence of neuropathy, cryoglobulinemia, and hyperviscosity.

Current treatment options for WM include alkylating agents (e.g. chlorambucil, and cyclophosphamide), nucleoside analogue (fludarabine and cladribine), the monoclonal antibody rituximab, and the proteasome inhibitor bortezomib [80-83]. Consensus-based response criteria for WM are found in table 2 [84].

Plasma exchange is a treatment technique used for hyperviscosity associated with WM. Symptoms of hyperviscosity are generally present when IgM exceeds 4000mg/dL and includes epistaxis, gingival bleeding and visual changes due to retinal hemorrhages. Plasma exchange is a temporary solution that is used for short-term management of hyperviscosity until initiating systemic chemotherapy, which treats the underlying cause of the hyperviscosity by reducing the IgM [85,86].

**Rituximab**

One of the most widely available agents used for the treatment of WM is rituximab, which is used either as a single agent or in combination regimes. It is a chimeric monoclonal antibody that binds to the CD20 antigen on B-cell lymphocytes. As a single agent it gives response rates of 35-48% [87-90], which is inferior than response rates seen with combination regimens. In addition, single agent rituximab can cause a “rituximab flare” phenomenon, which results in a transient rise in IgM level producing symptoms of hyperviscosity and requiring plasma exchange. This phenomenon is infrequently seen when rituximab is used in combination with chemotherapy. Some of the combination agents studied with rituximab include cyclophosphamide, bendamustine, and bortezomib [55-57,91,92].

In a study by Dimopoulos et al, 72 patients with newly diagnosed WM received rituximab, oral cyclophosphamide and dexamethasone. Overall response rate (ORR), which includes minimal response or better, was 83%, including 7%
complete response (CR), 67% partial remission (PR). The median time to progression was 35 months with a median overall survival (OS) of 95 months. Only 9% of patients experienced grade 3 or 4 neutropenia and none experienced grade 3 or 4 thrombocytopenia [91,93].

A phase III trial conducted by the Study group Indolent Lymphomas (StiL) included 549 patients with low grade lymphomas of which 162 were newly diagnosed WM patients who were received 6 cycles of bendamustine and rituximab (BR). Responding patients were randomized to rituximab every 2 months for 2 years or observation. In the 116 evaluable patients, ORR was 86%. The results of the maintenance arm of this study remain to be reported. No uncommon toxicities were observed during BR induction [94].

Maintenance therapy with rituximab is being used more commonly in patients with WM. A retrospective study showed improvement in progression free and overall survival among patients receiving maintenance rituximab when used at 375mg/m2 every 3 or 6 months over 2 years. A prospective study on maintenance regimen of rituximab given every 2 months for 2 years is now being studied [95].

**Nucleoside analogues**

Nucleoside analogs are conventional chemotherapeutic agents that inhibit DNA synthesis through interfering with different steps in the process of DNA synthesis.

Fludarabine and cladribine are nucleoside analogues studied as single agent or in combination with rituximab or cyclophosphamide.

A recent phase III study by Leblond et al showed the superiority of fludarabine compared with chlorambucil in the treatment of WM. Three hundred and thirty nine WM patients were included in this trial and the study showed no difference in overall response rate between the two arms. However, the median PFS and duration of response were significantly higher in the fludarabine arm then chlorambucil with a median follow up of 36 months (PFS 36.3 vs 27.1 months...
Median overall survival was not reached in the fludarabine arm versus 69.8 months in the chlorambucil arm (p=0.014). Secondary malignancies were more frequent in the chlorambucil arm with a 6-year cumulative incidence rate of 20.6% versus 3.7% in the fludarabine arm (p=0.001) [96]. This rate is inconsistent with prior retrospective studies, which have shown higher rates of secondary malignancies with fludarabine [97].

Adding rituximab to fludarabine can significantly enhance response without added toxicity [98]. Combinations of fludarabine and rituximab or fludarabine, cyclophosphamide and rituximab have shown response rates of 70-85% [99,100].

**New developments in WM**

Many novel agents have promising results and are at various stages of study in WM. These include everolimus, an mTOR inhibitor; perifosine, an AKT inhibitor; enzastaurin, a PI3K/AKT inhibitor; panobinostat, a histone deacetylase inhibitor; bortezomib, first in class of proteasome inhibitor and carfilzomib, a new irreversible proteasome inhibitor; pomalidomide, a new immunomodulatory agent; and ibrutinib, a Bruton tyrosine kinase inhibitor [101,102].

**Proteasome Inhibitors**

Bortezomib is a reversible proteasome inhibitor that specifically acts on the 20S of the proteasome. Blocking proteasomal degradation causes protein accumulation, which can alter the normal progression of the cell cycle, which is necessary for cell survival. Bortezomib in combination with rituximab with or without dexamethasone is being commonly used in either newly diagnosed or relapsed WM. The overall response rates with these combinations are more than 80%. Peripheral neuropathy is one of the adverse effects of bortezomib therapy, which is less when bortezomib is given weekly instead of the twice-weekly regimen [55-57].
Carfilzomib, an irreversible proteasome inhibitor, was approved by the FDA in July of 2012 for the treatment of patients for relapsed/refractory myeloma and is also being studied in WM. Antitumor activity of carfilzomib was validated in vivo by Sacco et al who demonstrated that carfilzomib targeted the chymotrypsin-like (CT-L) activity of both constitutive-(c20S) and immuno-(i20S) proteasome. This led to the induction of toxicity in primary WM cells, as well as in other IgM-secreting lymphoma cells [103].

The results recently reported from the CaRD trial by Treon et al in 31 patients. Eighty seven percent of patients were previously untreated. Symptomatic patients received carfilzomib in combination with rituximab and dexamethasone, and were eligible for maintenance therapy with all three agents if they had stable disease or better. The overall response rates and major response rates were 81% and 65% with 9 very good partial responses, 11 partial responses, and 4 minor responses. With a median follow-up of 9 months, 20 patients are free of disease progression. Most common toxicities which were hyperglycemia related to steroids; asymptomatic elevation of lipase and hypersensitivity to rituximab. All toxicities were reversible, and no grade ≥2 peripheral neuropathy was observed [104].

Oprozomib, an oral proteasome inhibitor, is in clinical development for MM and other hematologic malignancies. A phase 1b/2 trial of once-daily, modified-release oprozomib tablets was studied in 24 patients with hematologic malignances. Dose escalation of oprozomib is currently being implemented using two dosing schedules: oprozomib was given at 2 different dosing schedules; once-daily, modified-release tablets (150 mg/d), on either days 1, 2, 8, and 9 (QDx2) or on days 1 to 5 (QDx5), both on a 14-day cycle. From the data that Dr. Kaufman presented at the EHA meeting 2013, 24 patients were enrolled in the study (16 with MM; 8 with WM) in 7 cohorts. WM patients on the QDx2 schedule have shown no response while patients on the QDx5 schedule have shown 80% ORR. The MTD of oprozomib had not been reached for either schedule. Most of
the adverse effects seen were gastrointestinal. Dose escalation will continue in this study until the MTD of oprozomib is reached. This data

**Everolimus**

Everolimus (RAD001) is an inhibitor of MTORC1, a component of the Akt-MTOR pathway, which regulates growth, and survival of lymphoplasmacytic cells in WM. A phase II trial of single agent everolimus was studied in 50 patients with relapsed/ refractory WM. The overall response rate was 70% with a PR of 40% and MR of 30%. The most common adverse events were cytopenias. Pulmonary toxicity, which is a serious adverse effect of everolimus, was seen in 10% of patients [105]. Another phase I/II study explored the use of everolimus in combination with rituximab and/or bortezomib in 23 relapsed/refractory patients. From the 19 patients who were evaluable for response, 1 (5%) patient very good partial response (VGPR) and 9 (47%) minimal response (MR), for an overall response rate including MR of 10/19 (53%) in this relapsed/refractory population [106]. A prospective, multicenter study of everolimus as primary therapy was conducted in 33 WM patients. The best overall response rate utilizing consensus criteria was 72.2% (2 Very Good Partial Responses, 18 Partial Responses, 4 Minor Responses, and 9 Stable Disease), and the major response rate (PR or better) was 60.6%. However, discordance between serum IgM levels upon which consensus criteria for response are based, and BM disease response were common and complicated response assessment. At 6-month assessments, 10 of 22 (45.5%) patients for whom both serum IgM and BM assessments were performed, discordance between serum IgM and BM disease involvement were observed. Among these patients, 2 had no change, and 8 had increased bone marrow disease involvement despite decreases in serum IgM levels. Grade ≥2 hematologic and non-hematologic toxicities possibly, probably or definitively associated with everolimus included anemia (39.4%), thrombocytopenia (12%), neutropenia (18.2%), hyperglycemia (9.1%), oral ulcerations (27.3%), and pneumonitis (15%) with a median follow-up of 9 months (range 1-45 months), 6 patients remain on study [107].
Enzastaurin

Enzastaurin is an oral serine/threonine kinase inhibitor that targets the PKC and PI3/AKT pathway. A phase II trial of enzastaurin was studied in 42 patients with WM who were previously treated with 1 to 5 regimens. Oral enzastaurin was given at 250 mg twice daily (500 mg total) after a single loading dose (day 1, cycle 1) of 375 mg 3 times daily (1,125 mg total) for 8 cycles of 28 days each or until progressive disease. The objective response rate was 38.1%, with 2 patients achieving PR and 14 achieving minor response. Grade 3 adverse events seen were leukopenia in one patient and septic shock in another one [107]. These results warrant further investigation of enzastaurin for the treatment of WM.

Perifosine

Perifosine is an Akt inhibitor that belongs to a class of lipid-related compounds called alkylphospholipids. A phase II trial was conducted with perifosine and included 37 patients with WM who were previously treated with 1 to 5 regimens. Oral perifosine was given at 150 mg daily until progression. The objective response rate (MR or better) was 35% with 11% achieving PR and 24% achieving MR. Progressive disease was observed in only 11% of patients since 54% had stable disease. Median PFS was 12.6 months. The most common adverse events were GI toxicities including diarrhea, nausea and vomiting [108].

Immunomodulatory Drugs

Thalidomide and lenalidomide are immunomodulatory agents that are studied in combination with Rituximab in symptomatic WM patient [109,110]. A phase 2 study included 25 patients, 20 of whom were previously untreated. They received thalidomide (200 mg for 2 weeks, then 400 mg for a total of 1 year) and Rituximab. Overall and major response rate were 72% and 64%, respectively, with complete response (n = 1), partial response (n = 15), and major response (n = 2). Median time to progression for all evaluable patients was 34.8 months. Peripheral neuropathy was the most common adverse event (grade ≥2 in 44%),
and therefore limits the use of thalidomide in WM [109]. Lenalidomide was also poorly tolerated in WM patients. It was tested in combination with rituximab in 16 patients, 12 of whom were previously untreated. The overall response and major response (<50% decrease in serum IgM) rates were 50% and 25%, respectively, with a median time to progression of 18.9 months. Premature discontinuation of lenalidomide therapy occurred in 14 of 16 (88%) patients, which was most commonly due to an acute decrease in hematocrit in 13 of 16 patients, which resulted in 4 patients requiring hospitalizations due to anemia complications. This was attributable to lenalidomide and led to cessation of further enrollment on this study [110].

Pomalidomide, a 3rd generation immunomodulatory agent, has demonstrated efficacy, as a single agent and with dexamethasone for the treatment of relapsed/refractory multiple myeloma and as a single agent for myelofibrosis. A phase I trial was conducted on 6 patients with relapsed and/or refractory WM. Patients received daily oral pomalidomide (28 day cycles), starting with a dose of 1 mg. The median number of prior lines of therapy was 1-4. No dose limiting toxicities (DLTs) were encountered at the 1mg dose. At the 2 mg dose level, 2 patients experienced DLTs (grade 4 dizziness and grade 3 syncope in 1 and grade 4 neutropenia in the second); a 3rd withdrew from the study after experiencing mild fever, headaches and blurred vision, which resolved after plasma exchange and discontinuing pomalidomide. Other grade 3-4 adverse events have been neutropenia and infection. The best response to date for the 5 evaluable patients was stable disease in 3 patients, and progressive disease in 2 [111]. A study looking into the combination of pomalidomide, dexamethasone and rituximab was reported recently. Patients were enrolled on a dose escalating Phase 1 study (3+3 design). Following amendment, dose level one was rolled back to 0.5 mg daily, with intended escalation to 1, 2, 4 mg daily. Therapy consisted of 52 weeks of daily POM with rituximab (375 mg/m²/wk) IV and dexamethasone (40 mg) IV on weeks 1-4 and 12-15. Patients were required to be on aspirin (325 mg) prophylaxis. Patients with IgM >4,000 mg/dL had prophylactic pheresis to avoid symptomatic rituximab-related IgM flare.
patients were enrolled (3 at 0.5 mg, 4 at 1.0 mg of POM) before closure for rituximab-related IgM flare events. The best overall response rate is 42.8% (1 VGPR; 2 PR). Grade ≥2 treatment related toxicities include neutropenia (43%), symptomatic hyperviscosity from IgM flare with 3 patients (43%) all of whom had a baseline IgM >4,000 requiring emergent pheresis for IgM flaring while on therapy. Median response duration was 15.1 months, and all 3 patients continue to respond [112].

**Histone-Deacetylase Inhibitors (HDACi)**

Preclinical studies have shown that epigenetic regulators, including histone acetylation, regulate tumor progression. In vitro studies have confirmed the antitumor activity of panobinostat (HDACi) in primary tumor cells and cell lines [113] A phase II trial of panobinostat was studied in 36 patients with relapsed or relapsed/refractory WM. Patients received oral panobinostat at 30 mg 3 times a week. Overall response rate (MR or better) was achieved in 17 (47%) of patients, with 22% partial remissions and 25% MR. In addition, 18 (50%) patients achieved stable disease and none showed progression while on therapy. The median progression-free survival was 6.6 months. Grade 3 and 4 toxicities included thrombocytopenia, neutropenia, anemia, leukopenia, and fatigue [114]. Further studies are under way to better define the efficacy of HDAC inhibitors in WM.

**Bruton Tyrosine Kinase (BtK) inhibitor**

Bruton’s tyrosine kinase (BtK) is an essential element of the BCR signaling pathway, which regulates apoptosis, adhesion, and cell migration and homing. BTK activation is facilitated by MYD88 pathway signaling in L265P expressing WM cells, and participates in MYD88 downstream signaling. Ibrutinib (previously PCI-32765) is an oral agent designed to specifically target and selectively inhibit the enzyme Bruton’s tyrosine kinase (BTK) leading to tumor cell killing of MYD88 L265P expressing WM cells [115].
A phase I study of ibrutinib was conducted in 56 patients with a variety of B-cell malignancies including chronic lymphocytic leukemia/small lymphocytic lymphoma, mantle cell lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, Waldenstrom’s macroglobulinemia and multiple myeloma. Objective response rate in 50 evaluable patients was 60%, including complete response of 16%. Median progression-free survival in all patients was 13.6 months. Partial responses were seen in 3 of the 4 patients with WM and the drug was well tolerated [116].

63 patients including 17 with refractory disease were enrolled on a dedicated ongoing phase II trial (ClinicalTrials.gov Identifier: NCT01614821) in patients with relapsed/refractory WM disease. Patients had a median of 2 prior therapies, hemoglobin of 10.5 g/dL, serum IgM of 3,610 mg/dL, and a median BM disease involvement of 65%. At best response, median serum IgM level declined to 1,340 mg/dL, and median hemoglobin rose to 12.6 g/dL. With a median follow-up of 6 (range 2-15 cycles), the best overall response rate i.e. minor response (MR) or better using consensus criteria adapted from the 3rd International Workshop on WM is 81% (4 VGPR; 32 PR, 15 MR), with a major response rate (PR or better) of 57.1% and a median time to response of 4 weeks. 11 patients have stable disease, and 1 patient was a non-responder. The most common grade >2 treatment related toxicities were thrombocytopenia (14.3%); neutropenia (19.1%); stomatitis (1.6%); atrial fibrillation (1.6%); diarrhea (1.6%); herpes zoster (1.6%); hematoma (1.6%); and epistaxis (1.6%). Attainment of major responses was impacted by mutations in CXCR4. The major response rate was 77% for patients with wild-type CXCR4 vs. 30% in those with WHIM-like CXCR4 mutations.

**Conclusion**

Waldenstrom macroglobulinemia is a distinct lymphoproliferative disorder with specific epigenetic/genetic aberrations, and characterized by production IgM monoclonal secretion and lymphoplasmacytic cells in the BM. With the change in understanding the pathogenesis of WM, many new targets and therapies have
emerged within the past couple of years. Current therapies used in the upfront or relapsed setting include alkylating agents, bendamustine, nucleoside analogues, bortezomib and the monoclonal antibody rituximab. Newer options include everolimus and ibrutinib. However, targeted therapies are still warranted and recent revelations from whole genome sequencing have provided opportunities for their development.

**Expert commentary & five-year view**

To date there are no FDA approved therapeutic agents for treatment of WM. Recently, whole genome sequencing helped us identify specific mutations in subgroups of patients with WM (eg. MYD88 L265P mutation which is present in over 90% of WM) which provides the framework for the investigation of BTK inhibitors like ibrutinib. These newer agents show improved responses and lower long term toxicities. In addition, many of the newer agents are orally administered which makes them more convenient for patients. Some of the challenges for the future of WM include combining of agents to achieve higher response rates, and prolonged survival for patients, but with less toxicity.

**Key Issues**

- Recent findings demonstrated that MYD88/IRAK signaling pathway might have a critical role in pathogenesis of WM.

- Somatic mutations in MYD88 are found in over 90% of WM patients, which may help to differentiate WM from other B cell malignancies.
Current research efforts in inhibition of BTK and IRAK signaling might provide better treatment strategy for WM.

Many treatment options are available for WM patients and some of these include rituximab, bortezomib, cyclophosphamide, and nucleoside analogues fludarabine and cladribine which are less commonly used.

Novel agents under study are showing promising results as a single agent or in combination. Some of these include everolimus, ibrutinib, as well as the newer proteasome inhibitors carfilzomib and oprozomib.

There is a need for studies with PFS and OS as endpoints combined with quality of life assessment as well as randomized phase III studies to evaluate the benefit of different treatments for patients with WM.
### Table 2: Response Definitions

<table>
<thead>
<tr>
<th>Response Category</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete response (CR)</td>
<td>Absence of serum monoclonal IgM protein by immunofixation</td>
</tr>
<tr>
<td></td>
<td>Normal serum IgM level</td>
</tr>
<tr>
<td></td>
<td>Complete resolution of extramedullary disease*</td>
</tr>
<tr>
<td></td>
<td>Morphologically normal bone marrow aspirate</td>
</tr>
<tr>
<td>Very good partial response (VGPR)</td>
<td>Monoclonal IgM protein is detectable</td>
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<tr>
<td></td>
<td>≥90% reduction in serum IgM level from baseline</td>
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<tr>
<td>Response Type</td>
<td>Criteria</td>
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<tr>
<td>-------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Partial response (PR)</strong></td>
<td>Complete resolution of extramedullary disease*</td>
</tr>
<tr>
<td></td>
<td>No new signs or symptoms of active disease</td>
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<tr>
<td></td>
<td>Monoclonal IgM protein is detectable</td>
</tr>
<tr>
<td></td>
<td>≥50% but &lt;90% reduction in serum IgM level from baseline</td>
</tr>
<tr>
<td></td>
<td>Reduction in extramedullary disease*</td>
</tr>
<tr>
<td></td>
<td>No new signs or symptoms of active disease</td>
</tr>
<tr>
<td><strong>Minor response (MR)</strong></td>
<td>Complete resolution of extramedullary disease*</td>
</tr>
<tr>
<td></td>
<td>No new signs or symptoms of active disease</td>
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<tr>
<td></td>
<td>Monoclonal IgM protein is detectable</td>
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<tr>
<td></td>
<td>≥25% but &lt;50% reduction in serum IgM level from baseline</td>
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<tr>
<td></td>
<td>No new signs or symptoms of active disease</td>
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<tr>
<td><strong>Stable disease (SD)</strong></td>
<td>Complete resolution of extramedullary disease*</td>
</tr>
<tr>
<td></td>
<td>No new signs or symptoms of active disease</td>
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<tr>
<td></td>
<td>Monoclonal IgM protein is detectable</td>
</tr>
<tr>
<td></td>
<td>&lt;25% reduction and &lt;25% increase in serum IgM level from baseline</td>
</tr>
<tr>
<td></td>
<td>No new signs or symptoms of active disease</td>
</tr>
<tr>
<td></td>
<td>No progression in extramedullary disease*</td>
</tr>
<tr>
<td><strong>Progressive disease (PD)</strong></td>
<td>Complete resolution of extramedullary disease*</td>
</tr>
<tr>
<td></td>
<td>No new signs or symptoms of active disease</td>
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<tr>
<td></td>
<td>≥25% increase in serum IgM level from lowest nadir (requires confirmation) and/or progression in clinical features attributable to the disease</td>
</tr>
</tbody>
</table>

*i.e. lymphadenopathy and splenomegaly*
Financial and competing interests disclosure

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Papers of special note have been highlighted as:

• of interest
•• of considerable interest

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**The presence of a widely expressed somatic mutation (MYD88 L265P) was described in patients with Waldenstrom’s macroglobulinemia.**


• The study showed that combined inhibition of BTK and IRAK in MYD88 L265P expressing WM cells provides synergistic inhibition of NF-κB signaling and WM cell killing.


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- Phase I study of ibrutinib, BTK inhibitor, showed activity in patients with relapsed/refractory B-cell malignancies including Waldenström’s macroglobulinemia.