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# Somatic mutations in MYD88 and CXCR4 are determinants of clinical presentation and overall survival in Waldenstrom's Macroglobulinemia.

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**Keywords :** Waldenström's Macroglobulinemia, MYD88-L265P, CXCR4, WHIM, hyperviscosity, overall survival.

## Keypoints

- **Activating MYD88, as well as nonsense and frameshift WHIM-like CXCR4 somatic mutations are common in Waldenstrom's Macroglobulinemia.**
- **CXCR4 nonsense mutations are present in aggressive cases including hyperviscosity syndrome, and MYD88 status is a determinant of survival.**

## Abstract

Whole genome sequencing has revealed activating somatic mutations in MYD88 (L265P) and CXCR4 in Waldenstrom's Macroglobulinemia (WM). CXCR4 somatic mutations in WM are the first ever reported in human cancer, and similar to nonsense (NS) and frameshift (FS) germline mutations found in WHIM syndrome. We genotyped lymphoplasmacytic cells from 175 WM patients, and observed significantly higher bone marrow (BM) disease involvement, serum IgM levels, symptomatic disease requiring therapy including hyperviscosity syndrome in those patients with $MYD88^{L265P}CXCR4^{WHIM/NS}$ mutations ($p<0.03$). Patients with $MYD88^{L265P}CXCR4^{WHIM/FS\ or\ WILDTYPE\ (WT)}$ had intermediate BM and serum IgM levels, and those with $MYD88^{WT}CXCR4^{WT}$ showed lowest BM disease burden. Fewer patients with $MYD88^{L265P}$ and $CXCR4^{WHIM/FS\ or\ NS}$ versus $MYD88^{L265P}CXCR4^{WT}$ presented with adenopathy ($p<0.01$), further delineating differences in disease tropism based on CXCR4 status. Neither MYD88 nor CXCR4 mutations correlated with SDF-1a (RS1801157) polymorphisms in 54 patients who were genotyped for these variants. Unexpectedly, risk of death was not impacted by CXCR4 mutation status, but by $MYD88^{WT}$ status (Hazard ratio 10.54; 95% CI 2.4-46.2 $p=0.0018$). Somatic mutations in

MYD88 and CXCR4 are important determinants of clinical presentation, and impact overall survival in WM. Targeted therapies directed against MYD88 and/or CXCR4 signalling may provide a personalized treatment approach to WM.

## Introduction

Waldenstrom's Macroglobulinemia (WM) is an incurable B-cell neoplasm characterized by accumulation of malignant lymphoplasmacytic cells (LPC) in the bone marrow (BM), lymph nodes and spleen, and excess production of serum IgM which can produce symptoms related to hyperviscosity, tissue infiltration and autoimmune related pathology.[1] The most striking findings from recent whole genome sequencing in WM is the discovery of two activating somatic mutations effecting Toll-like (TLR) and CXCR4 receptor signalling.[2,3] Approximately 90-95% of WM patients carry MYD88 L265P ($MYD88^{L265P}$), an activating mutation that triggers Interleukin-1 Receptor Associated (IRAK) and Bruton's Tyrosine (BTK) Kinases which in turn activate NF-kB-p65 dependent nuclear translocation and malignant cell growth. The first ever reported somatic mutations in CXCR4 in cancer were identified by Hunter et al[3] in 30% of WM patients, and involve the C-terminus that contains serine phosphorylation sites that regulate signalling of CXCR4 by its only known ligand SDF-1a (CXCL12). The location of somatic mutations in the C-terminal domain of WM patients are similar to those observed in the germline of patients with WHIM (Warts, Hypogammaglobulinemia, Infections, and Myelokathexis) syndrome, a congenital immunodeficiency disorder characterized by chronic noncyclic neutropenia.[4,5] Germline mutations in the C-terminus of CXCR4 in WHIM patients block receptor internalization following SDF-1a stimulation in myeloid cells which results in persistent CXCR4 activation and BM myeloid cell trafficking.[6]

In WM patients, two classes of CXCR4 mutations occur in the C-terminus. These include non-sense ($CXCR4^{WHIM/NS}$) mutations that truncate the distal 15-20 amino acid region, and frameshift ($CXCR4^{WHIM/FS}$) mutations that compromise a region of up to 40 amino acids in the C- terminal domain.[3] Non-sense and frameshift mutations are almost equally divided among WM patients with CXCR4 somatic mutations. Preclinical studies with the most common $CXCR4^{WHIM/NS}$ mutation in WM (S338X) have shown enhanced and sustained AKT, ERK and BTK signalling following SDF-1a relative to $CXCR4^{WT}$, as well increased cell migration, adhesion, growth and survival of WM cells.[7-9] The clinical

implications for MYD88 and CXCR4 somatic mutations in WM remain to be delineated, and are the focus of this report.

## Patients and Methods

BM aspirates and peripheral blood (PB) samples were collected after informed written consent from 175 untreated patients with the clinicopathological diagnosis of WM as defined by the Second International Workshop on WM.[10] Consensus criteria were used to delineate patients as having symptomatic versus smoldering disease.[11] The study was approved by the DFCI/Harvard Cancer Center Institutional Review Board. This study was conducted in accordance with the Declaration of Helsinki. $MYD88^{L265P}$ status was determined using allele-specific PCR (AS-PCR), and Sanger sequencing of the C-terminal domain of CXCR4 was performed using sorted ($CD19^{+}$) lymphoplasmacytic cells (LPC) from BM aspirates as previously reported.[2,11] The somatic nature of these mutations was confirmed by sequencing CD19-depleted peripheral blood cells.[2,11] SDF-1a tumor sequencing and germline polymorphisms at RS1801157 were available for 54 patients in this study who underwent whole genome sequencing using previously reported methods.[3]

## Statistical Analysis

Data sets were analyzed by ANOVA and non-parametric comparisons made by the Fisher's exact probability test. Tukey's HSD was used for pairwise analysis. Ordinal data was analyzed using pairwise Wilcoxon Rank Sum Test with Holm Bonferroni correction. A p-value $< 0.05$ was deemed to be significant. A multivariate analysis to estimate risk of death was performed using the Cox proportional hazard regression model. The Fleming-Harrington test was used to assess differences in Kaplan Meier survival curves. Calculations were performed with R (R Foundation for Statistical Computing, Vienna, Austria).

## Results

### MYD88 and CXCR4 status in WM patients

$MYD88^{L265P}$ was detected in BM LPC from 158/175 (90.3%) patients, and mutations in the C-terminus of CXCR4 were present in 51/175 (29.1%) patients. Fifty of 51 patients (98%) who were $CXCR4^{WHIM}$ mutated also exhibited the $MYD88^{L265P}$ mutation (**Table 1**). Among the 51 $CXCR4^{WHIM}$ mutated patients, 25 (49%) and 26 (51%) had non-sense and frameshift mutations, respectively. One patient with a $CXCR4^{WHIM/FS}$ mutation was $MYD88^{WT}$. Two patients with $CXCR4^{WHIM/NS}$ demonstrated subclonal populations with other CXCR4 C-terminal mutations. One patient who had a K333X non-sense mutation had a subclonal population with a 1013C>G (S338X) non-sense mutation. Another patient with a S338X non-sense mutation demonstrated a subclonal population with 993_995insA (G332fs) frameshift mutation. All mutations were confirmed to be somatic in nature by sequencing of corresponding normal paired tissues.[2] The CXCR4 somatic mutations that were identified following Sanger sequencing are annotated for the first time in this report and presented in **Table 1**.

### MYD88 and CXCR4 Mutation Status and Clinical Presentation of WM patients

We first sought to delineate the impact of MYD88 and CXCR4 mutation status on the initial clinical presentation of WM patients. We limited our analysis to three subgroups of patients $MYD88^{WT}CXCR4^{WT}$ (n=15); $MYD88^{L265P}CXCR4^{WT}$ (n=109); and those with $MYD88^{L265P}CXCR4^{WHIM}$ mutations which included 50 patients with $MYD88^{L265P}CXCR4^{WHIM}$. One patient with $MYD88^{WT}CXCR4^{WHIM}$ who had a frameshift mutation in CXCR4 was not included in the initial analysis due to the singular nature of their presentation. This patient presented with 50% BM disease involvement, a serum IgM level of 550 mg/dL, adenopathy, and renal failure attributed to symptomatic WM disease.

Among the 174 patients included in the analysis, those with MYD88$^{WT}$ presented at an older age and had less BM involvement versus patients with the MYD88$^{L265P}$ mutation, regardless of CXCR4 mutation status; for age comparisons, p=0.01 and 0.08 versus MYD88$^{L265P}$CXCR4$^{WT}$ and MYD88$^{L265P}$CXCR4$^{WHIM}$, and for BM comparisons, p=0.03 and 0.01 versus MYD88$^{L265P}$CXCR4$^{WT}$ and MYD88$^{L265P}$CXCR4$^{WHIM}$ patients (**Table 2**). By multivariate analysis, a higher percentage (72.7%) of patients with MYD88$^{WT}$ also had a $B_2$-Microglobulin level of >3.0 mg/L versus the two other subgroups (p=0.003). Patients with MYD88$^{WT}$ also showed differences in absolute lymphocyte and platelet count versus the two other subgroups, but these differences were not clinically meaningful. Of interest, patients with MYD88$^{L265P}$CXCR4$^{WHIM}$ mutations exhibited a lower incidence of adenopathy versus patients with MYD88$^{L265P}$CXCR4$^{WT}$ (p<0.01). No differences in gender, absolute neutrophil counts, hemoglobin levels, serum IgA, IgG and IgM levels, $B_2$-microglobulin levels, presence of splenomegaly, familial history for B-cell malignancies, prior history of monoclonal gammopathy of unknown significance (MGUS), or symptomatic status at diagnosis was observed among these three subgroups.

We next examined if differences in clinical presentation existed when non-sense versus frameshift CXCR4$^{WHIM}$ mutation status was delineated (**Table 3**). As shown in **Figure 1**, patients with CXCR4$^{WHIM/NS}$ demonstrated significantly higher levels of BM disease versus CXCR4$^{WHIM/FS}$ and MYD88$^{WT}$ patients (p=0.05, and 0.005, respectively). Patients with MYD88$^{L265P}$CXCR4$^{WHIM/NS}$ also had significantly higher serum IgM levels versus MYD88$^{L265P}$CXCR4$^{WHIM/FS}$, MYD88$^{L265P}$CXCR4$^{WT}$and MYD88$^{WT}$patients; p=0.05, 0.01, and 0.01, respectively (**Figure 1**). As before, fewer patients with MYD88$^{L265P}$CXCR4$^{WHIM}$, regardless of frameshift or non-sense mutation status, exhibited adenopathy when compared to patients who were MYD88$^{L265P}$CXCR4$^{WT}$ mutated (p=0.04 and 0.05, respectively). As shown in **Table 3**, more patients with MYD88$^{L265P}$CXCR4$^{WHIM/NS}$ also presented with symptomatic disease requiring therapy at diagnosis, which contrasted particularly against MYD88$^{L265P}$CXCR4$^{WHIM/FS}$ patients (p=0.007). Patients with MYD88$^{L265P}$CXCR4$^{WHIM/NS}$ were also more likely to present with symptomatic hyperviscosity, reflecting higher serum IgM levels observed in these

patients at diagnosis (**Table 3**). No differences in gender, absolute lymphocyte counts, hemoglobin levels, serum IgA and IgG levels, $B_2$-microglobulin levels, presence of splenomegaly, familial history for B-cell malignancies, or prior history of monoclonal gammopathy of unknown significance (MGUS) were observed among the four subgroups. Clinically not meaningful differences in absolute neutrophil and platelet counts were also observed (**Table 3**). For 54 patients, both tumor and germline sequencing data for SDF-1a was available. No somatic mutations in SDF-1a were observed in these patients. The distribution for SDF-1a polymorphisms at RS RS1801157 which are associated with increased SDF-1a transcriptional activity did not vary from those reported in healthy donors,[12,13] nor did MYD88 (p=1.0) or CXCR4 mutations (p=0.763) show any correlation with RS1801157 SDF-1a polymorphisms in these patients.

**MYD88 and CXCR4 Mutation Status and Overall Survival in WM**

The median follow-up for all patients was 4.84 (range 0.65-23.45) years from time of diagnosis, and did not differ between the four subgroups (p=0.51). There were no significant differences in the number of therapies, or the percentage of patients who received rituximab, alkylators, proteasome inhibitors or immunomodulatory (IMID) agents among the four subgroups (**Table 4**). However, intergroup comparison showed that a higher number of patients with $MYD88^{L265P}CXCR4^{WHIM/NS}$ versus $MYD88^{L265P}CXCR4^{WHIM/FS}$ received proteasome inhibitor therapy (p=0.009).

Fifteen patients died during follow-up, with cause of death attributed to progressive or transformed disease (n=11), other cancers (n=2), treatment related myelodysplasia (n=1), and amyotrophic lateral sclerosis (n=1). The Kaplan Meier curve for overall survival for patients with $MYD88^{WT}CXCR4^{WT}$, $MYD88^{L265P}CXCR4^{WT}$, and $MYD88^{L265P}CXCR4^{WHIM}$ is shown in **Figure 2**. Differences in survival curves based on CXCR4 and MYD88 mutation status were significant (p<0.0001), as was the analysis based on MYD88 status alone (p<0.0001), with the highest mortality (5/15; 30%) observed in the $MYD88^{WT}CXCR4^{WT}$ cohort. One additional patient who was $MYD88^{WT}CXCR4^{WHIM/FS}$ also succumbed to progressive disease. Hence 6/16 (38%) of

patients with MYD88$^{WT}$ died during the follow-up period, and included 5 of 11 (45.5%) patients in the entire cohort who died of progressive disease. By comparison, lower incidences of mortality were observed in patients with MYD88$^{L265P}$ including 7/109 (6.4%) with CXCR4$^{WT}$, and 2/50 (4%) with CXCR4$^{WHIM}$ mutations (1 frameshift, 1 nonsense) (p=0.0007) during the follow-up period. By Cox proportional hazard, the age and $B_2M$ (>3 mg/L) adjusted risk of death associated with MYD88$^{WT}$ was 10.54 (95% CI 2.40-46.2; p=0.002), whereas the risk of death associated with CXCR4$^{WHIM}$ was 0.96 (95%CI 0.16-6.7; p=0.96).

## Discussion

This is the first study to address the clinical findings associated with two newly discovered somatic mutations (MYD88 L265P and CXCR4) in WM. A paucity of knowledge for these mutations in cancer exists due to the low prevalence for the MYD88 L265P mutation in other B-cell diseases, as well as the fact that CXCR4 somatic mutations have not been previously described in any malignant condition despite the often reported dysregulation of this gene in other cancers.[4] Important strengths of this study are the relatively large genotyped population of WM patients, and the long median follow-up (almost 5 years) from diagnosis. The key findings that emerged from this study are that distinct subsets of patients can be identified based on mutational analysis of MYD88 and CXCR4. Patients with $MYD88^{L265P}CXCR4^{WHIM/NS}$ showed higher BM disease burden, higher serum IgM levels, and more likely to have symptomatic disease requiring therapy at presentation. In contrast, patients with $MYD88^{WT}CXCR4^{WT}$ showed the lowest BM disease burden, whereas patients with either $MYD88^{L265P}CXCR4^{WT}$ or $MYD88^{L265P}CXCR4^{WHIM/FS}$ showed intermediate levels of BM disease involvement. Patients with $CXCR^{WHIM}$, regardless of whether they had a non-sense or frameshift mutation were also less likely to have adenopathy. These findings suggest an increased tropism for $CXCR4^{WHIM}$ mutated WM cells for the BM stroma, which promotes homing and adhesion of CXCR4 expressing WM cells by elaboration of SDF-1a.[14] Adhesion to the BM stroma promotes malignant cell survival and IgM release which could account for the more pronounced bone marrow involvement and serum IgM levels that were particularly observed in $CXCR4^{WHIM/NS}$ mutated patients.

Surprising in this study were the differences observed in the clinical presentation of $CXCR4^{WHIM/NS}$ and $CXCR4^{WHIM/FS}$ mutated WM patients. Patients with $CXCR4^{WHIM/NS}$ showed more aggressive disease features at presentation including higher bone marrow disease burden, serum IgM levels (which were nearly double), and symptomatic status including hyperviscosity syndrome requiring therapy versus $CXCR4^{WHIM/FS}$ mutated patients. These findings likely contributed to the finding of higher proteasome

inhibitor use in $CXCR4^{WHIM/NS}$ mutated patients, since bortezomib is recommended by consensus and NCCN guidelines in patients presenting with symptomatic hyperviscosity.[15,16] The findings also suggest that non-sense and frameshift mutations involving the C-terminus of CXCR4 behave differently. In WHIM syndrome patients, very few patients have been identified with frameshift mutations, and nearly all of these patients have non-sense mutations (R334X or S338X).[4,17] As such, little or no preclinical modeling has been done to understand the activating nature of CXCR4 C-terminal domain frameshift mutations. In the C-terminus, at least 6 serines are phosphorylated in response to SDF-1a beginning with serine 321.[5] Both G protein-coupled receptor kinases (GRK 2 and 6) and arrestins (β1 and β2) bind to the C-terminal domain of CXCR4 at different serine residues, resulting in both positive and negative modulation of CXCR4 signalling. Rapid attenuation of CXCR4 signalling follows SDF-1a ligation, which is mediated by recruitment of arrestins, and dependent on the phosphorylation of the terminal 5 serines that include serines 346/347 that modulate the phosphorylation of more proximal serines.[5,18] As such non-sense mutations such as S338X and R334X that truncate the more distal portions of the C-terminus may effect more proximal serine phosphorylation, as well as remove a scaffold critical for arrestin binding thereby promoting prolonged CXCR4 signalling. Preclinical modeling comparing $CXCR4^{WHIM/NS}$ to $CXCR4^{WHIM/FS}$ will be required to clarify any signalling differences in response to SDF-1a, which might also be amenable to therapeutic exploitation.

The potential to translate the findings of MYD88 and CXCR4 mutations into therapeutic gains for WM patients are noteworthy. Both IRAK 1 and 4, as well as BTK signal for MYD88 L265P, while BTK is activated by CXCR4.[8,19] Inhibitors to IRAK are currently in clinical development, whereas the BTK inhibitor ibrutinib has shown impressive activity in relapsed and refractory WM patients.[18] Major responses to ibrutinib therapy are higher in $MYD88^{L265P}$ and $CXCR4^{WT}$ patients, the later being highly associated with ibrutinib response.[20] In preclinical studies, WM cells engineered to express the S338X CXCR4 non-sense mutation show resistance to the suppressive effects of ibrutinib on AKT and ERK 1/2 signalling, which could be restored by use of the CXCR4 specific inhibitor plerixafor.[8] Taken together, these studies highlight the importance of

understanding both MYD88 and CXCR4 mutation status in WM, and may provide the basis for a more personalized treatment approach including the use of relevant inhibitors for MYD88 mutated patients, and the use of CXCR4 inhibitors in CXCR4 mutated WM patients. In addition to plerixafor, several other antagonists to CXCR4 have been developed and are in clinical trials including BMS-936564, AMD-070, TG-0054 and could be investigated for use either alone, or in combination treatment strategies for WM patients with CXCR4 mutations.

Despite the aggressive presentation of patients harboring $CXCR^{WHIM/NS}$ mutations, overall survival for these patients was not adversely impacted. A similar number of deaths occurred in both CXCR4 WT (6%) and WHIM mutated (4%) patients who harbored the MYD88 L265P mutation. Poulain et al[13] reported that a germline polymorphism in SDF-1a (−801GG) which is at RS1801157 was associated with shorter survival following initiation of therapy in WM patients. We observed no somatic mutations in SDF-1a for 54 patients in this study whose tumor cells were sequenced, nor did MYD88 or CXCR4 mutations correlate with RS1801157 SDF-1a polymorphisms determined by germline sequencing in these patients. Prospective studies incorporating SDF-1a polymorphisms and CXCR4 mutation status in assessing treatment response, progression-free and overall survival in WM patients could nonetheless be illuminating, and help better clarify the predictive and prognostic roles of SDF-1a and CXCR4 variants alone, and together.

Unexpectedly, patients who were $MYD88^{WT}$ showed significantly higher mortality (38%) versus patients with $MYD88^{L265P}$ (6%) during the follow-up period. Patients with $MYD88^{WT}$ were older, and more demonstrated a $B_2M$ of >3.0 mg/L, a poor prognosis marker in WM.[1] However, accounting for both age and $B_2M$ by multivariate analysis, $MYD88^{WT}$ status remained a significant risk for death. Important genomic differences between $MYD88^{WT}$ and $MYD88^{L265P}$ have previously been described including presence of MLL2 mutations in $MYD88^{WT}$ patients that are usually found in follicular and aggressive NHL, as well decreased IGHV3–23 rearrangements and somatic hyper-mutation in $MYD88^{WT}$ patients.[2,21,22] These studies highlight the urgency in better

understanding the pathogenesis underlying $MYD88^{WT}$ WM disease, and also development of targeted and more effective treatments for this patient population.

In summary, we report that MYD88 and CXCR4 mutation status denote important differences in disease presentation for patients with WM, including BM disease burden, presence of extramedullary disease, serum IgM levels, symptomatic status at diagnosis including presentation with hyperviscosity syndrome, and overall survival. These studies also provide the first ever reporting of clinical differences in cancer patients associated with CXCR4 somatic mutations, including non-sense and frameshift mutation status. These studies also provide a framework for the investigation of MYD88 and CXCR4 mutations as prognostic and predictive markers, as well as the development of targeted therapeutics for use in personalized treatment approaches to WM.

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## AUTHOR CONTRIBUTIONS

SPT and ZRH designed the study wrote the manuscript. SPT and ZRH performed the data analysis. ZRH and XL designed AS-PCR and Sanger sequencing primers. GY, YC, XL, and LX prepared the samples and performed the sequencing studies. SPT provided patient care, obtained consent and samples.

## AUTHOR DISCLOSURES

The authors have no competing interests to declare.

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## Table Legend

**Table 1. Somatic mutations in C-terminus of CXCR4 identified by Sanger sequencing, and stratified by MYD88 L265P mutation status in WM patients.**

**Table 2. Disease characteristics at presentation for WM patients stratified by MYD88 and CXCR4 mutation status.** Denotes comparisons of patient characteristics at diagnosis stratified by all $CXCR^{WHIM}$ mutated patients.

**Table 3. Disease characteristics at presentation for WM patients stratified by MYD88 and non-sense and frameshift CXCR4 mutation status.** Denotes comparisons of patient characteristics at diagnosis delineated by non-sense and frameshift CXCR4 mutation status.

**Table 4. Treatment characteristics for studied WM patients stratified by MYD88 and non-sense and frameshift CXCR4 mutation status.**

## Table 1.

| N= | MYD88 Status | Mutation Type | Nucleotide change | Amino acid change |
|---|---|---|---|---|
| 1 | L265P | Nonsense | r.997 A>T[1] | K333X[1] |
| 3 | L265P | Nonsense | r.1000C>T | R334X |
| 7 | L265P | Nonsense | r.1013C>A | S338X |
| 15 | L265P | Nonsense | r.1013C>G[2] | S338X[2] |
| 1 | WT | Frameshift | r.931_933insT | T311fs |
| 3 | L265P | Frameshift | r.952_954insA | T318fs |
| 2 | L265P | Frameshift | r.951_953delACCTC | T318fs |
| 1 | L265P | Frameshift | r.954_956insC | S319fs |
| 1 | L265P | Frameshift | r.958_960delTG | V320fs |
| 1 | L265P | Frameshift | r.963_965insC | R322fs |
| 1 | L265P | Frameshift | r.969_971insG | S324fs |
| 1 | L265P | Frameshift | r.978_980insT | K327fs |
| 1 | L265P | Frameshift | r.984_986insT | L329fs |
| 1 | L265P | Frameshift | r.993_995insA | G332fs |
| 1 | L265P | Frameshift | r.1005_1007insT | G336fs |
| 2 | L265P | Frameshift | r.1013_1015delATCT | S338fs |
| 1 | L265P | Frameshift | r.1013_1015delATCTGTTTCCACTGAGT | S338fs |
| 3 | L265P | Frameshift | r.1012_1014insT | S338fs |
| 1 | L265P | Frameshift | r.1015_1017delCT | S339fs |
| 1 | L265P | Frameshift | r.1020_1022delT | S341fs |
| 1 | L265P | Frameshift | r.1024_1026delCT | S342fs |
| 1 | L265P | Frameshift | r.1030_1041CTGAGTCTTC>GT | S344fs |
| 1 | L265P | Frameshift | r.1033_1035delAG | E345fs |

[1]Patient also had subclonal population with 1013C>G (S338X) non-sense mutation.
[2]One patient had subclonal population with 993_995insA (G332fs) frameshift mutation.

## Table 2.

| | MYD88$^{WT}$CXCR4$^{WT}$ | MYD88$^{L265P}$CXCR4$^{WT}$ | MYD88$^{L265P}$CXCR4$^{WHIM}$ | p= |
|---|---|---|---|---|
| N= | 15 | 109 | 50 | |
| Gender (M/F) | 7/8 | 73/36 | 31/19 | ns |
| Age (years) | 66 (42-82) | 59 (40-88) | 60 (34-88) | 0.02 |
| BM Involvement (%) | 15 (5-65) | 40 (5-95) | 50 (5-95) | 0.02 |
| Abs Neutrophil (k/uL) | 2.93 (0.72-3.7) | 3.5 (1.04-8.94) | 3.2 (1.52-8.2) | ns |
| Abs Lymphocyte (k/uL) | 1.61 (0.8-17.3) | 1.73 (0.48-11.1) | 1.69 (0.51-3.98) | 0.03 |
| Hemoglobin (g/dL) | 11.3 (8-14.4) | 11.5 (6-15.5) | 11.6 (4.8-15.6) | ns |
| Platelet (k/uL) | 218 (105-378) | 274 (75-512) | 251 (42-441) | 0.01 |
| Serum IgA (mg/dL) | 140 (27-324) | 61 (0-1240) | 45 (7-864) | ns |
| Serum IgG (mg/dL) | 734 (441-1480) | 686 (138-2920) | 595 (168-1330) | ns |
| Serum IgM (mg/dL) | 2720 (134-5810) | 3190 (345-8720) | 3490 (416-8767) | ns |
| $B_2$ Microglobulin (mg/L) | 3.1 (2-5.5) | 3.1 (1.4-10.4) | 2.3 (1-9.2) | ns |
| Adenopathy | 6 (40%) | 59 (54.1%) | 15 (30%) | 0.01 |
| Splenomegaly | 6 (40%) | 18 (16.5%) | 8 (16%) | ns |
| Family History | 4 (26.7%) | 31(28.4%) | 20 (40%) | ns |
| Prior MGUS History | 4 (26.7%) | 18 (16.5%) | 8 (16%) | ns |
| Symptomatic Disease | 9 (60%) | 69 (63.3%) | 34 (66.6%) | ns |

## Table 3.

| | MYD88$^{WT}$CXCR4$^{WT}$ | MYD88$^{L265P}$CXCR4$^{WT}$ | MYD88$^{L265P}$CXCR4$^{WHIM/FS}$ | MYD88$^{L265P}$CXCR4$^{WHIM/NS}$ | p= |
|---|---|---|---|---|---|
| N= | 15 | 109 | 24 | 26 | |
| Gender (M/F) | 7/8 | 73/36 | 14/10 | 17/9 | ns |
| Age (years) | 66 (42-82) | 59 (40-88) | 62 (34-88) | 59 (41-75) | 0.05 |
| BM Involvement (%) | 15 (5-65) | 40 (5-95) | 37 (5-90) | 50 (15-95) | <0.01 |
| Abs Neutrophil (k/uL) | 2.93 (0.72-3.7) | 3.5 (1.04-8.94) | 3.54 (1.52-8.2) | 3.1 (1.56-6.14) | 0.01 |
| Abs Lymphocyte (k/uL) | 1.61 (0.8-17.3) | 1.73 (0.48-11.1) | 1.77 (0.96-3.55) | 1.63 (0.51-3.88) | ns |
| Hemoglobin (g/dL) | 11.3 (8-14.4) | 11.5 (6-15.5) | 11.8 (4.8-15.6) | 11.5 (6.2-14.2) | ns |
| Platelet (k/uL) | 218 (105-378) | 274 (75-512) | 275 (101-441) | 208 (42-314) | <0.01 |
| Serum IgA (mg/dL) | 140 (27-324) | 61 (0-1240) | 45 (20-202) | 45 (7-864) | ns |
| Serum IgG (mg/dL) | 734 (441-1480) | 686 (138-2920) | 612 (267-978) | 569 (168-1330) | ns |
| Serum IgM (mg/dL) | 2720 (134-5810) | 3190 (345-8720) | 2895 (416-8320) | 5200 (804-8767) | <0.01 |
| $B_2$ Microglobulin (mg/L) | 3.1 (2-5.5) | 3.1 (1.4-10.4) | 2.45 (1.4-9.2) | 2.4 (1-5) | ns |
| Adenopathy | 6 (40%) | 59 (54.1%) | 7 (29.2%) | 8 (30.1%) | 0.04 |
| Splenomegaly | 6 (40%) | 18 (16.5%) | 2 (8.3%) | 6 (23.1%) | ns |
| Family History | 4 (26.7%) | 31(28.4%) | 9 (37.5%) | 11 (42.3%) | ns |
| Prior MGUS History | 4 (26.6%) | 18 (16.5%) | 5 (20.8%) | 3 (11.5%) | ns |
| Symptomatic Disease | 9 (60%) | 69 (63.3%) | 11 (45.8%) | 22 (84.6%) | 0.03 |
| Hyperviscosity Syndrome | 0 (0%) | 10 (9.2%) | 2 (8.3%) | 8 (30.7%) | 0.01 |

## Table 4.

| | MYD88$^{WT}$CXCR4$^{WT}$ | MYD88$^{L265P}$CXCR4$^{WT}$ | MYD88$^{L265P}$CXCR4$^{WHIM/FS}$ | MYD88$^{L265P}$CXCR4$^{WHIM/NS}$ | p= |
|---|---|---|---|---|---|
| N= | 15 | 109 | 24 | 26 | |
| Number of therapies | 2 (0-8) | 2 (0-7) | 1 (0-4) | 2 (1-8) | ns |
| Rituximab | 11 (68.8%) | 89 (81.7%) | 18 (75.0%) | 22 (84.6%) | ns |
| Alkylator | 7 (43.8%) | 63 (57.8%) | 13 (54.2%) | 13 (50.0%) | ns |
| Nucleoside analogue | 3 (18.8%) | 20 (18.4%) | 2 (8.3%) | 3 (11.5%) | ns |
| Proteasome Inhibitor | 6 (37.5%) | 41 (37.6%) | 4 (17.0%) | 14 (53.9%) | ns |
| IMID | 1 (6.3%) | 6 (5.5%) | 0 (0%) | 2 (7.7%) | ns |

## Figure Legend

**Figure 1. Bone marrow disease involvement and serum IgM levels at diagnosis for WM patients stratified by MYD88 and CXCR4 mutation status.** $MYD88^{WT}CXCR4^{WT}$(n=15); $MYD88^{L265P}CXCR4^{WT}$(n=109); $MYD88^{L265P}CXCR4^{WHIM/FS}$ (n=24); $MYD88^{L265P}CXCR4^{WHIM/NS}$ (n=26). Box plots with interquartile ranges are shown with an overlay of the individual data points.

**Figure 2. Kaplan Meier plot for overall survival of 175 WM patients from time of diagnosis stratified by MYD88 and CXCR4 mutation status.** Differences in survival curves based on CXCR4 and MYD88 mutation status were significant ($p<0.0001$), as was the analysis based on MYD88 status alone ($p<0.0001$) by Fleming-Harrington log-rank analysis.

**Figure 1.**

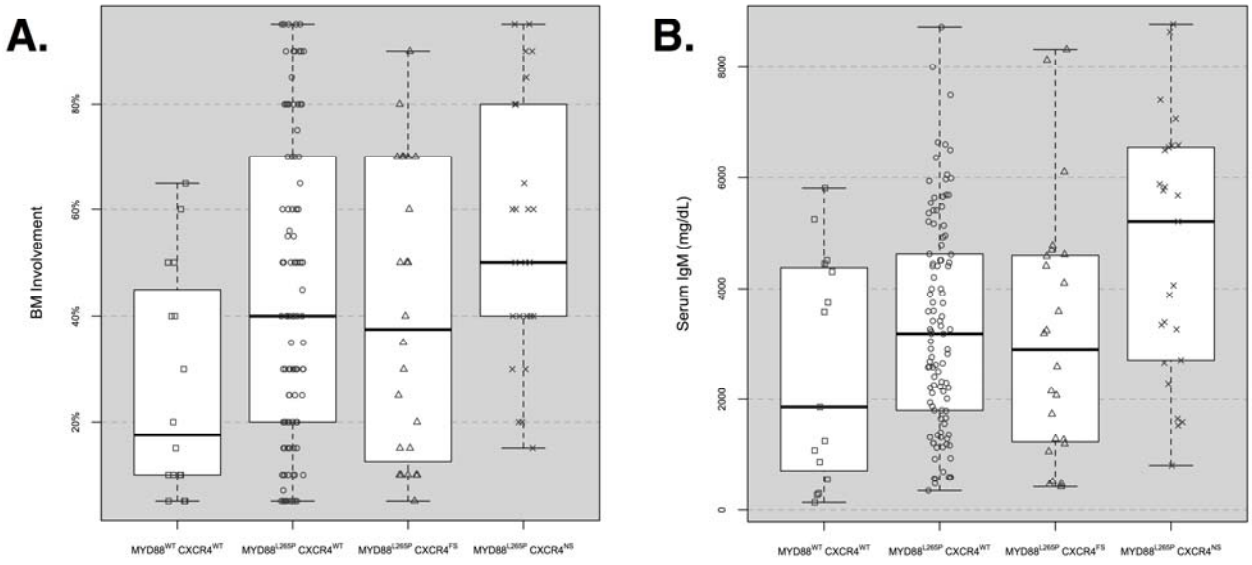

**Figure 2.**