Mutated MYD88 homozygosity is increased in previously treated patients with Waldenstrom’s Macroglobulinemia and associates with CXCR4 mutation status and ibrutinib exposure.

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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 (Mutant allele frequency (MAF) >0.50). Mutated MYD88 homozygosity (i.e. MAF >0.50) has been observed in 10-15% 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 clarified. In this study, we evaluated 236 (130 untreated and 106 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. Notably, our findings show that mutated MYD88 homozygosity is increased in previously treated patients with Waldenstrom’s Macroglobulinemia, and associates with CXCR4 mutation status and ibrutinib exposure.

MYD88 Copy Number

For the 42 patients thought to have a MYD88 MAF >0.50 by Sanger, a Taqman copy number assay (Hs18816352_m1; 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. To see how copy number alterations and aUPDs might affect the MYD88 expression, Taqman gene expression probe (Hs01073787_g1; ThermalFisher) was used. Significant differences were seen between the MYD88 amplification group vs. healthy donors (p < 0.05), and all WM patients vs. healthy donors (p < 0.05; data not shown). In other case, MYD88 expression was higher in the WM cohort.

aUPD Analysis and Results

Clinical Characteristics

A) CXCR4 mutations were similarly distributed between untreated patients with MYD88 MAF >50% (61/66, 76.5% and patients with MAF ≤50% (66/114, 49%). Among previously treated patients, 26/106 (24.5%) presented with MYD88 MAF >50% (p=0.039 vs. untreated patients). CXCR4 mutations were more prevalent among previously treated patients with MYD88 MAF >50% (19/26, 73%) versus MAF ≤50% (29/80, 36%) patients who were previously treated (p=0.081). Nine out of 10 (90%) previously treated patients with aUPDs were also CXCR4-mutated versus 23/80 (36%) patients with a MYD88 MAF >50% who were CXCR4-mutated (p=0.005). Only 1/5 (20%) untreated patients were CXCR4-mutated (p=0.017 versus previously treated patients with aUPD). B) Patients who had been treated with the BTK inhibitoribrutinib had a higher MAF >50% (7/15, 46.7%) versus patients not treated withibrutinib (3/21, p=0.049).

Summary

Our findings show that a significantly higher percentage of patients with a MYD88 MAF >50% were previously treated. Within the previously treated WM cohort, CXCR4 mutations were much more prevalent for patients with increased MYD88 MAF than for those who MAF ≤50%. Our results also indicate that a MYD88 MAF >50% was more often caused by aUPDs than through copy number alterations. Lastly, when comparing patients who had been exposed toibrutinib versus those who had not, it was found that a significantly larger percentage of the former group had a MAF >50%. The findings suggest that MYD88 homozygosity may contribute to altered survival signaling.

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