Clinical Implications of Genomic Profile in Waldenström Macroglobulinemia

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ABSTRACT

With the increasing availability of sequencing techniques and new polymerase chain reaction-based methods, data regarding the genomic profile of Waldenström macroglobulinemia (WM) are being continuously analyzed and reproduced. *MYD88* and *CXCR4* mutations are highly prevalent in all the stages of WM, including the early IgM monoclonal gammopathy of undetermined significance or a more advanced stage, such as smoldering WM. Translating this knowledge into the clinic is becoming crucial, given the improvement in diagnostic tools and the importance of redefining the risk of progression in the asymptomatic stages. Moreover, treatment response in symptomatic WM can vary according to genotypes. Thus, there is a need to define genotypes before starting either standard treatment regimens or clinical trials. Here, we review the genomic profile of WM and its clinical implications while focusing on recent advances.

Introduction

Waldenström macroglobulinemia (WM) is characterized by highly recurrent somatic mutations in the myeloid differentiation factor 88 (*MYD88)* and the C-X-C motif chemokine receptor 4 (*CXCR4)* genes (1,2). More than 90% of patients with WM harbor *MYD88* mutations, while *CXCR4* mutations account for up to 40% (3). A change from leucine to proline at position 265 in *MYD88*, also known as *MYD88* L265P, accounts for almost all mutations identified in that gene (2). On the other hand, a wide spectrum of nonsense and frameshift mutations in *CXCR4* has been reported. However, the most prevalent are those found at position 338, causing a stop codon (*CXCR4* S338*) (1). Pre-symptomatic stages such as IgM monoclonal gammopathy of undetermined significance (MGUS) or smoldering WM (SWM) also show a similar profile (4,5). Various reports have described the three most common genotypes in WM: *MYD88* mutated (mut) *CXCR4* wild type (wt), *MYD88*mut *CXCR4*mut, and *MYD88*wt *CXCR4*wt (6). With the advent of new sequencing techniques and highly sensitive polymerase chain reaction (PCR) assays, there is now an increasing number of options available to assess the genotypes of WM patients. The availability of new methods to detect somatic mutations in WM has also made it possible to characterize and diagnose the disease, draw correlations with treatment responses, design new clinical trials, and redefine the prognostic risk in both asymptomatic and symptomatic patients.

Here, we review the current knowledge on the genomic landscape of WM and the clinical implications regarding diagnosis, treatment, and prognosis. We focus on recent advances using targeted or whole genome sequencing (WGS), novel PCR methods such as droplet digital PCR (ddPCR), the use of new potential sample sources like cell-free DNA (cfDNA), and the importance of defining the genotype to improve and personalize treatment.

Advances in the biology of Waldenström macroglobulinemia

The clinical need to analyze the genome of samples from patients with WM has been increasing during the last few years. Taking advantage of the wide availability of sequencing methods, we can now describe the mutation profile using bone marrow, peripheral blood, or even cfDNA samples. Moreover, recent novel approaches to assess the genome, such as single-cell sequencing, have brought us a wider picture of the clonal heterogeneity of this disease.

In this sense, there has been novel advances regarding the biology of WM. For example, detecting *MYD88* L265P early in B cell development gave more insight into the origin of the malignant clone. Cellular indexing of transcriptome and epitopes followed by sequencing (CITE-seq) has revealed that pre-B cells from WM had transcriptionally enriched pathways such as IL6-STAT3, similarly found in B cells. Furthermore, exome sequencing identified the *MYD88* L265P mutation in pre-B progenitors in the bone marrow of patients with WM and IgM MGUS (7). Another study using single-cell DNA sequencing showed that *MYD88* L265P has been found not only in tumor B cells, but also in B cell precursors and normal B lymphocytes from WM samples. Although *MYD88* L265P was detected early in B-cell development, the sole presence of the mutation could not drive lymphomagenesis in a mice model (8). Altogether, these data demonstrated that small clonal alterations might arise during

lymphopoiesis in WM. Still, the acquisition of *CXCR4* mutations, copy number alterations (CNAs), and del(6q) would confer a greater risk of clonal expansion, with the development of a phenotypically overt WM. This evolutionary model has also been proposed using a large targeted sequencing panel, by which CNAs were increasingly found in relapsed WM compared to stable WM. Leveraging WGS data, the same study identified gains in chromosome 12 early in the *MYD88*wt WM development, while other chromosomal gains occurred later (9). These findings were similar to those reported in chronic lymphocytic leukemia and can serve as a basis for discovering potential biomarkers in a subset of WM patients.

More recently, whole exome sequencing (WES) identified Spi1 proto-oncogene (*SPI1*) Q226E mutation in 6% of WM patients. These results were later confirmed by either targeted or RNA sequencing on sorted tumor cells. *SPI1* encodes a transcription factor that activates gene expression in B-cell and myeloid-cell development. The mutant transcription factor binds to other promoter regions, which results in increased B-cell proliferation. *SPI1* Q226E mutation was associated with worse overall survival than the wild-type *SPI1* counterpart. Moreover, the study also reported a preclinical potential activity of BET inhibitors or lenalidomide in *SPI1* Q226E WM, as MYC and IRF4-enriched signatures were associated with the mutant cases (10).

Regarding the epigenome, the emerging methods to analyze it have also been applied in WM, giving more insights into the biology of the disease. By leveraging both a DNA methylation array and RNA sequencing in WM samples, a study could classify patients in two different subgroups, memory B-cell and plasma-cell-like showing different features. For instance, the memory B-cell-like group showed more *CXCR4* mutations and del(13q), while the plasma-cell-like group harbored more del(6q) (11).

The fact that highly prevalent somatic mutations in WM are also observed in early asymptomatic stages and the reported predisposition in family clusters make WM a model to study cancer biology and establish potential causal factors. In that context, genome-wide association studies (GWAS) can help understand the relationship between genetic variants and predisposition to cancer. In the case of WM, two loci (6p25.3 and 14q32.13) were associated with an increased risk of WM in a familial cluster of cases, later confirmed in a non-familial set. Both loci were closely related to previously known genes dysregulated in other lymphoid neoplasms, making them potential susceptibility regions (12). Functional studies are needed to analyze the biological impact of these findings further.

After all these technological advances, the application of high throughput technologies in WM has enabled to further disentangle the biology of the disease and to identify new biomarkers of disease progression with potential use in future clinical trials.

Impact on clinical phenotypes

From a clinical perspective, *MYD88* and *CXCR4* mutations can describe phenotypical characteristics in patients with WM. For instance, more than 60% of patients with WM harbor *MYD88*mut *CXCR4*wt genotype, associated with a moderate bone marrow lymphoplasmacytic infiltrate and high serum IgM level. *MYD88*mut *CXCR4*mut is the second most common genotype (approximately 30-40% of WM patients). It has been associated with higher bone marrow involvement and serum IgM level, thus observing a higher incidence of hyperviscosity

(3). Different gene expression profiling methods confirmed that a *CXCR4* signature was associated with this clinical phenotype (13,14).

Among the *CXCR4* S338* mutations reported in WM, the conversion of C>G is the most frequently reported. This mutation has been associated with both bone marrow homing of lymphoplasmacytic cells and extramedullary dissemination, explaining in part the clinical phenotype of *CXCR4*mut patients (15).

As previously mentioned, the epigenetic characterization of WM into a memory B-cell and plasma-cell-like groups also revealed differential clinical characteristics. Memory B-cell-like WM cases were associated with increased thrombocytopenia and splenomegaly. These patients also exhibited a higher variant allele frequency of *CXCR4* mutations. On the other hand, plasma-cell-like cases showed differences in the morphology of tumor cells and CNAs, but no clinical phenotype was distinctive (11).

Lastly, less than 10% of WM patients are *MYD88*wt *CXCR4*wt. This genotype is associated with an increased prevalence of lymphadenopathy and a higher risk for transformation to a diffuse large B-cell lymphoma (DLBCL) (16). Using WES, *MYD88*wt cases harbored mutations that are known to trigger NF-Kb activation or are involved in epigenomic dysregulation and DNA damage repair. For instance, mutations in *TBL1XR1* were identified in *MYD88*wt WM cases, reported in other B-cell lymphomas with aggressive presentation (17). Yet caution is needed in *MYD88*wt WM cases, as a proportion of them might still show a different diagnosis, which include IgM-secreting marginal zone lymphoma or IgM multiple myeloma (16).

Treatment response according to the mutation status

Given that there are associations between the genotypes and the clinical presentation of patients with WM, efforts have been made to include the mutation status in the design of clinical trials. Although there are no meta-analyses to disentangle the impact of genotypes on treatment effect, the general overview is that *MYD88*wt patients achieved lower overall response rate (ORR), followed by those with concomitant *MYD88* and *CXCR4* mutations. A plausible explanation is that *MYD88*wt WM cases showed a mutation profile similar to some aggressive B-cell lymphomas (17). On the other hand, *CXCR4* mutations confer a higher disease burden in the *MYD88*mut cases (15).

In fact, it has been reported that ORR to ibrutinib was higher in *MYD88*mut patients with or without concomitant *CXCR4* mutations compared to *MYD88*wt (18). Moreover, *MYD88*wt cases achieved lower ORR than the other two genotypes in the study that combined ibrutinib with rituximab (19). When using second-generation Bruton tyrosine kinase (BTK) inhibitors, acalabrutinib also showed higher ORR in *MYD88*mut patients compared to wild-type (20). Retrospective studies analyzing the role of *CXCR4* mutations on ibrutinib response have also reported that nonsense *CXCR4*mut patients achieved lower major response rates compared to frameshift *CXCR4*mut (21). These results again favored the importance of testing for *CXCR4* S338* mutations, as the nonsense mutations might associate with a less functional protein. Moreover, the mutation burden of *CXCR4* S338* has been also associated with reduced very good partial response rates in patients who received ibrutinib monotherapy (21). The lower responses in *MYD88*mut *CXCR4*mut showed in the trials using ibrutinib monotherapy could be partially improved by incorporating rituximab (19).

Similarly, patients treated with zanubrutinib achieved a higher partial response or better rates in *MYD88*mut *CXCR4*wt and *MYD88*mut *CXCR4*mut patients compared to wild-type (22). Venetoclax, a B-cell lymphoma 2 (BCL2) inhibitor, had a similar trend to better ORR in *MYD88*mut *CXCR4*wt patients compared to those harboring *CXCR4* mutations (23). Among other new agents, the combination of ixazomib, dexamethasone, and rituximab reported higher ORR in *MYD88*mut *CXCR4*wt and *MYD88*mut *CXCR4*mut, with a slightly inferior proportion of response in the patients of the latter group (24).

As we previously exposed, *CXCR4* mutations might confer not only a greater disease burden, but also a trend to lower response rates. To mitigate this problem, new agents against *CXCR4* (ulocuplumab or mavorixafor) have shown promising results in *MYD88*mut *CXCR4*mut patients (25,26). Non-covalent BTK inhibitors (pirtobrutinib) and the combination of acalabrutinib or venetoclax with rituximab are being evaluated in clinical trials in WM patients that might unravel the impact of genotypes on treatment effects.

Prognosis according to genotypes in Waldenström macroglobulinemia

Classification of WM patients according to genotype has been able to draw associations with overall survival (OS). An early analysis doing both AS-PCR and Sanger sequencing for *MYD88* L265P and *CXCR4* mutations in bone marrow samples from 174 WM patients showed that OS was shorter in *MYD88*wt patients compared to those harboring the L265P mutation (3). Moreover, the updated analysis after a longer median follow-up confirmed these results. Thus, the 10-year estimated OS for *MYD88*wt and *MYD88*mut patients was 73% and 90%, respectively (16). In both studies, the co-occurrence of *CXCR4* mutations in *MYD88*mut patients did not affect OS. As explained above, *MYD88*wt patients share a certain mutation profile with other aggressive B-cell lymphomas (17). These findings were also translated into a higher incidence of transformation to DLBCL with an ominous prognosis (16).

Regarding progression-free survival (PFS), *MYD88*wt patients had shorter PFS followed by *MYD88*mut *CXCR4*mut patients treated with ibrutinib monotherapy (18,27). Similar PFS was observed in the case of ibrutinib with rituximab regardless of mutation status (19). Patients treated with venetoclax and ixazomib, dexamethasone and rituximab reported *MYD88* mutations in all patients, and the co-occurrence of *CXCR4* mutations did not impact PFS in both studies (24). Similarly, the combination of obinutuzumab and idelalisib showed no differences in PFS regarding the *CXCR4* mutation status; however, 24% of the whole series harbored *TP53* mutations, and they had a trend to worse impact (28).

On the other hand, the availability of genotyping early asymptomatic stages has increased in the last years, further elucidating the risk of progression to symptomatic disease. The interest remains in the fact that *MYD88* and/or *CXCR4* mutations can model the progression in IgM MGUS and SWM. In this scenario, *MYD88* L265P was found in 54% of a series of IgM MGUS patients assessed by AS-PCR showing a shorter PFS after a median follow-up of 83 months (29). Likewise, other study using ddPCR reported 64% and 35% of *MYD88* L265P and *CXCR4* S338* mutations prevalence in IgM MGUS patients, showing an increased incidence of progression to symptomatic disease in those cases with high allele burden (30). In the case of SWM, two studies using AS-PCR showed that *MYD88*wt patients had shorter PFS than *MYD88*mut (31,32). More recently, it was reported that the co-occurrence of MYD88 L265P

and CXCR4 S338* mutations and a high allele burden increased the probability of progression in SWM leveraging ddPCR technology (30).

Another biomarker that negatively affects outcomes in WM patients is del(6q). A study reported a prevalence of 4%, 9%, and 30% of del(6q) in IgM MGUS, SMW, and symptomatic WM patients, respectively. Patients harboring del(6q) had a shorter time to disease progression in the asymptomatic stages. Meanwhile, symptomatic WM patients with del(6q) had a shorter time to next treatment. Irrespective of diagnosis, OS was shorter in all groups with del(6q) (33).

Thus, the absence of mutations in *MYD88* confers a higher risk of transformation to an aggressive B-cell lymphoma to patients with WM and is associated with a shorter OS compared to the other two genotypes. *CXCR4*mut status apparently impacts treatment response, especially after BTK inhibitors, but with no clear impact on OS. In case of the asymptomatic stages, as most samples have a relatively lower disease burden, there is a great dependency on the technology used to analyze somatic mutations. It seems that higher tumor burden could be associated with shorter time to symptomatic disease

New technologies to assess the genotypes of Waldenström macroglobulinemia

Since the detection of *MYD88* L265P in WM using WGS in selected CD19+ bone marrow cells (2), designing PCR-based methods has helped expand and reproduce the mutation detection among different centers around the world. Figure 1 summarizes the most common sample sources used to analyze mutations or other genomic alterations in WM. Using CD19+ selected samples, unselected bone marrow, or even peripheral blood samples, AS-PCR has proven to achieve high sensitivity and specificity to detect *MYD88* L265P (4). In the case of *CXCR4*, the attention has been focused on the nonsense S338* c.1013C>G and c.1013C>A mutations. AS-PCR assays to test both mutations have also been developed and reproduced; however, as most of them are subclonal, sorting CD19+ bone marrow cells was an important step before PCR amplification (5). More recently, sequencing targeted genes using different platforms has enabled to assess other mutations already described before by WGS or WES. This technology partially compensates for cost issues to sequence a genome, achieving high sensitivity. However, very small subclones are difficult to detect. In this sense, customizable features before sequencing can achieve deeper resolution, thus increasing the costs (34). Table 1 summarizes studies where AS-PCR was used in IgM MGUS and WM to detect *MYD88* L265P and *CXCR4* S338* mutations. As observed, there is a wide variation of mutation detection rates when using unselected or selected CD19+ bone marrow or peripheral blood samples in IgM MGUS.

Recently, based partially on the somehow patchy bone marrow infiltration and the need to detect mutations bypassing a bone marrow biopsy, cfDNA is becoming an important source under investigation. Although not yet standardized internationally, several studies have reported the detection of both *MYD88* L265P and *CXCR4* S338* mutations with AS-PCR. Table 2 also summarizes studies that used cfDNA as a source of material.

More lately, and because of the need to achieve an even deeper resolution to detect somatic mutations, some groups have focused on ddPCR. This technology has already achieved higher sensitivity than AS-PCR, thus replacing the prior CD19+ sorting step. This step is

particularly problematic in three situations: very small clones in IgM MGUS or minimal residual disease assessment in WM, subclonality of *CXCR4* mutations, and using cfDNA as samples. Since ddPCR can achieve absolute quantification of a mutation, it has shown promising results as a mutation detection method and a biomarker of disease progression in its early stages. For instance, a study reported that *MYD88* L265P mutation was detected in more cases in IgM MGUS (up to 64%) using ddPCR compared to AS-PCR in unsorted bone marrow samples (30). Moreover, a comparative study that analyzed sorted CD19+ versus unsorted samples described no differences in detecting the *MYD88* L265P mutation using ddPCR (35). Regarding *CXCR4* mutations, ddPCR detected S338* mutations in up to 35% of IgM MGUS patients without prior CD19+ sorting step (30). Previously, others reported *CXCR4* mutations in 17% (5) and 29% (36) of CD19+ sorted IgM MGUS samples using AS-PCR and/or Sanger sequencing.

Another recent PCR-based method reported is competitive allele-specific PCR (Cast-PCR). Cast-PCR can work with very low amounts of DNA either on tumoral or cfDNA samples, and to properly quantify the mutation burden. Although the limit of detection of Cast-PCR to detect *MYD88* L265P was 0.1%, between the canonical AS-PCR (1%) and ddPCR (0.05%), the ability to work with low amounts of DNA made it a potential candidate to assess cfDNA samples (37).

As we rely on the continuous advances of high-throughput technologies to detect somatic mutations in cancer, promising tools are guaranteed regarding deeper sensitivity for *MYD88* L265P and *CXCR4* S338* mutations. The increasing availability of new methods also necessitates standardizing mutation detection in WM. Further studies will confirm the reproducibility of the results for *CXCR4* mutations using ddPCR in WM.

Conclusions

Genotype testing is an important step to a better diagnosis of patients with WM, for predicting disease progression in IgM MGUS or SWM, evaluating treatment response, and designing clinical trials. The genotypes based on *MYD88* and *CXCR4* mutations are translated into clinical characteristics and can affect the PFS and OS of WM patients. Advances to increase depth of mutation detection is critical, as well as a need to standardize methods among different centers. ddPCR has proven to be a reliable technique to analyze *MYD88* and *CXCR4* mutations in WM using either bone marrow or cfDNA samples and can obviate the CD19+ sorting step.

CLINICAL CARE POINTS

* *MYD88* and *CXCR4* mutations are critical in the biology of WM. *MYD88* L265P is an early event during the evolution of the lymphoplasmacytic clone. *CXCR4* S338* mutations are subclonal to MYD88 and confer greater risk of disease progression.

* More than 90% and up to 40% of WM patients harbor *MYD88* and *CXCR4* mutations, respectively. Caution should be taken in *MYD88*wt patients, as some of them might have an alternative diagnosis or are in greater risk of transformation to aggressive lymphomas.

* Novel techniques can achieve higher sensitivity to detect somatic mutations, along with precise quantification of the mutation burden. AS-PCR have demonstrated reproducible results among different studies, while ddPCR is an emerging technology that can overtake detection issues in samples with very low tumor burden (i.e. IgM MGUS, minimal residual disease follow-up, use of cfDNA, among others).

* *MYD88* and *CXCR4* mutations translate into a clinical phenotype in patients with WM. *CXCR4*mut patients show more serum IgM, increased prevalence of hyperviscosity, and higher bone marrow infiltration.

* Genotype testing is crucial in the inclusion for clinical trials and may also contribute to evaluate treatment response. *MYD88*mut patients show higher overall response rates to BTK inhibitors compared to *MYD88*wt, with a longer median OS.

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TABLES

Table 1. Summary of studies using bone marrow samples as a source of material for testing *MYD88* and *CXCR4* mutations. The percentage is the mutation detection prevalence reported. AS-PCR: allele-specific polymerase chain reaction; ASO-PCR: allele-specific oligonucleotide polymerase chain reaction; ddPCR: droplet digital polymerase chain reaction; MGUS: monoclonal gammopathy of undetermined significance; WM: Waldenström macroglobulinemia; SWM: smoldering Waldenström macroglobulinemia

Studies using bone marrow samples	***MYD88*^{mut}**	***CXCR4*^{mut}**

	IgM MGUS	WM	IgM MGUS	WM
Treon et al, 2012 Sanger on CD19+ cells	2/21 (10%)	49/54 (91%)	-	-
Xu et al, 2013 AS-PCR on CD19+ cells	13/24 (54%)	97/104 (93%)	-	-
Jiménez et al, 2013 ASO-PCR on unsorted cells	27/31 (87%)	101/117 (86%)	-	-
Treon et al, 2014 AS-PCR on CD19+ cells for *MYD88* Sanger on CD19+ cells for *CXCR4*	-	158/175 (90%)	-	51/175 (29%)
Varettoni et al, 2014 AS-PCR on unsorted cells	36/77 (47%)	58/58 (100%)	-	-
Xu et al, 2015 AS-PCR on CD19+ cells Sanger on CD19+ cells	6/12 (50%)	97/102 (95%)	2/12 (17%)	44/102 (43%)
Varettoni et al, 2017 AS-PCR on CD19+ cells for *MYD88* Sanger on CD19+ cells for *CXCR4*	78/130 (60%)	112/130 (86%)	5/130 (4%)	29/130 (22%)
Drandi et al, 2018 ddPCR on unsorted cells	-	109/112 (97%)	-	-
Ferrante et al, 2021 ddPCR on unsorted cells	54/62 (87%)	93/97 (96%)	-	-
Moreno et al, 2022* ddPCR on unsorted cells	54/84 (64%)	45/55 (82%)	21/54 (39%)	21/42 (50%)

*These data refer only to smoldering Waldenström macroglobulinemia patients.

Table 2. Summary of studies using cell-free DNA as source of material for testing *MYD88* and *CXCR4* mutations. The percentage is the mutation detection prevalence reported. AS-PCR: allele specific polymerase chain reaction; ddPCR: droplet digital polymerase chain reaction; MGUS: monoclonal gammopathy of undetermined significance; WM: Waldenström macroglobulinemia; SWM: smoldering Waldenström macroglobulinemia

Studies using cell-free DNA samples	***MYD88*^{mut}**		***CXCR4*^{mut}**	
	IgM MGUS	**WM**	**IgM MGUS**	**WM**
Drandi et al, 2018 ddPCR	-	53/60 (88%)	-	-
Wu et al, 2020 AS-PCR	-	23/27 (85%)	-	1/27 (4%)
Demos et al, 2021 AS-PCR	-	20/28 (71%)	-	4/23 (17%)
Ferrante et al, 2021 ddPCR	3/4 (75%)	25/32 (78%)	-	-
Moreno et al, 2022* ddPCR	8/21 (38%)	10/16 (63%)	0	1/10 (10%)

*These data refer only to smoldering Waldenström macroglobulinemia patients.

FIGURES

Figure 1. Samples commonly used for the analysis of genomic alterations of Waldenström macroglobulinemia patients. Direct tumor DNA can be obtained from bone marrow samples, followed by CD19+ sorting or enrichment and DNA extraction. Another approach is to use bone marrow mononuclear cells (figure above). Peripheral samples can be processed to obtain peripheral blood mononuclear cells (PBMC) followed by DNA extraction, or plasma samples can be processed to obtain cell-free DNA (figure below). AS-PCR: allele-specific polymerase chain reaction; ddPCR: droplet digital polymerase chain reaction; NGS: next-generation sequencing; WES: whole exome sequencing; WGS: whole genome sequencing.