New Treatment Strategies for Waldenström Macroglobulinemia

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Abstract: The development of high-throughput technologies has allowed us to characterize the molecular landscape of hematologic neoplasms and identify somatic mutations. As a result, we can now use these technologies to screen for and diagnose neoplastic disease, model risk factors for progression, make treatment decisions, track response to treatment, and design clinical trials. Waldenström macroglobulinemia (WM), which is a lymphoplasmacytic lymphoma, serves as a good example of how genomic data collected at the bench can be applied at the bedside. *MYD88* L265P and *CXCR4* nonsense and frameshift mutations are the most common recurrent variants observed in patients who have WM, with detection rates of 90% and 40%, respectively. Knowing about these mutations has made it possible to develop agents that target the underlying signaling pathways. In this review, we describe the various treatment strategies for WM and detail the genotype of the malignant WM cell.

Keywords
CXCR4, lymphoplasmacytic lymphoma, *MYD88*, treatment, Waldenström

Introduction

Waldenström macroglobulinemia (WM) is a lymphoplasmacytic lymphoma in which malignant cells produce an immunoglobulin M (IgM) monoclonal protein, with subsequent accumulation in the serum.[1,2] The most common disease manifestations, specifically anemia and thrombocytopenia, are related to involvement of the bone marrow. Other frequent symptoms are caused by enlargement of the lymph nodes and spleen. The IgM paraprotein can also result in significant comorbidities, including symptomatic hyperviscosity, neuropathy, and autoimmune-related complications.[3]

With the advent of high-throughput technologies, it is now known that patients with WM harbor 2 highly recurrent somatic mutations in the *MYD88* and *CXCR4* genes.[4,5] This knowledge has dramatically changed the landscape of disease management. First, assessment of the *MYD88* L265P mutation (with a prevalence of more than 90% in patients with WM) has overcome diagnostic challenges.[6] Second, both the *MYD88* L265P and *CXCR4* mutations have been associated with specific clinical manifestations and correlated with prognosis.[7] Third, knowledge of the mutation status of

patients with WM makes it possible to conduct genomically driven clinical trials and also can be used to assess treatment responses.[8]

Here, we review the role of the mutational landscape in WM, describe treatment options based on molecular targets in both treatment-naive patients and those with relapsed or refractory WM, analyze how responses vary according to the genomic status, and offer insights regarding emerging treatments and ongoing clinical trials.

The Genomic Landscape of Waldenström Macroglobulinemia

The MYD88 L265P Mutation

The somatic variant in which leucine changes to proline at amino acid position 265 in the *MYD88* gene (*MYD88* L265P) was first described with RNA interference screening and sequencing and was shown to be recurrent in 29% of cases of activated B-cell–like diffuse large B-cell lymphoma (ABC DLBCL) and other lymphoproliferative disorders.[9] In vitro and in vivo experiments later confirmed that the MYD88 protein is an adaptor that assembles a complex known as the myddosome, which contains interleukin-1 receptor-associated kinase (IRAK1), IRAK4, and Bruton tyrosine kinase (BTK). After an immune physiologic response or mutation, the myddosome signals through nuclear factor–kappa B (NF-κB), increasing its activity and inducing cell proliferation and survival.[10]

The *MYD88* L265P mutation was first described in WM with whole-genome sequencing in isolated CD19+ bone marrow cells,[5] and an allele-specific polymerase chain reaction (AS-PCR) assay was then developed to detect the mutation.[6] Subsequently, other groups used different technologies to replicate and validate these data. Now, the overall agreement is that more than 90% of patients with WM patients harbor the *MYD88* L265P mutation.[11-15]

CXCR4 Mutations

Following the discovery of the *MYD88* mutation, researchers also identified recurrent mutations in the *CXCR4* gene in samples from patients with WM. Frameshift and nonsense mutations were described; the most frequent and pathogenic of these were located at nucleotide position 1013 and caused a stop codon (*CXCR4* S338* C1013G and C1013A).[4] Upon binding to its ligand, CXCL12, the CXCR4 surface protein initiates a signaling process that activates phosphatidylinositol 3-kinase (PI3K) and the JAK/STAT pathways, finally regulating cell migration and chemotaxis.[16-18] The mutations were also identified with Sanger sequencing in CD19+ sorted bone marrow cells or with 2 AS-PCR assays.[19] Similar results were reported by other studies, in which the prevalence of *CXCR4* mutations in patients with WM was as high as 40%. More recently, *CXCR4* nonsense mutations have been described by means of high-throughput PCR (droplet digital PCR [ddPCR]) without a CD19+ sorting step.[20]

Given these results, the use of *MYD88* L265P and *CXCR4* nonsense/frameshift mutations allowed the differentiation of clinical phenotypes and prognostic groups in patients with WM. For instance, 15 of 174 patients who had WM with wild-type *MYD88* (*MYD88*^{wt}) and wild-type *CXCR4* (*CXCR4*^{wt}) had the shortest median overall survival (OS), whereas the patients who harbored *MYD88* L265P and *CXCR4* mutations had a better prognosis compared with those who had *MYD88*^{wt} and *CXCR4*^{wt}. The patients with *MYD8* and *CXCR4* mutations also had more bone marrow involvement, lower platelet counts, higher serum levels of IgM, and a higher rate of hyperviscosity syndrome and acquired von Willebrand disease in comparison with the other groups.[7]

Other Molecular Abnormalities

Among other mutations found in WM, *ARID1A* mutations were described in 17% of patients with the use of whole-genome sequencing. ARID1A is involved in the regulation of chromatin remodeling, thus regulating gene expression, and it is reported that ARID1A can bind to P53 and modulate the cell cycle. Additionally, *CD79A* and *CD79B* mutations were found in up to 12% of patients with WM. Both of these genes encode proteins that are components of the B-cell receptor (BCR) and cooperate to activate a transduction signal.[4] A study reported that mutations in *CD79A* and *CD79B* were found only in samples of *CXCR4*-mutated WM,[17] although another study found that co-expression of mutations in both *CD79B* and *MYD88* was associated with transformation from WM to DLBCL.[21]

Recently, a somatic mutation in the transcription factor coding for the *SPI1* gene was identified with whole-exome sequencing in 6% of a series of patients with WM.[13] SPI1 is part of the erythroblast transformation specific (ETS) family of transcription factors, and studies have reported abnormal regulation of SPI1 in other blood neoplasms.[22,23] During B-cell development, *SPI1* expression is associated with negative plasma cell differentiation, which explains in part its presence in WM. The *SPI1* Q226E somatic mutation was associated with shorter OS among patients with WM.[13]

Regarding copy number alterations (CNAs), 6q deletion is the most prevalent of these in patients with WM.[4,24,25] Genes affected include *PLEKHG1*, *ARID1B*, *FOXO3*, *IBTK*, *BCLAF1*, *TNFAIP3*, and *HIVEP2*. These genes are known to regulate cell growth in B-cell lymphomas, NF-κB signaling, apoptosis, and plasma cell differentiation. CNAs outside chromosome 6 include deletion of *ETV6*, *BTG1*, *LYN*, *PRDM2*, and *TOP1*.

Table 1. Summary of Clinical Trials for Waldenström Macroglobulinemia With Reported Genomic Data

Study (Sample Size)	Molecular Assay	Genomic Group	ORR, %	Rate of PR or Better, %	Rate of VGPR or Better, %	PFS
Ibrutinib in previously treated patients (N=63)	CD19+ selected BM cells: AS-PCR for *MYD88*^{mut} Sanger sequencing for *CXCR4*^{mut} AS-PCR for *CXCR4* S338* C>G/A	*MYD88*^{mut} *CXCR4*^{wt}	100	97	47	5-y: 70%
		MYD88^{mut} *CXCR4*^{mut}	86	68	9	5-y: 38%
		MYD88^{wt} *CXCR4*^{wt}	50	0	0	Median: <2 y
Ibrutinib in treatment-naive patients (N=30)	CD19+ selected BM cells: AS-PCR for *MYD88*^{mut} Sanger sequencing for *CXCR4*^{mut} AS-PCR for *CXCR4* S338* C>G/A	*MYD88*^{mut} *CXCR4*^{wt}	100	94	44	4-y: 92%
		MYD88^{mut} *CXCR4*^{mut}	100	78	14	4-y: 59%
Ibrutinib plus rituximab (N=75)	Whole BM cells: Targeted exome sequencing	*MYD88*^{mut} *CXCR4*^{wt}	94	81	44	54-mo: 72%
		MYD88^{mut} *CXCR4*^{mut}	100	77	23	54-mo: 63%
		MYD88^{wt} *CXCR4*^{wt}	82	73	27	54-mo: 70%
Acalabrutinib (N=50)	*MYD88* testing not standardized *CXCR4*^{mut} not assessed	*MYD88*^{mut}	94	78	28	NR
		MYD88^{wt}	79	57	0	
Zanubrutinib (N=102)	Whole BM cells: Sanger sequencing for *CXCR4*^{mut}	*MYD88*^{mut} *CXCR4*^{wt}	NR	82	34	NR
		MYD88^{mut} *CXCR4*^{mut}	NR	70	18	
		MYD88^{wt} *CXCR4*^{wt}	81	50	27	
Venetoclax (N=32)	CD19+ selected BM cells: AS-PCR for *MYD88*^{mut} Sanger sequencing and AS-PCR for *CXCR4*^{mut}	*MYD88*^{mut} *CXCR4*^{wt}	86	86	29	Median: 30 mo No difference between groups
		MYD88^{mut} *CXCR4*^{mut}	82	77	12	
Ulocuplumab (N=13)	CD19+ selected BM cells: AS-PCR for *MYD88*^{mut} Sanger sequencing and AS-PCR for *CXCR4*^{mut}	*MYD88*^{mut} *CXCR4*^{mut}	100	100	33	2-y: 90%
Ixazomib, dexamethasone, and rituximab (N=26)	CD19+ selected BM cells: AS-PCR for *MYD88*^{mut} Sanger sequencing and AS-PCR for *CXCR4*^{mut}	*MYD88*^{mut} *CXCR4*^{wt}	99	81	36	Median: 36 mo
		MYD88^{mut} *CXCR4*^{mut}	94	75	7	Median: 40 mo
Obinutuzumab and idelalisib (N=49)	Whole BM cells: Targeted NGS and ddPCR for *MYD88* and *CXCR4*^{mut}	*MYD88*^{mut} *CXCR4*^{wt}	71	67	67	Median: 25 mo No difference between groups
		MYD88^{mut} *CXCR4*^{mut}	74	59	NR	

AS-PCR, allele-specific polymerase chain reaction; BM, bone marrow; ddPCR, droplet digital PCR; mo, month(s); mut, mutated; NGS, next-generation sequencing; NR, not reported; ORR, overall response rate; PFS, progression-free survival; PR, partial response; VGPR, very good partial response; wt, wild-type; y, year(s).

These mutations can be involved in *BCR* and *TP53* signaling as well as glucocorticoid resistance, as described in other lymphoproliferative disorders.[4]

Modeling the Development of WM Progression

Lately, the development of single-cell sequencing methods has been a major advance in understanding cancer biology, while overcoming tumor heterogeneity. In this sense, the presence of *MYD88* L265P was considered to be an early event in the development of the lymphoplasmacytic clone, not only in mature CD19+ B-cells but also in hematopoietic progenitor cells from samples of IgM monoclonal gammopathy of undetermined significance (MGUS).[26,27] However, other somatic mutations, such as those found in *CXCR4*, or CNAs are involved later in WM progression and are required for malignant transformation. These molecular abnormalities were found throughout B-cell evolution up to plasma cell differentiation. For instance, the genes affected in 6q deletion were described to be associated with blocking B-cell

differentiation to plasma cells.[27] Therefore, we are now able to propose a more precise model of disease evolution, in which *MYD88* L265P is the earliest clonal event and other somatic mutations and CNAs emerge as secondary "hits" that finally drive WM progression.

BTK Inhibitors in Waldenström Macroglobulinemia

As previously mentioned, the genomic landscape of WM has been correlated with disease biomarkers used in the clinic, particularly the presence and/or co-occurrence of the 2 most recurrent somatic mutations (those in *MYD88* and *CXCR4*).[7] Moreover, the availability of Sanger sequencing in many centers or specific probes for AS-PCR to identify *MYD88* L265P and *CXCR4* S338* C>A/G has made it possible to describe associations between genomic data and outcomes of treatment in patients with WM.[6,19] A number of ongoing studies and clinical trials have been designed to target specific molecular abnormalities observed in the disease. In this section, we discuss current molecularly based treatment options in WM.

Ibrutinib Monotherapy

Earlier studies have shown that phosphorylated BTK forms complexes with MYD88 protein in WM cells with the *MYD88* L265P mutation. Inhibition of this pathway decreases coupling of these molecules, finally inducing apoptosis in WM cells.[5,9,10] Ibrutinib (Imbruvica, Pharmacyclics/Janssen), an orally administered BTK inhibitor, was first assessed in a series of patients with previously treated WM. The regimen consisted of 420 mg of ibrutinib daily, given as monotherapy until progression or unacceptable toxic effects. In this study, the genomic analyses were carried out with AS-PCR for *MYD88* L265P and *CXCR4* S338* C>G/A and with Sanger sequencing for other *CXCR4* mutations. The overall response rate (ORR) was 90% in the entire group, 100% in the mutated *MYD88* (*MYD88*^{mut}) *CXCR4*^{wt} group, 86% in the *MYD88*^{mut} mutated *CXCR4* (*CXCR4*^{mut}) group, and 71% in the *MYD88*^{wt} *CXCR4*^{wt} group. The 2-year progression-free survival (PFS) and OS rates were 69% and 95%, respectively. The most frequent adverse events were hematologic (neutropenia, thrombocytopenia, and anemia), cardiac (atrial fibrillation), and gastrointestinal (gastroesophageal reflux). Grade 3/4 atrial fibrillation was noted in 1 patient, and manifestations of bleeding occurred in 1 patient.[28]

Recently, an updated analysis of this cohort after 59 months of follow-up revealed an ORR of 90.5%. According to genotype, the highest ORR was again observed in the *MYD88*^{mut} *CXCR4*^{wt} patients (100%), followed by the *MYD88*^{mut} *CXCR4*^{mut} (86.4%) and *MYD88*^{wt} *CXCR4*^{wt} patients (50%; Table 1). The median time to achieve a major response was shorter in the *MYD88*^{mut} *CXCR4*^{wt} patients than in the *CXCR4*^{mut} patients, at 1.8 vs 4.7 months, respectively. Moreover, the 5-year PFS rates were 70% and 38% for the *MYD88*^{mut} *CXCR4*^{wt} and *MYD88*^{mut} *CXCR4*^{mut} patients, respectively. In a subgroup analysis of the patients with *CXCR4* mutations, the 5-year PFS rate was 50% for those with frameshift mutations and 36% for those with nonsense mutations. All 4 *MYD88*^{wt} *CXCR4*^{wt} patients had disease progression during a 2-year period of treatment.[29] Overall, ibrutinib monotherapy was proved to be highly effective in patients with relapsed or refractory WM.

Ibrutinib monotherapy as first-line treatment for treatment-naive WM patients has also showed impressive results; 100% achieved at least a minor response, whereas a major response or more was observed in 83% of patients. Although both the *MYD88*^{mut} *CXCR4*^{wt} group and the *MYD88*^{mut} *CXCR4*^{mut} group achieved ORRs of 100%, major response rates were higher in the first group (94% vs 78%, respectively). The 1.5-year PFS rate was 92%, and the safety profile was acceptable (no grade 4 toxicities).[30] The updated analysis after 4 years of follow-up showed similar rates of overall and major responses. Particularly, a trend toward a reduced very good partial response (VGPR) rate was observed in the patients with *CXCR4* mutations. The 4-year PFS was 76%, with a trend toward the worst PFS rate in the patients with *CXCR4* mutations. Here, the incidence of atrial fibrillation and bleeding symptoms was low, with no grade 3 or 4 toxicities reported.[31]

The efficacy of ibrutinib has been also assessed in patients who have WM with central nervous system involvement (also known as Bing Neel syndrome). A retrospective study showed that ibrutinib monotherapy decreased symptoms in 81% of patients, and radiologic improvement occurred in 60%.[32] Regarding peripheral neuropathy related to WM, response data were available for patients with relapsed/refractory WM who were treated with ibrutinib. Here, all 9 patients with peripheral neuropathy who received ibrutinib monotherapy showed a clinical response.[28] In another study, 3 patients who had WM with anti-MAG (myelin-associated glycoprotein) neuropathy (all *MYD88*^{mut} *CXCR4*^{wt}) and received ibrutinib monotherapy experienced clinical benefit and improvement over a 12-month follow-up.[28] Overall, ibrutinib has shown a high degree of efficacy, either as first-line treatment or in the relapsed/refractory setting. Although results have been promising in WM-related neuropathy, this remains a field of ongoing research.

Ibrutinib Plus Rituximab

The anti-CD20 agent rituximab is frequently used in the treatment of B-cell lymphoproliferative neoplasms. Given either as monotherapy or in combination with other

alkylating agents or proteasome inhibitors, rituximab has been proved to achieve acceptable response rates in WM.[33-37] Moreover, rituximab is easily administered and has a low toxicity rate. Thus, the rationale for testing a combination of rituximab and ibrutinib was the basis of the iNNOVATE clinical trial. The initial results from a subcohort of 31 patients with rituximab-refractory disease included an ORR of 90% and an 18-month PFS rate of 86%.[38] Thereafter, 150 patients were randomized to receive ibrutinib plus rituximab or placebo and rituximab. In this trial, next-generation sequencing (NGS) of targeted genes, including variants of *MYD88* and *CXCR4*, in bone marrow samples was used to evaluate genotype. The ORRs were 92% and 47%, respectively, and the major response rates were 72% and 32%, respectively, for the ibrutinib-plus-rituximab group vs the placebo-plus-rituximab group. Benefit was also correlated with genotype; the *MYD88*^{mut} *CXCR4*^{wt} group and the *MYD88*^{mut} *CXCR4*^{mut} group showed a trend to higher response rates. The most frequent adverse events in the ibrutinib-plus-rituximab group were atrial fibrillation and hypertension (grade ≥3 in 9 patients [12%]), and the most common adverse events in the placebo-plus-rituximab group were infusion-related reactions (grade ≥3 in 12 patients [16%]).[39]

More recently, an updated analysis showed similar results after a median follow-up of 50 months. The ORRs were 92% and 44%, respectively, and the major response rates were 76% and 31%, respectively, in the ibrutinib-plus-rituximab group vs the placebo-plus-rituximab group. According to genotype, the ORRs in the *MYD88*^{mut} *CXCR4*^{mut} group and the *MYD88*^{mut} *CXCR4*^{wt} group were 100% and 94%, respectively, with ibrutinib plus rituximab vs 48% and 43%, respectively, for placebo plus rituximab. In the *MYD88*^{wt} *CXCR4*^{wt} patients, the ORRs were 82% and 56%, respectively.[40] Thus, response rates were higher with ibrutinib plus rituximab, regardless of mutational status, than with rituximab monotherapy. Given the absence of an ibrutinib monotherapy arm in the iNNOVATE study, the benefit of adding rituximab to ibrutinib is unclear.

Acalabrutinib

Given the concerns about ibrutinib-related cardiac toxicity, new BTK inhibitors were developed to try to minimize the problem. Acalabrutinib (Calquence, AstraZeneca), which inhibits BTK covalently, has been shown to have a more selective profile than ibrutinib, with less off-target activity. Whereas ibrutinib inhibits Src family kinases and increases the risk for atrial fibrillation and other cardiac effects, acalabrutinib has not shown this activity in vitro.[41,42] Furthermore, less inhibition of the TEC family of kinases, which results in manifestations of bleeding, occurs with acalabrutinib than with ibrutinib.[43] Efficacy was demonstrated in a single-arm phase 2 clinical trial in which 106 patients (14 treatment-naive and 92 with relapsed or refractory WM) received acalabrutinib monotherapy at 200 mg daily. *MYD88 L265P* mutation was evaluated according to the protocol of each center; however, *CXCR4* mutations were not analyzed. With a median follow-up of 27.4 months, the ORR was 93% in both the treatment-naive patients and those with relapsed or refractory disease, and the 24-month PFS rates were 90% and 82%, respectively. Atrial fibrillation and grade 3/4 bleeding were observed in 1 and 3 patients, respectively. According to the presence of molecular abnormalities, the ORR was 94% in the *MYD88*^{mut} patients and 79% in the *MYD88*^{wt} patients.[44] To summarize, acalabrutinib achieved a durable response with a high degree of efficacy and an acceptable safety profile.

Zanubrutinib

Zanubrutinib (Brukinsa, BeiGene), another second-generation BTK inhibitor, has shown greater BTK selectivity and caused fewer off-target effects in comparison with ibrutinib. In WM, a randomized open-label phase 3 trial (ASPEN) compared zanubrutinib at 160 mg twice daily vs ibrutinib at 420 mg once daily. Here, *MYD88* L265P and *CXCR4* mutations were analyzed with AS-PCR and Sanger sequencing. The limit of detection of Sanger sequencing was 10% to 15% of mutant alleles. In addition, a targeted NGS platform was used to detect *CXCR4* mutations, covering all exonic regions. After a median follow-up of 18 months, the ORRs were similar in the ibrutinib and zanubrutinib cohorts (93% and 94%, respectively). The VGPR rate was 28% with zanubrutinib and 19% with ibrutinib. This difference, however, was not significant. As VGPR attainment was the main outcome of the study, the ASPEN study was considered negative.

Regarding safety issues, the cumulative incidence rates of atrial fibrillation/flutter and hemorrhage were significantly lower, but the incidence of neutropenia was higher, in the zanubrutinib arm.[45] Given the lower response rates observed with ibrutinib in the *MYD88*^{wt} patients, the ASPEN trial undertook a sub-study in 26 of the *MYD88*^{wt} patients treated. ORR and VGPR were 81% and 27%, respectively. Although this single-arm cohort showed a high degree of efficacy for zanubrutinib in the *MYD88*^{wt} patients, the techniques used did not allow an analysis of the mutations with low allelic frequency.[46]

New Agents Targeting Other Molecular Abnormalities or Antigens

Venetoclax

An analysis of the transcriptome of WM samples with bulk RNA sequencing found that the anti-apoptotic

BCL2 gene is upregulated in WM, regardless of *CXCR4* mutation status.[47] Moreover, *BCL2* overexpression acts with *MYD88* L265P in the development and progression of WM, as recently shown with single-cell sequencing.[27] In vitro, venetoclax (Venclexta, AbbVie) induced apoptosis in WM cell lines, also regardless of *CXCR4* mutation status.[48] Given these data and the high degree of efficacy observed in the treatment of chronic lymphocytic leukemia,[49] venetoclax was evaluated in a phase 2 clinical trial in patients with previously treated WM. A total of 32 patients received venetoclax at up to 800 mg daily for 2 years. AS-PCR was used to assess *MYD88* L265P in previously sorted CD19+ bone marrow cells, and either the same approach or Sanger sequencing was used to analyze *CXCR4* mutations. All patients had the *MYD88* L265P mutation, and 17 (53%) had a *CXCR4* mutation. The ORR for the entire cohort was 84%; however, the ORR was higher in the patients who had received 1 to 2 prior lines of therapy (ORR, 95%) than in those who had received 3 or more lines (ORR, 63%). According to genotype, the ORRs were similar in the patients with *CXCR4*^{mut} and those with *CXCR4*^{wt} (82% and 86%, respectively). Moreover, the median PFS of 30 months did not differ between the genotype subgroups. The most frequent grade 3 or higher adverse event was neutropenia (45%); grade 3 laboratory tumor lysis syndrome was observed only in 1 patient.[50] In summary, venetoclax showed a high degree of efficacy in the treatment of patients with relapsed or refractory WM regardless of *CXCR4* mutational status.

CXCR4 Inhibitors

As previously mentioned, *CXCR4* mutations occur in up to 40% of patients with WM.[7,17,19,20] Given the high prevalence of *CXCR4* mutations and their role in the development of the lymphoplasmacytic clone, the *CXCR4* mutation is an attractive target. For instance, in vivo experiments have assessed a fully human monoclonal IgG_4 against CXCR4 (ulocuplumab), which was able to inhibit the proliferation and dissemination of WM cells.[16] This finding led to the design of a phase 1 clinical trial that included 13 patients with *CXCR4*^{mut} WM. *MYD88* and *CXCR4* mutational status was analyzed in CD19+ sorted bone marrow cells. Patients were started on ibrutinib at 420 mg daily until progression or drug intolerance along with ulocuplumab from cycles 1 to 6. Ulocuplumab was administered intravenously every week according to a dose-escalation design. The ORR and major response rate were both 100%, and a VGPR was achieved in 4 patients (33%). After a median follow-up of 22.4 months, the median time to a major response was 1.2 months, and the 2-year PFS rate was 90%. The most common grade 2 or higher adverse events were thrombocytopenia, rash, and skin infections. The administration of ulocuplumab was well tolerated in all patients, with no infusion-related adverse events.[51]

Another antagonist of CXCR4 is mavorixafor, an oral agent that inhibits CXCL12 binding to CXCR4. Preliminary data on mavorixafor were evaluated in a phase 1b clinical trial of 18 (9 already dosed) *MYD88*^{mut} *CXCR4*^{mut} patients. Treatment consisted of mavorixafor at 200 mg and ibrutinib at 420 mg, both orally administered daily. Among 8 evaluable patients, the ORR and the major response rate were 100% and 50%, respectively. A VGPR occurred in 1 patient. Most adverse events (79%) were grade 1. Dose-limiting toxicity was observed in 1 patient (grade 3 hypertension).[52]

The results of these studies show the efficacy of combining ibrutinib with CXCR4 antagonists, and the potential for the development of other anti-CXCR4 drugs in WM. Another promising antagonist molecule is the endogenous peptide EPI-X4, which binds to CXCR4 of WM cells in competition with CXCL12, thereby impairing the migration toward CXCL12 and the proliferation of WM cells.[53]

Ixazomib

Ixazomib (Ninlaro, Takeda) is the first oral second-generation proteasome inhibitor. Its relatively high affinity for a specific residue of the 20S proteasome (in contrast to bortezomib) makes it less likely to cause peripheral neuropathy.[54] A combination of ixazomib, rituximab, and dexamethasone was evaluated in a phase 2 clinical trial of 26 treatment-naive patients with WM. *MYD88* and *CXCR4* mutations were evaluated in CD19+ sorted bone marrow cells. All patients had the *MYD88* L265P mutation, and 58% were *CXCR4*^{mut}. The study reported an ORR of 96% and a major response rate of 77%. The median time to response was longer in the *CXCR4*^{mut} than in the *CXCR4*^{wt} patients (12 and 8 weeks, respectively). Grade 3 peripheral neuropathy developed in 1 patient, and 5 patients had grade 1 peripheral neuropathy.[55] The updated follow-up of this study showed the same trend toward a longer time to achieve a response in the *CXCR4*^{mut} patients than in the *CXCR4*^{wt} patients. Moreover, the VGPR rate was lower in the *CXCR4*^{mut} patients than in the *CXCR4*^{wt} patients (7% vs 36%, respectively). However, the median PFS values were similar regardless of *CXCR4* mutational status (40 and 36 months).[56]

More recently, another group showed results of a phase 1/2 clinical trial of ixazomib in combination with rituximab and dexamethasone in 59 patients with relapsed WM. *MYD88* and *CXCR4* mutations were assessed with targeted NGS in whole bone marrow samples. The prevalence of *MYD88* mutations was 93%, and the prevalence of *CXCR4* mutations was 27%. Here, the ORR was 71% after 8 cycles of treatment. The *MYD88*^{mut} *CXCR4*^{wt} patients and the *MYD88*^{wt} *CXCR4*^{wt} patients achieved

VGPR rates of 47% and 33%, respectively. However, no *MYD88*^{mut} *CXCR4*^{mut} patient achieved a VGPR. After a median follow-up of 24 months, the median PFS was not achieved for the entire cohort, in neither the *MYD88*^{mut} *CXCR4*^{wt} subgroup nor the *MYD88*^{wt} *CXCR4*^{wt} subgroup. In the *MYD88*^{mut} *CXCR4*^{mut} patients, the median PFS was 36 months, although the difference was not significant. The safety profile was quite similar to that in the previously reported study, showing mostly grade 1 or 2 neurotoxicity.[57] Overall, the combination of ixazomib with rituximab and dexamethasone showed a high degree of efficacy, with deeper responses and shorter times to response in the *CXCR4*^{wt} patients.

Noncovalent BTK Inhibitors

Covalent BTK inhibitor therapy (eg, ibrutinib, acalabrutinib, zanubrutinib) is of indefinite duration until disease progression or unacceptable toxicity. Disease progression during active covalent BTK inhibitor therapy is associated with the acquisition of a recurrent mutation in *BTK* (ie, *BTK* C481S).[58] The best therapeutic approach for a patient with WM progression on a covalent BTK inhibitor has not yet been well defined, but chemoimmunotherapy, proteasome inhibitors, and venetoclax have shown efficacy in this setting, especially if the patient was not previously exposed to these agents.[59] However, a patient previously exposed to all these agents and whose disease is progressing on a covalent BTK inhibitor represents a therapeutic challenge.

Noncovalent BTK inhibitors have been shown to be effective in patients who cannot tolerate or whose disease is progressing on covalent BTK inhibitors. They exert their effect by binding to BTK without interacting with the 481 locus. Pirtobrutinib is a highly selective, oral noncovalent BTK inhibitor with restricted off-target effects.[60] In a phase 1/2 study (BRUIN), 26 patients with WM received pirtobrutinib therapy.[61] Of these, 19 were evaluable for response and 13 had previously received a covalent BTK inhibitor. The rate of response to pirtobrutinib in the patients who had previously received a covalent BTK inhibitor was 69%, suggesting a high level of activity of pirtobrutinib in this setting.

Other Molecules

Given the activation of the PI3K pathway in WM,[48,62] the oral inhibitor idelalisib (Zydelig, Gilead) was evaluated in 2 clinical trials for indolent lymphoma. The first study was a phase 2 clinical trial and included 10 patients with WM[63]; the other was a phase 1 study with 9 patients who had WM.[64] The planned treatment was idelalisib at 150 mg twice daily. The ORR was 80% in the first study and 56% in the second study.[63,64] Concerns regarding the safety profile arose, however, when a study reported significant liver toxicity with idelalisib (grade ≥3 in 75% of patients).[65] Later, the combination of idelalisib plus 6 cycles of obinutuzumab (Gazyva, Genentech) followed by idelalisib maintenance led to an ORR of 71% in 43 patients with relapsed or refractory WM. Interestingly, *CXCR4* mutations (evaluated by targeted NGS or ddPCR) did not affect response rates or PFS. Nonetheless, grade 3 or higher hematologic and nonhematologic adverse events were again reported in 45% and 24% of patients, respectively.[66] Table 1 summarizes the molecular targets reported in the previously mentioned clinical trials.

Another potential agent with a target antigen that has been evaluated in WM is daratumumab (Darzalex, Janssen Biotech), a monoclonal antibody against CD38. Daratumumab has been shown to decrease the expression of WM cell signaling molecules, including BTK.[67] A phase 2 study assessed the outcomes of 13 patients with previously treated WM. Daratumumab monotherapy was administered intravenously at dose of 16 mg/kg once weekly during cycles 1 and 2 (8 doses), then every 2 weeks during cycles 3 to 6, and then once every 4 weeks during cycles 7 to 18. Here, the ORR and major response rate were 23% and 15%, respectively. Changes in the CD38 median fluorescence intensity in plasma cells and B cells suggested that daratumumab did not alter the B-cell compartment.[68] The combination of daratumumab with ibrutinib is currently undergoing evaluation.

Chimeric Antigen Receptor T-Cell Therapy

New advances in immunotherapy for WM are on the horizon. Chimeric antigen receptor (CAR) T-cell therapy against CD19 has shown impressive activity in other lymphoid neoplasms, such as acute lymphoblastic leukemia and DLBCL.[69,70] CAR T cells targeting the B-cell maturation antigen (BCMA) have also demonstrated clinical activity in patients with multiple myeloma and very advanced disease, achieving deep responses with prolonged survival, although a clear survival plateau has not been observed.[71,72]

Some preliminary evidence of CAR T-cell activity against CD19 in WM has been reported. In vitro and in vivo experiments have confirmed the activity of CAR T cells against a human *MYD88* L265P–positive WM cell line, BCWM.1. A series of 3 patients treated with CAR T-cell products against CD19 demonstrated early signs of safety and clinical activity; 2 patients were treated with 19-28z CAR T-cell therapy and 1 was treated with truncated human epidermal growth factor receptor (EGFRt)/19-28ζ/4-1BBL, an "armored" modified CAR. Treatment was well tolerated, with only grade 1/2 toxicities observed. All patients showed a clinical response, from stable disease with a hematologic response to complete remission. However, progression occurred in all 3 patients.[73] Similarly

Table 2. General Characteristics of the 3 Most Common Genotypes in Waldenström Macroglobulinemia

	MYD88^{mut} *CXCR4*^{wt}	*MYD88*^{mut} *CXCR4*^{mut}	*MYD88*^{wt} *CXCR4*^{wt}
Prevalence	>60%	30%-40%	<10%
Clinical presentation			
BM involvement	++	+++	+
Serum IgM	++	+++	+
Adenopathy	++	+	+++
Hyperviscosity	+	+++	+
Acquired von Willebrand disease	+	+++	+
Risk for DLBCL transformation	+	+	+++
Response to novel targeted agents			
ORR, 1st-gen BTK inhibitors	94%-100%	86%-100%	50%-82%
VGPR rate, 2nd-gen BTK inhibitors	34%	18%	27%
ORR, BCL2 inhibitors	86%	82%	–
ORR, CXCR4 inhibitors	–	100%	–

BM, bone marrow; BTK, Bruton tyrosine kinase; BCL2, B-cell lymphoma 2; CXCR4, C-X-C motif chemokine receptor 4; DLBCL, diffuse large B-cell lymphoma; gen, generation; IgM, immunoglobulin M; mut, mutated; ORR, overall response rate; VGPR, very good partial response; wt, wild-type.

encouraging results have been reported in another patient, in whom histologic transformation to DLBCL arising from WM was treated with CAR T-cell therapy. The patient is still in complete remission from both the large cell transformation and WM at 1 year after CAR T-cell infusion.[74]

Future Perspectives

We have reviewed the genomic landscape of WM in regard to the biology and diagnosis of this disease (Table 2), as well as the design and evaluation of outcomes in clinical trials. From a diagnostic point of view, it is imperative to analyze *MYD88* and *CXCR4* mutations before treatment initiation. As high-throughput technologies become widely used, new assays will be available that can precisely analyze somatic mutations in cancer. In this sense, ddPCR offers an enormous advantage because it can provide absolute quantification of a mutation without a previous sorting preparation step. This technology has the potential to be implemented easily in many centers and make molecular data more reproducible across studies. Promising results have been described in patients with IgM MGUS or WM.[20,75]

Regarding treatments, Table 3 summarizes the active clinical trials specifically designed for patients with WM. Formal comparisons of BTK inhibitors with chemoimmunotherapy, which is arguably the most commonly used therapeutic modality in WM, are great interest. For example, an important study compared the combination of ibrutinib and rituximab vs cyclophosphamide, dexamethasone, and rituximab. The goal of combining targeted agents is to deepen the response to therapy and prolong the duration of the response, as is the case with the combination of ibrutinib and venetoclax and the combination of ibrutinib and mavorixafor in *CXCR4*-mutated WM. Several studies will evaluate triple regimens in WM, including a Canadian study combining bendamustine, acalabrutinib, and rituximab and a US study combining ibrutinib, venetoclax, and rituximab. Of additional interest is the possibility of administering these regimens in a fixed-duration fashion, thereby minimizing exposure to therapy and toxicity. The role of immunotherapy in WM is unclear. The results of studies looking into antibody-drug conjugates, such as loncastuximab tesirine (Zynlonta, ADC Therapeutics), and CAR T-cell therapy are eagerly awaited.

Conclusions

The identification of highly recurrent somatic mutations in the *MYD88* and *CXCR4* genes has contributed to a better understanding of the biology of WM. Moreover, knowledge of the genomic landscape has facilitated the design and evaluation of treatment options based on molecular targets. In this sense, BTK, BCL2, and CXCR4 inhibitors have demonstrated a high degree of efficacy with good tolerability. Along with the availability and improvement of high-throughput technologies, treatment options have increased, and the development of further treatment strategies for patients with WM is assured in the coming years.

Disclosures

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Table 3. Selected Clinical Trials for Waldenström Macroglobulinemia

Agents	Design (Identifier)
Mavorixafor and ibrutinib	Phase 1 (NCT04274738)
APG-2575, as single agent or in combination with ibrutinib	Phase 1/2 (NCT04260217)
Ibrutinib and venetoclax	Phase 2 (NCT04273139)
Ibrutinib and rituximab vs ibrutinib, rituximab, and venetoclax	Phase 2 (NCT04840602)
Daratumumab and ibrutinib	Phase 2 (NCT03679624)
Bendamustine, rituximab, and acalabrutinib	Phase 2 (NCT04624906)
Zanubrutinib, ixazomib, and dexamethasone	Phase 2 (NCT04463953)
Pembrolizumab and rituximab	Phase 2 (NCT03630042)
Obinutuzumab monotherapy	Phase 2 (NCT03679455)
Orelabrutinib (ICP-022)	Phase 2 (NCT04440059)
Loncastuximab tesirine	Phase 2 (NCT05190705)
Iopofosine I131 (CLR 131)	Phase 2 (NCT02952508)
Acalabrutinib and rituximab	Phase 2 (NCT05065554)
Pirtobrutinib (LOXO-305)	Phase 2 (NCT03740529)
Venetoclax and rituximab vs CDR	Phase 2 (NCT05099471)
Ibrutinib and rituximab vs CDR	Phase 2/3 (NCT04061512)
Ibrutinib and carfilzomib vs ibrutinib	Phase 3 (NCT04263480)

CDR, cyclophosphamide, dexamethasone, and rituximab.

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