# Determination of MYD88 and CXCR4 Mutations for Clinical Detection and Their Significance in Waldenström Macroglobulinemia

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## ABSTRACT

**Purpose:** This study aims to explore the incidence and clinical features of MYD88 and CXCR4 mutations in patients with Waldenström macroglobulinemia (WM) and determine the optimal method for routine clinical practice. Additionally, we seek to evaluate the prognostic significance of these features across various therapeutic backgrounds [the cytotoxic group, the rituximab/bortezomib-based group, and the Bruton tyrosine kinase inhibitor (BTKi) group].

**Experimental Design:** A total of 385 symptomatic patients with WM were analyzed for MYD88 and CXCR4 mutations using Sanger sequencing, next-generation sequencing, allele-specific qPCR (AS-PCR), and/or droplet digital PCR (ddPCR).

**Results:** The overall MYD88 mutation rate was 87.8%, relatively lower than that in the Western cohort. Both AS-PCR and ddPCR demonstrated high sensitivity in unsorted samples, detecting 98.5% and 97.7% of mutations, respectively, including those with low tumor burdens. The total CXCR4 mutation rate was 30.9%, with next-generation sequencing exhibiting the highest sensitivity of 78.0%. CXCR4 mutation was significantly linked to shorter OS only within the BTKi treatment group. The multivariate analysis indicated that MYD88 and CXCR4 mutations were not independent prognostic factors in the non-BTKi group when considering the International Prognostic Scoring System for Waldenström macroglobulinemia (IPSSWM) clinical staging. However, in the BTKi treatment group, these mutations emerged as independent adverse prognostic factors, overshadowing the prognostic significance of the IPSSWM classification (MYD88: HR, 0.229; $P = 0.030$; CXCR4: HR, 3.349; $P = 0.012$).

**Conclusions:** Testing for MYD88 mutations using AS-PCR or ddPCR in unsorted samples is viable for routine clinical practice. Under BTKi treatment, MYD88 and CXCR4 mutations hold greater prognostic importance than IPSSWM staging in WM.

## Introduction

Lymphoplasmacytic lymphoma is characterized by the monoclonal proliferation of small B lymphocytes, plasmacytoid lymphocytes, and plasma cells. Waldenström macroglobulinemia (WM), a subtype of lymphoplasmacytic lymphoma, is marked by bone marrow (BM) involvement and the presence of an IgM monoclonal protein (1). Recent research advancements in WM have underscored the importance of two key somatic mutations: MYD88 and CXCR4 (2, 3). MYD88 mutation ($MYD88^{MT}$), present in approximately 93% to 97% of WM cases (2), is crucial for both diagnosis and prognosis. Mutations in the CXCR4 gene, the second most common somatic mutation, are found in about 30% to 40% of patients with WM (3). Identifying the status of these mutations is essential for evaluating the efficacy of Bruton tyrosine kinase inhibitor (BTKi) treatment (4–6). Despite these advancements, a standardized method for the detection of these mutations remains to be established.

Initial studies on the incidence of MYD88 mutations utilized AS-PCR on CD19-selected samples to optimize sensitivity (7). AS-PCR is an economical and practical assay that offers higher sensitivity compared with Sanger sequencing. Subsequent studies highlighted droplet digital PCR (ddPCR) as a superior technique that provides improved sensitivity, precision, and reproducibility over AS-PCR (8, 9). Next-generation sequencing (NGS) also delivers dependable results on CD19-selected samples, with the added benefit of detecting both non-L265P MYD88 mutations and other genetic alterations such as those in CXCR4 and TP53 (2, 3). However, the necessity of CD19 sorting for all specimens in current clinical practice remains debated. On one hand, sorting can be time-consuming and costly. An additional challenge is that for patients with lower tumor infiltration, at least 5 mL of BM fluid needs to be collected in order to isolate a sufficient quantity of $CD19^{+}$ cells for MYD88 detection. Moreover, our routine clinical assays, including immunotyping, FISH, and target sequencing, demand a considerable volume of BM sample in addition to MYD88 testing. On the other hand, some patients with WM encountered challenges such as hyperviscosity syndrome or dry tap during BM aspiration, further

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## Translational Relevance

The detection of MYD88 and CXCR4 mutations is crucial for Waldenström macroglobulinemia (WM), yet there is a lack of standardized and unified methods for their detection. This comprehensive study includes a large cohort to investigate the prevalence, clinical manifestations, and prognostic significance of MYD88 and CXCR4 mutations in Chinese patients with WM. We used various methods to simultaneously test for MYD88 and CXCR4 mutations in the same sample, directly comparing the sensitivity of different approaches. Additionally, we explored the prognostic value of MYD88 and CXCR4 mutations against diverse treatment backgrounds, including non–Bruton tyrosine kinase inhibitor (BTKi) therapy and BTKi therapy. Furthermore, the research integrates these mutations with the traditional International Prognostic Scoring System for Waldenström macroglobulinemia (IPSSWM) scoring system, assessing their independent prognostic values across various treatment protocols. It was discovered that under BTKi treatment, MYD88 and CXCR4 mutations carry greater prognostic impact than the IPSSWM staging system in WM.

complicating the collection of adequate BM fluid volume. This scenario could elevate the likelihood of experimental failure. Additionally, recent studies have highlighted the potential for false negatives that may arise from selecting $CD19^+$ cells in samples from treated patients with WM who have undergone extensive B cell–depletion treatments (10, 11). Acknowledging the impracticalities of CD19 enrichment in routine clinical settings, our study seeks to assess the sensitivity and specificity of direct MYD88 mutation detection on unsorted clinical samples using various methods. Our goal is to identify a testing approach that is not only more convenient, cost-effective, and efficient but also has a higher success rate. Ultimately, we aim to establish a method that can be seamlessly integrated into standard clinical testing protocols, addressing both the practical and technical challenges currently faced.

Our research not only evaluates the effectiveness of various techniques in identifying MYD88 and CXCR4 mutations but also aims to elucidate the mutation frequency of WM within the Chinese population. Previous research suggests that WM has a lower age-adjusted incidence rate in East Asians compared with Western populations (12, 13). The MYD88 mutation rate in China is reported to be relatively low, ranging from 60% to 90% (14–17). The occurrence of CXCR4 mutations among the Chinese WM population remains ambiguous, possibly due to the reliance on less standardized and accurate methods like Sanger sequencing in past studies. To address this, we have meticulously confirmed each MYD88-negative case using at least three distinct methodologies and reexamined some negative samples after enhancing them with CD19 cell enrichment. This rigorous approach aims to test the hypothesis that the mutation rate of MYD88 among the Chinese cohort is indeed lower than observed in Western populations. Furthermore, our study compiles and analyzes data on the clinical characteristics, genetic variances, and survival rates of patients with MYD88-negative WM in China, offering a detailed insight into this specific patient group.

This comprehensive study encompasses a large cohort to delve into the incidence, clinical manifestations, and prognostic implications of MYD88 and CXCR4 mutations in Chinese patients with WM. It offers a direct comparison of the efficiency across various detection methods and explores whether the prognostic significance of these mutations remains consistent across different therapeutic approaches, including non-BTKi therapy and BTKi therapy.

# Materials and Methods

### Patients and sample collection

In our study, 418 patients diagnosed with WM according to the Second International Workshop on WM criteria (18) were admitted from June 2013 to June 2023 at the Institute of Hematology and Blood Diseases Hospital. Of these, 33 patients did not require treatment during the follow-up period and were consequently excluded from the study. Thus, a total of 385 symptomatic patients with WM were included in the final analysis. All participants in our study underwent comprehensive BM evaluations, including immunophenotyping and biopsy. These examinations revealed infiltration of the BM by monoclonal B cells and plasma cells in all patients. Comprehensive clinical and biological data were collected for each patient at the time of diagnosis. Comprehensive clinical and biological data were collected at diagnosis. Of these, 336 patients were available for analysis of treatment response and outcome. They were categorized into three groups based on their first-line therapy: cytotoxic therapy group, rituximab/bortezomib-based therapy group, and BTKi therapy group. More details on these treatment regimens are in Supplementary Table S1.

Tumor cell samples were prospectively collected from unsorted specimens prior to the initiation of any therapeutic intervention. The collection encompassed BM (in 356 cases), peripheral blood (PB, in 22 cases), and lymph nodes (in seven cases). The processes for sample collection, storage, and nucleic acid extraction are detailed in a previous publication (14). Due to the potential significant underestimation of tumor burden by the multiparameter flow cytometry method, we utilized IHC from biopsies to estimate the tumor load. Because precise quantification is not feasible with IHC, we have categorized the tumor burden into four groups: 0.5% to 10%, 10% to 30%, 30% to 50%, and greater than 50%.

To be classified as MYD88 wild-type ($MYD88^{WT}$), each sample underwent at least three different detection methods, including Sanger sequencing, NGS, ddPCR, and AS-PCR. Given the absence of a universally acknowledged "gold standard" for MYD88 L265P mutation detection, we considered a positive result from any detection method as sufficient evidence to classify a patient as MYD88 mutation–positive ($MYD88^{MT}$). Given that the tumor burdens in most $MYD88^{WT}$ patients were relatively high, we supposed that CD19 sorting might deplete the sample material significantly while offering minimal improvement to the detection results. As such, we opted not to retest all MYD88-negative samples after sorting. Instead, we strategically selected and retested eight patients who were not only MYD88-negative but also had a notably low tumor infiltration rate of less than 5%. This selective approach allowed us to maximize the use of our limited sample material and enhance the reliability and specificity of our detection methods for MYD88 mutations.

All patient samples were obtained after written informed consent in accordance with the Declaration of Helsinki and approved by the Institute of Hematology and Blood Disease Hospital, Chinese Academy of Medical Sciences, and Peking Union Medical College Ethics Committee.

### Efficacy and outcomes

Assessment of the treatment response in patients with measurable WM was based on the latest response criteria consensus from 11th International Workshop on Waldenström Macroglobulinemia and National Comprehensive Cancer Network (NCCN) guidelines (version 2.2022; refs. 19, 20). Overall survival (OS) was calculated from diagnosis to either the date of death or the date of the last

follow-up examination. Progression free survival (PFS) was calculated from diagnosis to either the date of the first relapse, death, or the date of the last follow-up examination. The response to BTKi was evaluated at a minimum of 3 months in BTKi therapy. The overall response rate (ORR) encompassed the rates of minor response (MR), partial response, very good partial response, and complete response. The deep response rate was defined as the combined rate of complete response and very good partial response.

### Detection of MYD88 and CXCR4 mutations by Sanger sequencing, AS-PCR, ddPCR, and NGS

MYD88 mutations were identified by Sanger sequencing, AS-PCR, ddPCR, and NGS. CXCR4 mutations were identified by Sanger sequencing, AS-PCR, and NGS. The details of the methods are seen in Supplementary Materials.

### Statistical analysis

The association between two categorical variables was assessed using Pearson $\chi^2$ test and Fisher exact test. The comparison of continuous variables among two groups of patients was evaluated using Student *t* test. Survival curves were constructed using the Kaplan–Meier method, and differences were estimated through the log-rank test. Multivariate Cox regression analysis was used to assess the independent prognostic impact. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated. The concordance between different assays was investigated using the κ test, with agreement deemed almost perfect between 0.8 and 1. A *P* value less than 0.05 was considered statistically significant. All the statistical analyses were performed using IBM Statistical Package for the Social Sciences (SPSS) (RRID: SCR_002865); GraphPad Prism (RRID: SCR_002798), and/or R package version 3.5.1.

### Data availability

Data are available from the corresponding author on reasonable request. The original data (BAM files) have been deposited in the Genome Sequence Archive of the National Genomics Data Center from China and are accessible under BioProject ID PRJCA028747 (accessible at https://ngdc.cncb.ac.cn/bioproject/browse/PRJCA028747) and Accession ID HRA008237 (accessible at https://bigd.big.ac.cn/gsa-human/browse/HRA008237).

## Results

### Mutation rate and test performance of different methods for MYD88 and CXCR4

The cohort comprised 385 patients with WM with a median age of 62 years old, ranging from 20 to 86 years old. Clonal IgM protein was detected in all patients. The median serum IgM level was 3,430 mg/dL at diagnosis.

All the patients underwent assessment for MYD88 mutation. We performed AS-PCR in 322 patients, NGS in 246 patients, Sanger sequencing in 233 patients, and ddPCR in 214 patients. To enhance the sensitivity of MYD88 mutation detection, we simultaneously performed at least three testing methods in each $MYD88^{WT}$ sample. We defined a sample as $MYD88^{WT}$ only if all the assays yielded negative results. If any one of the testing methods produced a positive result, the sample was defined as a $MYD88^{MT}$ sample. In total, MYD88 mutation was observed in 338 (87.8%) of the 385 patients. The patients' clinical characteristics and treatment choices are detailed in Supplementary Table S2. The mutation detection rates were 87.6% (312/356) in BM samples and 90.9% (20/22) in PB samples. Only two of those mutations were situated at a non-L265P site identified by NGS. Examining the mutation rate revealed by each method, Sanger sequencing detected a rate of 61.4%, NGS of 74.4%, AS-PCR of 84.2%, and ddPCR of 79.0%, respectively (Supplementary Table S3). The detection rate of MYD88 using ddPCR was slightly lower than that of AS-PCR, but the difference was not statistically significant. This difference does not imply that ddPCR is less sensitive than AS-PCR; there was selection bias in the samples tested by the two methodologies. Therefore, we separately analyzed 196 patients who underwent MYD88 testing with both ddPCR and AS-PCR. The positive detection rates for ddPCR and AS-PCR were 78.6% (154/196) and 77.6% (152/196), respectively, showing similar detection rates for both methods.

We evaluated the test performance of the four methods in detecting MYD88 mutations (Supplementary Table S3). AS-PCR and dd-PCR exhibited the highest sensitivities (98.5%, 95% CI, 96.1%–99.5% and 97.7%, 95% CI, 93.8–99.3) and negative predictive values (91.7%, 95% CI, 79.1–97.3, and 91.1%, 95% CI, 77.9–97.1). AS-PCR and dd-PCR also demonstrated the lowest false negative rates (1.5% and 2.3%). However, Sanger sequencing and NGS failed to detect MYD88 mutation effectively in patients with low tumor load and exhibited a high false negative rate in patients with tumor burden less than 10% (55.6% and 31.3%, respectively, **Fig. 1**), whereas there was no significant difference in the MYD88 mutation rate detected by AS-PCR and ddPCR among patients with varying tumor infiltration fraction ($P = 0.149$ and 0.316, respectively, Supplementary Fig. S1). The eight MYD88-negative samples mentioned in the Methods section were retested using ddPCR after CD19 magnetic bead sorting, and the results remained negative. Subsequently, we analyzed the concordance among the four methods; the highest concordance was found between ddPCR and AS-PCR (κ 0.911), and the lowest concordance was found between Sanger sequencing and ddPCR (κ 0.200, Supplementary Table S4).

Due to the significant limitations of AS-PCR and Sanger sequencing in detecting CXCR4 mutations, especially because AS-PCR cannot detect non-hotspot or frameshift mutations, we did not include patients who were tested for CXCR4 using only one method (either AS-PCR or Sanger sequencing). As a result, our study included a total of 362 patients conducting CXCR4 mutation testing. All included patients were either tested with both AS-PCR and Sanger sequencing ($n = 273$), both AS-PCR and NGS ($n = 202$), or solely with NGS ($n = 44$), as illustrated in Supplementary Table S5. The observed overall mutation rate was 30.9%. Examining each method individually, NGS demonstrated the highest detection rate of CXCR4 mutation among the three methods, with a mutation-positive detection rate reaching up to 26.0%, followed by AS-PCR and Sanger sequencing with mutation rates of 18.2% and 12.8%, respectively. Due to the limitation of AS-PCR in detecting only point mutations and the low sensitivity of Sanger sequencing, we adopted a joint testing approach by combining both methods. A sample was defined as positive if either method detected a mutation and negative if both methods were negative. This combined approach increased the positive detection rate to 26.7%. Among the methods, NGS exhibited the highest sensitivity at 78.0% (95% CI, 67.3–86.1), followed by AS-PCR at 58.6% (95% CI, 48.2–68.3) and Sanger sequencing at 41.2% (95% CI, 30.8–52.4, Supplementary Table S6). The AS-PCR and Sanger sequencing combined approach reached a sensitivity of 85.9%, showcasing its feasibility as an alternative to NGS. Focusing solely on the two hotspot S338X mutations detectable by AS-PCR, we observed that AS-PCR outperformed NGS in sensitivity for these specific mutations, registering at 93.4% compared with

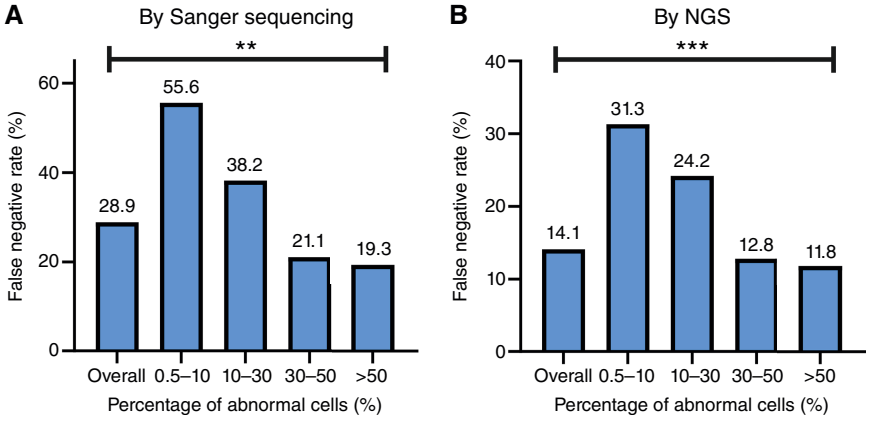


**Figure 1.**
False negative rates of MYD88 testing by Sanger sequencing (**A**) and NGS (**B**) in patients with different tumor burdens. The tumor burden is estimated by IHC of BM biopsies. **, $P < 0.01$; ***, $P < 0.001$.

NGS's 62.5%. Nonetheless, AS-PCR's capability was confined to identifying hotspot point mutations. The mutation detection results for CXCR4 via the three methods lacked uniformity, with concordance among the methods measuring below 0.5 (Supplementary Table S7). Additionally, Supplementary Table S5 presents the detail deletion results for MYD88 and CXCR4 across the entire cohort using different methods.

### The clinical and genetic characteristics of patients with mutated and WT MYD88

Of the 385 patients with WM examined, 47 (12.2%) were categorized as $MYD88^{WT}$ patients. $MYD88^{WT}$ patients exhibited significantly lower proportion of males (59.6% vs. 74.0%, $P = 0.039$) and lymphadenopathy (25.0% vs. 43.4%, $P = 0.035$) and higher proportion of hepatomegaly (33.3% vs. 19.3%,

**Table 1.** Clinical and genetic characteristics of MYD88-mutated and WT patients.

| Characteristic | $MYD88^{WT}$ (*N* = 47) | $MYD88^{MT}$ (*N* = 338) | *P* | $CXCR4^{WT}$ (*N* = 250) | $CXCR4^{MT}$ (*N* = 112) | *P* |
|---|---|---|---|---|---|---|
| Age, ≥65 years, *n* (%) | 16 (34.0) | 121 (35.8) | 0.814 | 79 (31.6) | 48 (42.9) | 0.038 |
| Gender, male, *n* (%) | 28 (59.6) | 250 (74.0) | 0.039 | 181 (72.4) | 79 (70.5) | 0.716 |
| Laboratory parameters, *n* (%) | | | | | | |
| Serum IgM > 40 g/L, | 16 (34.0) | 130 (38.5) | 0.559 | 87 (34.8) | 52 (46.4) | 0.035 |
| HGB ≤ 11 g/dL | 40 (83.1) | 257 (76.0) | 0.165 | 184 (73.6) | 94 (83.9) | 0.031 |
| PLT ≤ $100 \times 10^9$/L | 14 (29.8) | 92 (27.2) | 0.712 | 49 (19.6) | 52 (37.4) | 0.000 |
| Serum albumin < 35 g/L | 22 (47.8) | 166 (51.9) | 0.607 | 127 (52.9) | 47 (44.8) | 0.163 |
| Serum $\beta_2$-MG > 3 mg/L | 33 (78.6) | 205 (70.9) | 0.304 | 164 (74.5) | 57 (61.3) | 0.019 |
| LDH ≥ 250 U/L | 13 (28.3) | 37 (11.8) | 0.003 | 37 (15.6) | 11 (10.7) | 0.230 |
| Malignant cells fraction in BM detected by FCM, median (range) | 11.3 (0.50–71.5) | 9.4 (0.51–90.2) | 0.886 | 6.8 (0.50–84.5) | 12.2 (0.51–85.3) | 0.000 |
| Clinical manifestations, *n* (%) | | | | | | |
| Lymphadenopathy | 9 (25.0) | 112 (43.4) | 0.035 | 86 (45.7) | 23 (25.6) | 0.001 |
| Splenomegaly | 27 (64.3) | 141 (47.6) | 0.043 | 108 (49.5) | 51 (50.0) | 0.939 |
| Hepatomegaly | 14 (33.3) | 57 (19.3) | 0.037 | 48 (22.3) | 20 (19.4) | 0.554 |
| IPSSWM score, *n* (%) | | | | | | |
| Low risk | 9 (20.9) | 86 (27.4) | 0.369 | 72 (31.2) | 19 (18.4) | 0.016 |
| Intermediate risk | 17 (39.5) | 106 (33.8) | 0.455 | 76 (32.9) | 38 (36.9) | 0.477 |
| High risk | 17 (39.5) | 122 (38.9) | 0.932 | 83 (35.9) | 46 (44.7) | 0.130 |
| FISH examination, *n* (%) | | | | | | |
| Del (11q22-23) | 0 | 2 (1.0) | 1.000 | 2 (1.4) | 0 | 0.853 |
| Del (13q14) | 0 | 5 (2.8) | 0.991 | 2 (1.6) | 2 (3.4) | 0.783 |
| Trisomy 12 | 0 | 3 (3.0) | 1.000 | 1 (1.3) | 2 (6.1) | 0.458 |
| Del (17p13) | 0 | 16 (5.4) | 0.318 | 12 (5.7) | 2 (2.0) | 0.241 |
| Del (6q23) | 1 (9.1) | 19 (32.8) | 0.221 | 15 (32.6) | 5 (21.7) | 0.348 |
| G-banded metaphase karyotypes, *n* (%) | | | | | | |
| Normal karyotype | 30 (78.9) | 209 (73.1) | 0.415 | 165 (78.9) | 57 (60.6) | 0.001 |
| Complex karyotype | 7 (18.4) | 21 (7.3) | 0.047 | 18 (8.6) | 7 (7.5) | 0.761 |
| Treatment regimen, *n* (%) | | | | | | |
| Cytotoxic therapy | 7 (17.5) | 53 (17.9) | 0.950 | 40 (18.3) | 15 (15.0) | 0.300 |
| Rituximab/bortezomib-based therapy | 24 (60.0) | 148 (50.0) | 0.235 | 112 (51.4) | 49 (49.0) | 0.694 |
| BTKi-based therapy | 9 (22.5) | 95 (32.1) | 0.218 | 66 (30.3) | 36 (36.0) | 0.310 |

Abbreviations: FCM, flow cytometry; HGB, hemoglobin; LDH, lactate dehydrogenase; $\beta_2$-MG, $\beta_2$-microglobulin; PLT, platelet.

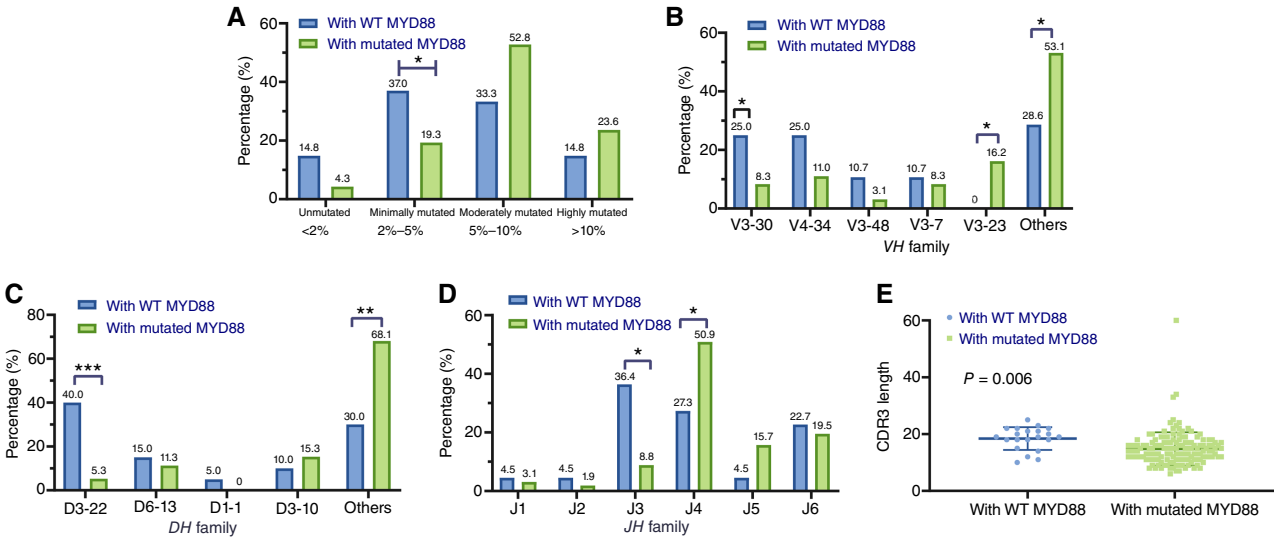


**Figure 2.**

IGHV-D-J gene repertoire of patients with mutated and WT MYD88. **A,** Distribution of patients with different identity to the germline *IGHV* gene. **B,** Distribution of major *VH* family gene segments. **C,** Distribution of major *DH* family gene segments. **D,** Distribution of major *JH* family gene segments. **E,** Distribution of CDR3 length. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

$P = 0.037$) and splenomegaly (64.3% vs. 47.6%, $P = 0.043$). Additionally, MYD88$^{WT}$ patients showed a notably higher proportion of elevated lactic dehydrogenase (28.3% vs. 11.8%, $P = 0.003$; **Table 1**).

Subsequently, we identified the differences in *IGHV* mutation status and VDJ rearrangements between MYD88$^{MT}$ and MYD88$^{WT}$ patients. Productive IGHV-D-J rearrangements were obtained and analyzed in 260 patients with WM. The majority of WM cases (94.6%) were found to carry mutated *IGHV* genes, as determined by an *IGHV* germline homology cutoff of 98%. The proportion of mutated *IGHV* was comparable between MYD88$^{MT}$ and MYD88$^{WT}$ patients (95.9% vs. 85.2%, $P = 0.055$). However, there was a notable difference in the distribution of the *IGHV* mutation percentage between the two groups (**Fig. 2A**). We observed that *IGHV3-30*, *IGHD3-22*, and *IGHJ3* were significantly overrepresented and *IGHV3-23* and *IGHJ4* were significantly underrepresented in the MYD88$^{WT}$ group compared with the mutated group ($P < 0.05$, **Fig. 2B–D**). Concurrently, CDR3 length was significantly longer in MYD88$^{WT}$ patients (median 19 vs. 14, $P = 0.006$; **Fig. 2E**). The detailed IGHV-D-J repertoire of the two groups is shown in Supplementary Fig. S2.

The cytogenetic features were assessed through FISH and G-banded metaphase karyotypes. No significant difference in cytogenetic features was observed between MYD88$^{MT}$ and MYD88$^{WT}$ patients. Complex karyotype was significantly more commonly detected in MYD88$^{WT}$ patients than in MYD88$^{MT}$ patients (18.4% vs. 7.3%, $P = 0.047$; **Table 1**).

### The clinical and genetic characteristics of patients with mutated and WT CXCR4

Given the individual sensitivities of AS-PCR and Sanger sequencing are relatively low, we used a combination of AS-PCR and Sanger sequencing as a unified detection strategy. We tested a total of 362 cases for CXCR4 mutations, identifying 114 mutations in 112 cases, representing a mutation rate of 30.9%. The majority of CXCR4 mutations were truncated mutations (112 of 114, 98.2%), located in the regulatory C-terminal domain that extends spanning amino acids 313 to 345 (Supplementary Fig. S3A). Nonsense and frameshift mutations accounted for 64.0% and 33.3%, respectively (Supplementary Fig. S3B). The most prevalent alteration was the amino acid change S338X as nonsense mutation at the nucleotide position 1,013, comprising 64 of the 114 mutations (56.1%). About the nucleotide changes contributing to this alteration, C > G was observed in 65.6% of cases, and C > A in 34.3%. The second most commonly affected site was at amino acids 341 and 318, accounting for 6.1% and 5.3% of the mutations, respectively (Supplementary Fig. S3C). This analysis highlights the diversity and prevalence of CXCR4 mutations in the examined patient population.

Patients with CXCR4 mutations demonstrated distinctive clinical characteristics compared with WT patients (**Table 1**). Notably, the mutated group exhibited a significantly higher proportion of patients older than 65 years (42.9% vs. 31.6%, $P = 0.038$) and higher proportions of anemia (83.9% vs. 73.6%, $P = 0.035$) and thrombocytopenia (37.4% vs. 19.6%, $P < 0.001$). Additionally, these patients displayed an elevated tumor burden in the BM, as evidenced by a median of 12.2% versus 6.8% determined by flow cytometry ($P < 0.001$). The CXCR4-mutated group had a significantly higher proportion of patients with serum IgM levels exceeding 40 g/L (46.4% vs. 34.8%, $P = 0.035$) and serum β2-MG more than 3 mg/L (74.5% vs. 61.3%, $P = 0.019$). Consequently, there was a notably lower percentage of patients in the CXCR4-mutated group classified as low-risk according to the IPSSWM staging (18.4% vs. 31.2%, $P = 0.016$). In contrast, patients with WT CXCR4 were more prone to develop lymphadenopathy (45.7% vs. 25.6%, $P = 0.001$).

*IGHV* mutation status and gene repertoire were also analyzed between CXCR4 WT and mutated groups. We observed that *IGHD3-22* genes were significantly overrepresented and *IGHD3-10*

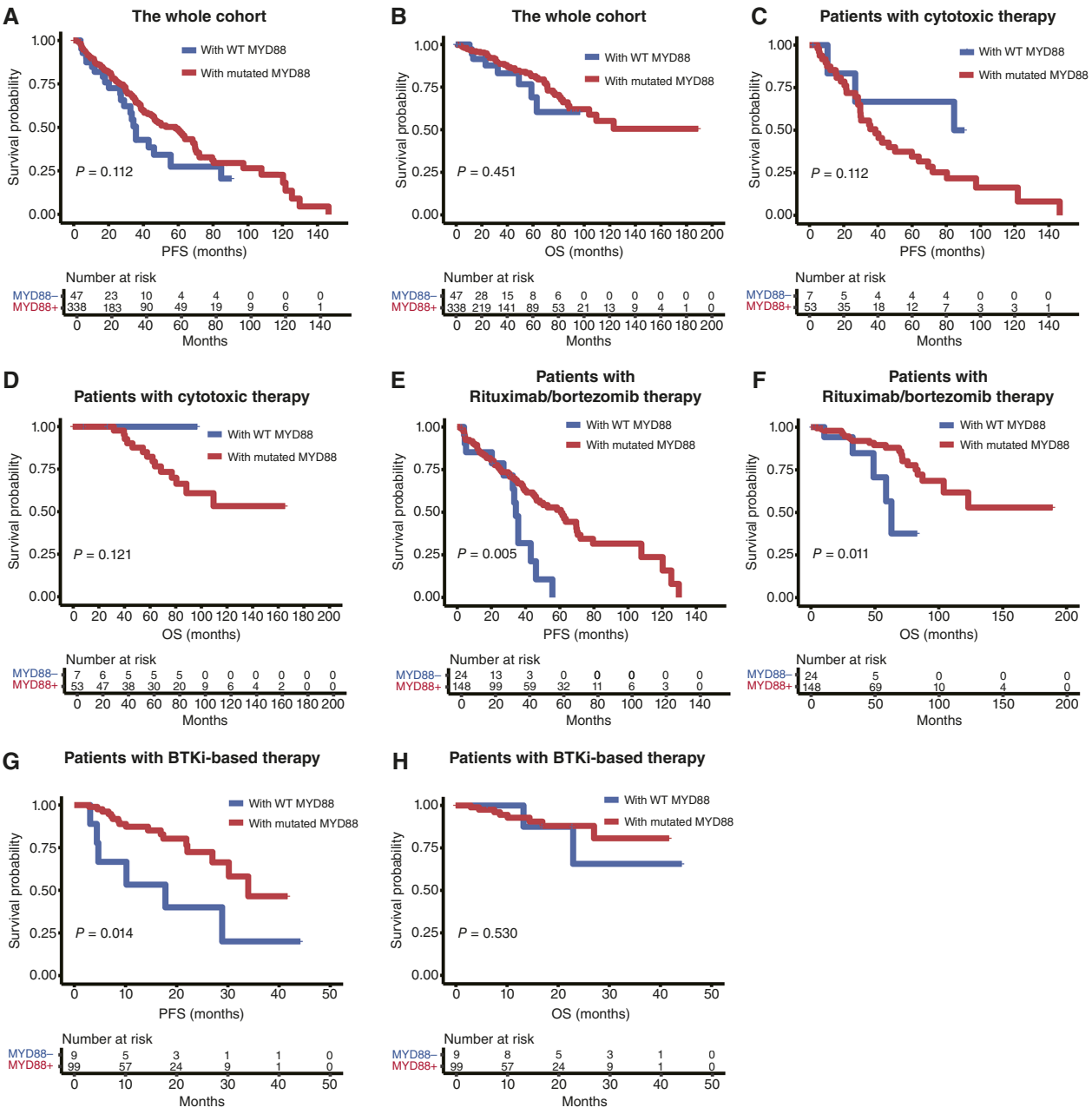


**Figure 3.**

Survival outcomes in patients with mutated and WT MYD88 according to the option of different first-line therapies. The PFS and OS in the whole cohort (**A** and **B**), in patients with cytotoxic therapy (**C** and **D**), in patients with rituximab/bortezomib-based therapy (**E** and **F**), and in patients with BTKi-based therapy (**G** and **H**).

genes were significantly underrepresented in the CXCR4 WT group compared with the mutated group ($P = 0.012$, $P = 0.008$). Besides, we observed no significant difference in the distribution of the *IGHV* hypermutation rate, *IGHVJ* gene repertoire, and CDR3 length between the CXCR4 WT and mutated groups (Supplementary Figs. S4 and S5).

We observed a significantly higher proportion of patients exhibiting abnormal karyotypes in the CXCR4 mutation group compared with the WT group (39.4% vs. 21.1%, $P = 0.001$; **Table 1**). Specifically, the mutation group displayed a significantly elevated incidence of abnormal karyotypes with -Y (6.5% vs. 1.0%, $P = 0.018$) and del 7 (5.4% vs. 0%, $P = 0.004$) in comparison with the CXCR4 WT group. This comprehensive analysis elucidates the varied clinical and genetic profiles between patients with CXCR4 mutations and those with WT CXCR4.

## Impact of MYD88/CXCR4 mutations on treatment outcomes

We initiated our analysis by examining the response rate and survival outcomes of patients based on their MYD88 and CXCR4 mutation

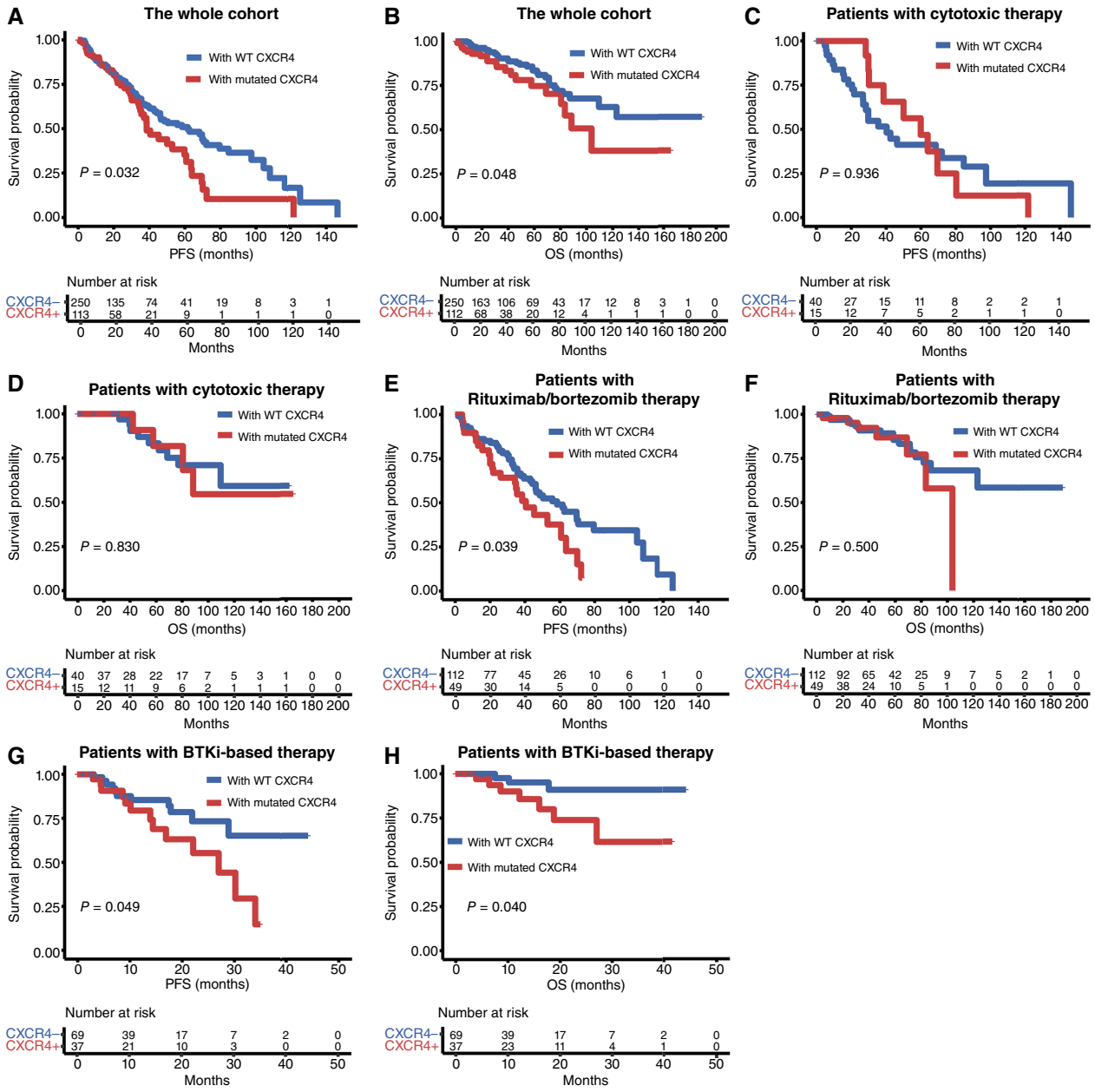


**Figure 4.**

Survival outcomes in patients with mutated and WT CXCR4 according to the option of different first-line therapies. The PFS and OS in the whole cohort (**A** and **B**), in patients with cytotoxic therapy (**C** and **D**), in patients with rituximab/bortezomib-based therapy (**E** and **F**), and in patients with BTKi therapy (**G** and **H**).

status across different therapeutic approaches. Among the 336 patients who received systemic treatment, 172 patients (51.1%) received rituximab/bortezomib-based therapy and 104 patients (31.0%) received BTKi-based regimens (Supplementary Table S1). There was no obvious difference in the option of treatment regimens among patients with different MYD88 and CXCR4 mutation status (**Table 1**). With a median follow-up of 33.3 months, the median PFS for the entire cohort was 50.0 months (95% CI, 38.3–61.7), and the median OS was not reached. The 5-year OS rate was 80.1% (Supplementary Fig. S6A and S6B).

Overall, there were no significant differences observed in either PFS or OS between $MYD88^{WT}$ and $MYD88^{MT}$ groups ($P = 0.112$ and 0.451, respectively; **Fig. 3A** and **B**). Then, we assessed the prognostic value of MYD88 mutation across various treatment regimens (**Fig. 3C–H**). Across the non-BTKi therapy groups, patients with $MYD88^{WT}$ exhibited similar ORR compared with those with MYD88 mutations in first-line therapy (Supplementary Tables S8 and S9). Notably, $MYD88^{WT}$ patients exhibited significantly lower ORR compared with those with MYD88 mutations under BTKi-based therapy (44.4% vs. 83.8%, $P = 0.019$, Supplementary Table S10). Interestingly, patients

treated with non-BTKi therapy displayed worse PFS and OS in the MYD88$^{WT}$ group compared with the mutated group (median PFS: 34.3 months vs. 60.7 months, $P = 0.005$; median OS: 62.9 months vs. not reached, $P = 0.011$; **Fig. 3E** and **F**). Moreover, the MYD88$^{WT}$ group exhibited inferior PFS as compared with the mutated group under BTKi-based therapy (median 17.8 months vs. 34.0 months, $P = 0.014$), but no difference in OS was observed (**Fig. 3G** and **H**).

Notably, the CXCR4-mutated group manifested significantly worse PFS and OS compared with the WT group (median PFS: 38.5 months vs. 61.6 months, $P = 0.032$; median OS: 103.8 months vs. not reached, $P = 0.048$; **Fig. 4A** and **B**). We then conducted a detailed analysis on response rates and survival outcomes based on CXCR4 mutation status across different therapeutic options in first-line therapy. We did not observe significant difference in treatment response between CXCR4 WT and mutated patients across varied treatment strategies (Supplementary Tables S8–10). CXCR4 WT and mutated patients exhibited analogous PFS and OS within the cytotoxic therapy group (**Fig. 4C** and **D**). However, under rituximab/bortezomib-based therapy, the CXCR4 mutation group demonstrated diminished PFS compared with the WT group (median 40.5 months vs. 58.4 months, $P = 0.039$; **Fig. 4E** and **F**). Likewise, under BTKi-based regimens, the CXCR4-mutated group exhibited significantly inferior survival outcomes compared with the WT group (**Fig. 4G** and **H**).

We further explored the outcomes of patients with different CXCR4 mutation types, amino acid sites, and cancer cell fraction (CCF; Supplementary Fig. S7). Our results indicated that neither types of mutation nor amino acid sites of CXCR4 mutation significantly influenced outcomes. Although a trend was observable suggesting that patients with higher CXCR4 clonality (CCF > 50%) experienced inferior PFS and OS outcomes compared with those with lower CXCR4 clonality (CCF < 50%), the impact of varying CCF did not reach statistical significance (PFS: $P = 0.741$; OS: $P = 0.521$; Supplementary Fig. S7E and S7F). This is likely due to the small number of patients with subclonal CXCR4 mutations. A larger sample size is warranted for more conclusive validation.

To sum up, both MYD88 and CXCR4 mutations had a prognostic impact on PFS in patients undergoing BTKi-based therapy, but only CXCR4 mutation exerted significant impact on OS. When MYD88 and CXCR4 mutations were combined in the survival curve, we did not observe any prognostic significance of MYD88 and CXCR4 mutations in patients treated with cytotoxic therapy (Supplementary Fig. S8A and S8B). CXCR4 mutation status emerged as an essential factor distinguishing outcomes in patients with rituximab/bortezomib-based therapy, whereas MYD88$^{WT}$ patients and those with both MYD88 and CXCR4 mutations exhibited comparably poor prognoses (Supplementary Fig. S8C and S8D). Notably, in the era of BTKi treatment, both MYD88 and CXCR4 mutations were significant determinants of patients' outcomes. If we categorized the patients into three groups, those with MYD88 mutations but without CXCR4 mutations had the most favorable prognosis regardless of PFS or OS (Supplementary Fig. 8E and S8F). It is worth noting that conducting multiple hypothesis testing could increase the risk of statistical errors. Consequently, we also applied the Bonferroni correction to the *P* values obtained from our survival analysis. This adjustment revealed that the presence of MYD88 mutations continued to show prognostic relevance for PFS in the rituximab/bortezomib-based group. However, OS ceased to maintain statistical significance after the application of multi-hypothesis correction. Furthermore, following the Bonferroni adjustment, the prognostic value of CXCR4 mutations for OS no longer held statistical significance across any of the subgroups. This may be attributed to the wide array of treatment options available to patients with WM following the failure of first-line therapies, including the opportunity to participate in diverse clinical trials. The advent of new therapeutic agents has markedly enhanced the survival prospects for these patients. Further validation of our findings will require more uniform treatment cohorts or clinical trial cohorts.

### Prognostic prediction under different treatment backgrounds

The IPSSWM system was developed and validated in cohorts treated with chemotherapy or immunotherapy (Supplementary Fig. S9A). However, we found that the IPSSWM system could not stratify the survival in patients treated with BTKi therapy ($P = 0.498$; Supplementary Fig. S9B). To integrate genetic mutations with the clinical prognostic index, we incorporated IPSSWM staging along with MYD88 and CXCR4 mutations for multivariate analysis of OS. The results elucidated that, across the entire cohort, CXCR4 mutation served as an independent adverse prognostic factor irrespective of IPSSWM staging, whereas MYD88 mutation did not exhibit significance in the multivariate analysis (**Table 2**). Subsequently, we segregated patients into BTKi and non-BTKi treatment groups. We found that in the BTKi treatment group, the IPSSWM classification did not hold prognostic significance in univariate and multivariate analyses (Supplementary Fig. S9B; **Table 2**). MYD88 and CXCR4 mutations serve as an independent adverse prognostic factor for OS (MYD88: HR, 0.229; 95% CI, 0.061–0.865; $P = 0.030$; CXCR4: HR, 3.349; 95% CI, 1.302–8.612; $P = 0.012$). In contrast, in the non-BTKi treatment group, the IPSSWM classification was an independent adverse prognostic factor (HR, 1.596, $P = 0.003$). Upon considering the IPSSWM classification, both CXCR4 and MYD88 mutations relinquished their prognostic significance in the non-BTKi treatment group (**Table 2**). Hence, prognostic factors varied under different treatment backgrounds. In the era of BTKi-based therapy, the detection of gene mutations has assumed an increasingly pivotal role in prognosis prediction.

## Discussion

MYD88 and CXCR4 mutations have been identified as highly recurrent somatic mutations in WM by whole-genome sequencing (2, 3). The direct comparison of the efficacy of different detection methods and the mutation rates in Chinese patients with WM remain unclear. Moreover, the clinical implications of MYD88 and CXCR4 mutations in the eras of immunochemotherapy and targeted therapy warrant further exploration. This study represents the largest integrative studies on the incidence, clinical characteristics, and prognostic significance of MYD88 and CXCR4 mutations. Additionally, it conducts subgroup analyses of survival data across different treatment regimens, shedding light on the real-world clinical significance of detecting MYD88 and CXCR4 mutations.

Previous research has highlighted the high sensitivity of CD19-selected BM with AS-PCR for detecting MYD88 mutations (7). However, the practicality of sorting B cells for this testing is limited in routine clinical settings. To ensure the accuracy of MYD88 testing on unsorted samples, we used a three-pronged approach. First, for patients identified as MYD88-negative, confirmation was sought using at least three distinct tests, with a consensus of negativity required across all tests. Second, we stratified patients based on tumor cell proportions, enabling us to compare mutation detection rates across different tumor loads and evaluate the influence of tumor cell purity on detection rates. Third,

**Table 2.** Univariate and multivariate Cox regression analyses for OS.

| Variable | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | HR (95% CI) | *P* | HR (95% CI) | *P* |
| The whole cohort ($N = 336$) | | | | |
| IPSSWM | 1.531 (1.112–2.107) | 0.008 | 1.425 (1.006–2.018) | 0.046 |
| MYD88 mutation | 0.788 (0.358–1.734) | 0.554 | 0.481 (0.291–1.254) | 0.176 |
| CXCR4 mutation | 1.795 (1.038–3.104) | 0.036 | 1.830 (1.023–3.271) | 0.042 |
| BTKi-based therapy cohort ($N = 104$) | | | | |
| IPSSWM | 1.388 (0.780–2.468) | 0.265 | 1.219 (0.686–2.164) | 0.500 |
| MYD88 mutation | 0.312 (0.087–1.117) | 0.073 | 0.229 (0.061–0.865) | 0.030 |
| CXCR4 mutation | 3.099 (1.228–7.825) | 0.017 | 3.349 (1.302–8.612) | 0.012 |
| Non-BTKi therapy cohort ($N = 232$) | | | | |
| IPSSWM | 1.596 (1.090–2.337) | 0.016 | 1.596 (1.039–2.450) | 0.003 |
| MYD88 mutation | 1.256 (0.491–3.213) | 0.634 | 0.859 (0.321–2.229) | 0.762 |
| CXCR4 mutation | 1.440 (0.689–3.014) | 0.333 | 1.268 (0.578–2.450) | 0.553 |

we performed CD19 magnetic bead sorting on eight $MYD88^{WT}$ samples characterized by low tumor loads. After enriching for tumor cells, we retested for MYD88 mutations using ddPCR to confirm their negative status. Through these comprehensive detection methods, we determined that the MYD88 mutation rate in Chinese patients with WM was 87.8% (338/385), which is notably lower than the 93.5% observed in a European WM cohort using comparable methods on unsorted samples ($\chi^2$ test $P = 0.003$; ref. 21). The distinction between Eastern and Western populations in WM extends beyond MYD88 mutation frequencies. Research has reported that age-standardized incidence rates in Asians are lower than those reported in the United States and Europe (12). Retrospective studies indicate that Asian populations present with WM at a younger age, exhibit a higher prevalence of anemia, and contain a larger proportion of IPSSWM high-risk patients (22). Furthermore, our previous research has identified differences in the usage of *IGHV* segments between Chinese and Western patients with WM, with a higher usage of V4 segments (14). These differences may be attributed to distinct microbiological and genetic backgrounds between Eastern and Western populations, possibly exposing them to different antigenic stimuli spectra. Further research is needed to elucidate these underlying mechanisms. However, the study does have certain limitations. Although we have used multiple methods to minimize the impact of unsorted samples on detection accuracy, the most direct and reliable approach would be to sort all samples and retest MYD88 using multiple methods. Comparing these results with those from unsorted samples would directly demonstrate whether sorting affects the detection results and further validate the conclusion that the MYD88 mutation detection rate is lower in China. On the other hand, to prove this rigorously, additional studies on other Chinese cohorts using the same methodology are needed.

Our study found that ddPCR and AS-PCR are highly sensitive for detecting MYD88 mutations in unsorted samples, outperforming NGS and Sanger sequencing (23). This supports the use of ddPCR and AS-PCR for routine clinical testing. However, it is important to note that 92.4% of our samples were from BM, and PB was tested only in cases with high white blood cell counts. Our study did not include samples with extremely low tumor loads (<0.5%), so our findings do not apply to such cases. Although AS-PCR is cost-effective, it is semi-quantitative and requires standard reference curves and strict standardization, which may limit its use. The variation in results between studies could be due to the small patient cohort and differing experimental conditions (8). Therefore, ddPCR, on the other hand, offers a more stable and quantitative approach for MYD88 mutation detection. Despite their lower sensitivity for MYD88 mutations, NGS and Sanger sequencing can detect non-L265P MYD88 mutations and CXCR4 mutations, which is a significant advantage. Even though the detection rate of non-L265P MYD88 mutations in our study was lower than in some other studies (3), this capability should not be underestimated.

CXCR4 mutations are essentially unique to WM and can influence disease presentation and therapeutic responses, particularly to classic BTKi treatment (5). Unlike the MYD88 mutation, individuals can have various CXCR4 mutations, with differences in type, location, and clonality. Prior research indicated that the subtype of CXCR4 mutation (nonsense or frameshift) influences treatment response and survival outcomes. Additionally, the allelic burden of CXCR4 has been found to impact the response to ibrutinib and PFS. A retrospective analysis showed that a clonality of ≥25% was linked to lower response rates and worse PFS than clonality <25% (24). In contrast, our study did not find any prognostic significance in the type or clonality of CXCR4 mutations. Considering the limited sensitivity of Sanger sequencing and the restrictive capability of AS-PCR in detecting only hotspot mutations, our results endorse NGS as the premier method for identifying CXCR4 mutations. The integrative strategy of combining Sanger sequencing with AS-PCR could offer a viable alternative for CXCR4 mutation detection in scenarios in which NGS is not accessible.

Patients with the $MYD88^{WT}$ genotype are rare due to the low prevalence of this genotype of WM. Given that patients with WT MYD88 have a lower response rate to BTKi, in our clinical strategy selection, we tend to opt immunotherapy or combination immunotherapy treatments for patients with WT MYD88, such as the BR regimen, and less frequently use BTKi monotherapy. Following the adaptation of treatment strategies based on MYD88 mutation status, we observed that the OS rates of patients with $MYD88^{WT}$ closely aligned with those harboring MYD88 mutations (**Fig. 3A** and **B**). A comparison of the clinical characteristics between patients with WT and mutant MYD88 revealed that $MYD88^{WT}$ patients had higher lactate dehydrogenase levels and a higher proportion of complex karyotypes, which may suggest a slightly poorer response to non-BTKi therapy compared with $MYD88^{MT}$ patients. However, the

limited number of patients with MYD88$^{WT}$, further reduced after stratification into treatment groups, along with variability in follow-up duration and the generally short follow-up periods across these groups, necessitates a cautious interpretation of the survival curves. To definitively determine the prognostic value of MYD88 mutations across various treatment modalities, larger-scale prospective studies with extended follow-up periods are essential for a more thorough elucidation.

Moreover, patients with CXCR4 mutations typically have higher IgM levels and lower platelet counts, which are also adverse indicators. We found that patients with CXCR4 mutations had significantly worse outcomes than WT patients, especially in the rituximab/bortezomib- and BTKi-treated groups. Although many studies have explored how MYD88 and CXCR4 mutations affect response to BTKi treatment and survival prognosis in patients with WM, few have combined these mutations with clinical prognostic factors in a multivariate analysis. This leads to the question of whether MYD88 and CXCR4 mutations are independent prognostic factors separate from these clinical features. To investigate, we included MYD88 mutations, CXCR4 mutations, and IPSSWM staging in our analysis. We found that IPSSWM staging and CXCR4 mutations were independent adverse prognostic factors for survival (**Table 2**). Adding CXCR4 mutation testing to IPSSWM stratification improves prognosis prediction. However, MYD88 mutations did not show prognostic significance in our multivariate model. Considering the known lesser response of patients with WT MYD88 and mutated CXCR4 to BTKi therapy, we divided patients into two groups: those undergoing BTKi therapy and those receiving traditional non-BTKi (cytotoxic drugs and rituximab/bortezomib) treatment. We discovered that in the non-BTKi group, CXCR4 mutation did not significantly impact survival after adjusting for IPSSWM staging in the analysis. This suggests that in patients with non-BTKi therapy, the prognostic value of MYD88 and CXCR4 mutations is not crucial, and the need for additional mutation testing beyond IPSSWM stratification is debatable. However, in the BTKi treatment era, the effects of MYD88 and CXCR4 mutations on survival are independent of clinical IPSSWM stratification. Therefore, testing for these mutations is crucial for determining prognosis in patients treated with BTKi therapy.

Our study concludes that both AS-PCR and ddPCR are highly effective for detecting MYD88 mutations in unsorted samples, providing high sensitivity suitable for routine clinical use. For CXCR4 mutations, NGS showed the highest sensitivity. In the context of non-BTKi treatments, neither MYD88 nor CXCR4 mutation emerged as independent prognostic factors when considering the IPSSWM clinical staging. However, in the era of BTKi treatment, the prognostic relevance of both MYD88 and CXCR4 mutations becomes significant, independent of the IPSSWM score. This indicates that testing for these mutations is crucial for predicting survival outcomes in patients receiving BTKi therapy. Our findings highlight the importance of understanding how disease risk factors can vary depending on the treatment context.

## Authors' Disclosures

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## Authors' Contributions

**Y. Yan:** Data curation, formal analysis, supervision, visualization, writing–original draft. **Y. Yu:** Data curation, formal analysis, visualization, writing–original draft. **W. Xiong:** Resources. **Jun Wang:** Resources, formal analysis, validation. **Y. Yao:** Resources, data curation. **Y. Jia:** Resources, data curation. **Y. Huang:** Resources. **Y. Li:** Resources. **T. Wang:** Resources. **R. Lyu:** Resources. **H. Sun:** Visualization. **H. Wang:** Resources, data curation. **Q. Wang:** Resources. **W. Liu:** Resources. **G. An:** Resources. **W. Sui:** Resources. **Y. Xu:** Resources. **W. Huang:** Resources. **Z. Yu:** Resources. **D. Zou:** Resources. **M. Hao:** Resources. **Z. Xiao:** Supervision. **Jianxiang Wang:** Supervision. **L. Qiu:** Conceptualization, resources, supervision, funding acquisition, writing–review and editing. **S. Yi:** Conceptualization, resources, supervision, funding acquisition, writing–review and editing.

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## Note

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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## References

1. Cazzola M. Introduction to a review series: the 2016 revision of the WHO classification of tumors of hematopoietic and lymphoid tissues. Blood 2016;127:2361–4.
2. Treon SP, Xu L, Yang G, Zhou Y, Liu X, Cao Y, et al. MYD88 L265P somatic mutation in Waldenström's macroglobulinemia. N Engl J Med 2012;367:826–33.
3. Hunter ZR, Xu L, Yang G, Zhou Y, Liu X, Cao Y, et al. The genomic landscape of Waldenstrom macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell lymphomagenesis. Blood 2014;123:1637–46.
4. Castillo JJ, Xu L, Gustine JN, Keezer A, Meid K, Dubeau TE, et al. CXCR4 mutation subtypes impact response and survival outcomes in patients with Waldenström macroglobulinaemia treated with ibrutinib. Br J Haematol 2019;187:356–63.
5. Treon SP, Tripsas CK, Meid K, Warren D, Varma G, Green R, et al. Ibrutinib in previously treated Waldenström's macroglobulinemia. N Engl J Med 2015;372:1430–40.
6. Treon SP, Xu L, Hunter Z. MYD88 mutations and response to ibrutinib in Waldenström's macroglobulinemia. N Engl J Med 2015;373:584–6.
7. Xu L, Hunter ZR, Yang G, Zhou Y, Cao Y, Liu X, et al. MYD88 L265P in Waldenström macroglobulinemia, immunoglobulin M monoclonal gammopathy, and other B-cell lymphoproliferative disorders using conventional and quantitative allele-specific polymerase chain reaction. Blood 2013;121:2051–8.
8. Drandi D, Genuardi E, Dogliotti I, Ferrante M, Jiménez C, Guerrini F, et al. Highly sensitive MYD88$^{L265P}$ mutation detection by droplet digital polymerase chain reaction in Waldenström macroglobulinemia. Haematologica 2018;103:1029–37.
9. Willenbacher E, Willenbacher W, Wolf DG, Zelger B, Peschel I, Manzl C, et al. Digital PCR in bone marrow trephine biopsies is highly sensitive for MYD88(L265P) detection in lymphomas with plasmacytic/plasmacytoid differentiation. Br J Haematol 2019;186:189–91.
10. Gustine J, Meid K, Xu L, Hunter ZR, Castillo JJ, Treon SP. To select or not to select? The role of B-cell selection in determining the MYD88 mutation status in Waldenstrom macroglobulinaemia. Br J Haematol 2017;176:822–4.
11. Paiva B, Montes MC, Garcia-Sanz R, Ocio EM, Alonso J, de Las Heras N, et al. Multiparameter flow cytometry for the identification of the Waldenstrom's clone in IgM-MGUS and Waldenstrom's macroglobulinemia: new criteria for differential diagnosis and risk stratification. Leukemia 2014;28:166–73.
12. Iwanaga M, Chiang C-J, Soda M, Lai MS, Yang YW, Miyazaki Y, et al. Incidence of lymphoplasmacytic lymphoma/Waldenström's macroglobulinaemia in Japan and

Taiwan population-based cancer registries, 1996–2003. Int J Cancer 2014;134:174–80.
13. Kyle RA, Larson DR, McPhail ED, Therneau TM, Dispenzieri A, Kumar S, et al. Fifty-year incidence of Waldenström macroglobulinemia in Olmsted county, Minnesota, from 1961 through 2010: a population-based study with complete case capture and hematopathologic review. Mayo Clin Proc 2018;93:739–46.
14. Wang J, Yan Y, Xiong W, Song G, Wang Y, Zhao J, et al. Landscape of immunoglobulin heavy chain gene repertoire and its clinical relevance to LPL/WM. Blood Adv 2022;6:4049–59.
15. Wu Y-Y, Jia M-N, Cai H, Qiu Y, Zhou D-B, Li J, et al. Detection of the $MYD88^{L265P}$ and $CXCR4^{S338X}$ mutations by cell-free DNA in Waldenström macroglobulinemia. Ann Hematol 2020;99:1763–9.
16. Cao X-X, Meng Q, Cai H, He T-H, Zhang C-L, Su W, et al. Detection of MYD88 L265P and WHIM-like CXCR4 mutation in patients with IgM monoclonal gammopathy related disease. Ann Hematol 2017;96:971–6.
17. Jia YC, Lu J, Qiang WT, Li L, Liu J, Jiang H, et al. Clinical characteristics and prognosis of Waldenstrom's macroglobulinemia: a clinical data report from a single center. Zhonghua Xue Ye Xue Za Zhi 2020;41:1020–4.
18. Owen RG, Treon SP, Al-Katib A, Fonseca R, Greipp PR, McMaster ML, et al. Clinicopathological definition of Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia. Semin Oncol 2003;30:110–5.
19. Treon SP, Tedeschi A, San-Miguel J, Garcia-Sanz R, Anderson KC, Kimby E, et al. Report of consensus Panel 4 from the 11th International Workshop on Waldenstrom's macroglobulinemia on diagnostic and response criteria. Semin Hematol 2023;60:97–106.
20. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: Waldenström macroglobulinemia/lymphoplasmacytic lymphoma version 2.2022, December 7, 2021. Available from: http://www.nccn.org/patients.
21. Krzisch D, Guedes N, Boccon-Gibod C, Baron M, Bravetti C, Davi F, et al. Cytogenetic and molecular abnormalities in Waldenström's macroglobulinemia patients: correlations and prognostic impact. Am J Hematol 2021;96:1569–79.
22. Cao X-x, Yi S-h, Jiang Z-x, He J-s, Yang W, Du J, et al. Treatment and outcome patterns of patients with Waldenström's macroglobulinemia: a large, multicenter retrospective review in China. Leuk Lymphoma 2021;62:2657–64.
23. Kofides A, Hunter ZR, Xu L, Tsakmaklis N, Demos MG, Munshi M, et al. Diagnostic next-generation sequencing frequently fails to detect $MYD88^{L265P}$ in Waldenström macroglobulinemia. Hemasphere 2021;5:e624.
24. Gustine JN, Xu L, Tsakmaklis N, Demos MG, Kofides A, Chen JG, et al. $CXCR4^{S338X}$ clonality is an important determinant of ibrutinib outcomes in patients with Waldenström macroglobulinemia. Blood Adv 2019;3:2800–3.