Mutated MYD88 homozygosity is increased in previously treated patients with Waldenstrom's Macroglobulinemia and associates with CXCR4 mutation status

Nickolas Tsakmaklis[1], Lian Xu[1], Guang Yang[1,2], Jie Chen[1], Xia Liu[1,2], Jiaji Chen[1], Maria Demos[1], Christopher J. Patterson[1], Jorge Castillo[1,2], Steven P. Treon[1,2] and Zachary R. Hunter[1,2]

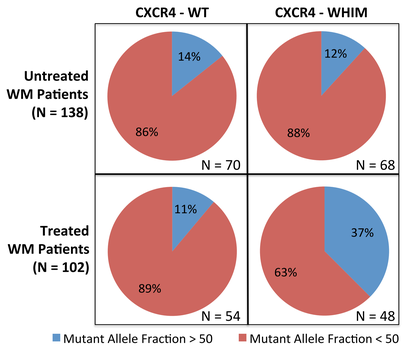
[1]Bing Center for Waldenström's Macroglobulinemia, Dana-Farber Cancer Institute, Boston, MA, USA; [2]Department of Medicine, Harvard Medical School, Boston, MA, USA

Abstract

MYD88 and CXCR4 WHIM-like mutations are present in >90% and 30-40% of untreated patients with Waldenstrom's Macroglobulinemia (WM), respectively. Nearly all MYD88 somatic mutations involve a single nucleotide mutation that results in a change from leucine to proline at amino acid position 265, and most are heterozygous. Mutated MYD88 homozygosity has been observed in 10-12% of untreated WM patients in small series, and was attributed to both acquired uniparental disomy (aUPD) and copy number alterations. CXCR4 WHIM-like mutations are almost always associated with mutated MYD88. The incidence, mechanism for MYD88 homozygosity, and association with CXCR4 mutations remain to be better clarified. In this study, we evaluated 240 (138 untreated and 102 previously treated) patients with the consensus diagnosis of WM. Sanger sequencing for MYD88, copy number (CNA) and aUPD analysis was performed on bone marrow CD19-selected cells. Mutated MYD88 homozygosity was determined to result from aUPDs that were confined to Chr. 3p, and less frequently due to copy number alterations that resulted in deletion of the wild-type MYD88 allele, and amplifications of the mutant MYD88 allele.

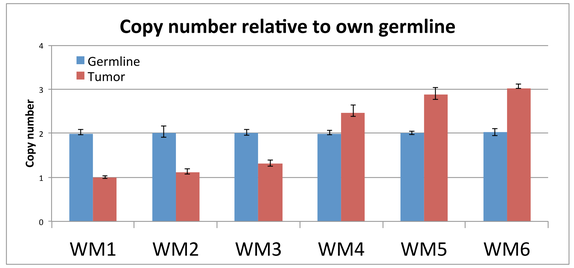
Untreated vs. Treated

	Treated	Untreated
N=	25	18
Deletions	3 (12%)	0 (0%)
Amplifications	2 (8%)	1 (5.5%)
Total number of CNAs	5 (20%)	1 (5.5%)

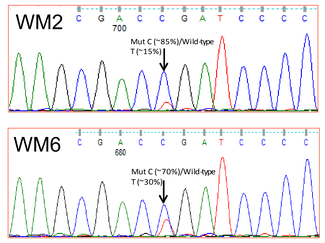


CXCR4 mutations were similarly distributed between untreated patients with MYD88 mutant allele frequency greater than 50% (8/18; 44.4%) and patients with heterozygous MYD88 mutations (60/120; 50.0%). Among previously treated patients, 24/102 (23.5%) presented with MYD88 mutant allele frequency greater than 50% (p=0.039 vs. untreated patients). CXCR4 mutations were more prevalent among previously treated patients with MYD88 mutant allele frequency greater than 50% (18/24; 75.0%) versus heterozygous (29/78; 37.2%) patients who were previously treated (p=0.001).

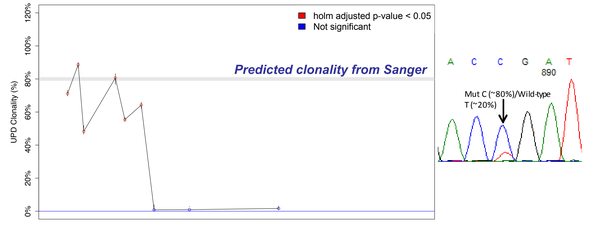
MYD88 Copy Number



For the 43 patients thought to have a MYD88 L265P allele frequency > 50% by Sanger, a Taqman copy number assay (Hs01838632_cn; MYD88 exon 1) was run to rule out the possibility that the increased frequency is due to a deletion of the wild-type allele or amplification of the mutant allele.

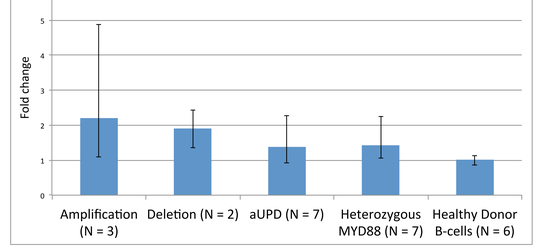


aUPD Results



In total, 21 homozygous patients who were found to have two copies of MYD88 were tested for the presence of an aUPD. 16/21 patients showed clear evidence of an aUPD, while the remaining 5 patients were thought to be below the panel of assay's threshold of detection. These 5 patients had between a 55-60% MYD88 L265P mutant allele fraction.

MYD88 Gene Expression

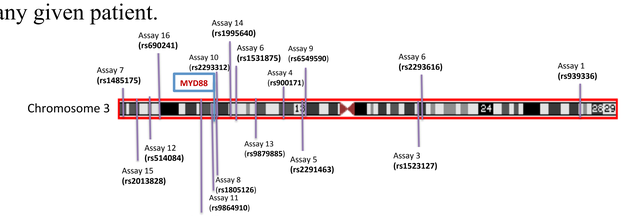


To see how copy number alterations and aUPDs might affect MYD88 expression, a TaqMan gene expression probe (Hs01573837_g1; ThermoFisher) was used. The only significant differences were between the Amplification group and healthy donors ($p < 0.05$), and all WM patients vs. healthy donors ($p < 0.05$).

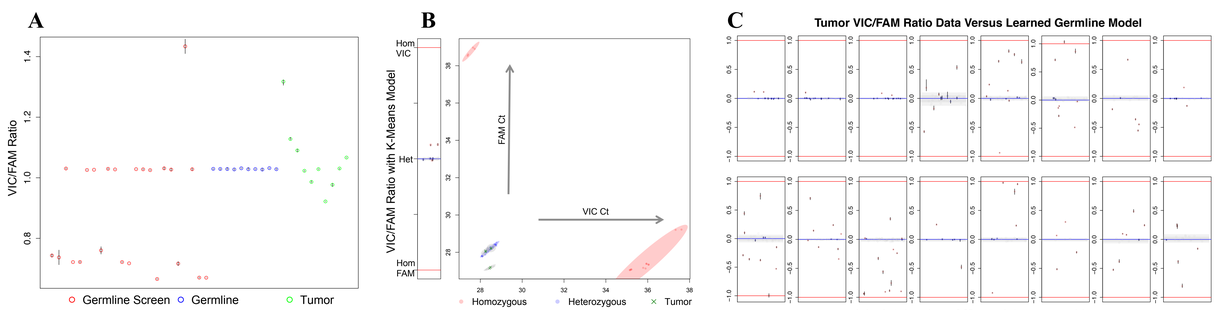
aUPD Panel

Up to 16 Taqman-based assays (ThermoFisher) were used to test for the presence of an aUPD. These assays, which are spread across Chr.3, were chosen for their high rates of heterozygosity. Germline DNA (CD19- cells from peripheral blood, PB19-) was first screened in order to determine the zygosity at each SNP. Tumor DNA (CD19+ cells from bone marrow, BM19+) was then run in quadruplicate on the same plate as PB19- DNA for all SNPs which were found to be heterozygous for any given patient.

Assay	SNP ID	Gene	Cytoband
1	**rs939336**	ABCC5	3q27
2	**rs2293616**	SLC15A2	3q13.33
3	**rs1523127**	NR1I2	3q12-q13.3
5	**rs2291463**	PDZRN3	3p13
6	**rs1531875**	PTPN23	3p21.3
7	**rs1485175**	GRM7	3p26.1-p25.1
8	**rs1805126**	SCN5A	3p21
9	**rs6549590**	CNTN3	3p12.3
10	**rs2293312**	WDR48	3p21.33
11	**rs9864910**	TRANK1	3p22.2
12	**rs514084**	NUP210	3p25.1
13	**rs9879885**	CACNA2D3	3p21.1
14	**rs1995640**	TGM4	3p22-p21.33
15	**rs2013828**	CNTN6	3p26.3
16	**rs690241**	RFTN1	3p24.3
17	**rs2293250**	UBP1	3p22.3



aUPD Analysis



A) Mean and range VIC/FAM cycle threshold (Ct) ratios were plotted for all of the samples in each assay for quality assurance. The ratio of Ct values were quite stable for germline data across multiple samples. **B**) For each assay, germline data was the fit into a k-means model to identify the mean Ct ratios and variance for homozygous VIC, heterozygous, and homozygous FAM genotypes. Tumor Ct ratios from patients who were heterozygous in their germline for a given assay were then compared to the heterozygous germline data to test for statistically significant Ct ratio shifts. **C**) The data for each assay was then rescaled so homozygous VIC and FAM were 1 and -1 respectively with the mean heterozgous signal centered at zero. This allowed for the assay data to be directly assessed for clonality and be plotted on the same scale. **D**) The rescaled data for a given patient was plotted relative to its position on chromosome 3 to demonstrate the extent of the potential aUPD.

Summary

Our findings show that a significantly higher percentage of patients with a MYD88 L265P mutant allele fraction greater than 50% were previously treated. Within the previously treated WM cohort, CXCR4 mutations were much more prevalent for patients with increased MYD88 mutant allele fraction than for those who were heterozygous. Our results also indicate that MYD88 homozygosity was more often caused by aUPDs than through copy number alterations. In addition, we demonstrate the feasibility of using Sanger sequencing, copy number assays, and SNP genotyping in combination in order to detect the presence of aUPDs.

Conflict of interest disclosure: There are no relevant conflicts of interest to disclose.