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

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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 aUPD that were confined to Chr.3, 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

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<thead>
<tr>
<th></th>
<th>Treated</th>
<th>Untreated</th>
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<tbody>
<tr>
<td>N</td>
<td>30</td>
<td>170</td>
</tr>
<tr>
<td>Deletions</td>
<td>3 (12%)</td>
<td>0 (0%)</td>
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<tr>
<td>Amplifications</td>
<td>2 (8%)</td>
<td>11 (5.5%)</td>
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</tbody>
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Total number of CNA

- 5 (20%) (16.5%) for untreated patients
- 1 (1%) (5.5%) for Treated patients

CXCR4 mutations were similarly distributed between untreated patients with MYD88 mutant allele frequency greater than 50% (6/120, 44.4%) and patients with heterozygous MYD88 mutations (60/120, 50.8%). 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% (14/24, 58.3%) 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 L265F allele frequency >50% by Sanger, a Taqman copy number assay (Hs01838632, MyD88 exon 1) was run to rule out the possibility that the increased frequency is due to a deletion of the wild-type allele as amplification of the mutant allele.

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. Germine DNA (CD19+ cells from peripheral blood, PB19+) was first screened in order to determine the homozygosity 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.

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 fit into a k-means model to identify the mean CT ratios and variance for homozygous VIC, heterozygous VIC, 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 a statistically significant CT ratio shift. C) The data for each assay was then rescaled so homozygous VIC and FAM were 1 and -1 respectively with the mean heterozygous signal centered at zero. This allowed for the assay data to be directly assessed for clonality and be plotted on the same C0. 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 L265F 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 aUPD 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.