Mutated MYD88 zygosity and CXCR4 mutation status are important determinants of ibrutinib response and progression free survival in Waldenstrom's Macroglobulinemia.

Treon SP, Tsakmaklis N, Meid K, Yang G, Chen JG, Liu X, Chen J, Demos M, Patterson CJ, Dubeau T, Gustine J, Castillo JJ, Advani R, Palomba M.L., Xu L, and Hunter ZR. Bing Center for Waldenstrom's Macroglobulinemia, Dana Farber Cancer Institute; Harvard Medical School, Boston MA USA; Stanford University Medical Center, Stanford CA; Memorial Sloan Kettering Cancer Center, New York NY.

Abstract

Background: Activating somatic mutations in MYD88 and CXCR4 are present in 90-95% and 30-40% of untreated WM patients, 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. In approximately 10-12% of untreated WM patients, MYD88 L265P is homozygous due to an acquired uniparenteral disomy (aUPD) or copy number alterations, is more common in previously treated WM patients and associated with CXCR4 mutations. (Treon et al, NEJM 2012; Poulain et al, Blood 2013; Tsakmaklis et al, IWWM-9, 2016). Over 30 different types of nonsense and frameshift mutations in CXCR4 have been described in WM, and almost always are associated with mutated MYD88 (Hunter et al, Blood 2014). Mutated MYD88 activates BTK, and the BTK inhibitor ibrutinib is highly active in WM (Yang et al, Blood 2013; Treon et al NEJM 2015; Dimopoulos et al, Lancet Oncology 2016). MYD88 mutations predict for response, while CXCR4 mutations are associated with slower response kinetics and lower response rates among mutated MYD88 WM patients on ibrutinib (Treon et al, NEJM 2015).

Methods and Materials: In this study, we sought to clarify the impact of MYD88 homozygosity on ibrutinib activity in WM. We evaluated 33 previously treated, symptomatic WM patients who received ibrutinib on a clinical trial (NCT01614821), and for whom baseline and serial bone marrow (BM) CD19-selected cells were available for sequencing, copy number (CNA) and aUPD analysis. Mutated MYD88 homozygosity was determined by establishing the ratio of mutant (Mut) versus wild-type (WT) allele expression by Sanger sequencing. CNA status was determined using TaqMan real-time PCR assays. For patients with unaltered copy number, the presence of aUPD was determined by analyzing the tumor/germline allele balance using established TaqMan genotyping assays.

Results: At baseline, 9/33 (27.3%) of patients were homozygous for mutated MYD88. Mutated MYD88 homozygosity was confirmed to be due to deletion of the wild-type MYD88 allele by CNA in one patient, and amplification of the mutant MYD88 allele in another patient. TaqMan genotyping assays confirmed the presence of an aUPD in 6 patients that were confined to Chr. 3p. CXCR4 mutations were highly prevalent (8/9; 88.8%) among mutated MYD88 homozygous versus heterozygous (7/24; 29.2%) patients (p=0.014). We next assessed the impact of mutated MYD88 zygosity and CXCR4 mutation status on ibrutinib treatment outcome. The baseline characteristics for patients based on mutated MYD88 zygosity and CXCR4 mutation status are shown in Table 1. Following ibrutinib treatment, serum IgM levels declined by -0.89%, -0.54%, and -0.61% among MYD88Mut/WTCXCR4WT, MYD88Mut/WTCXCR4Mut, and MYD88Mut/Mut patients, respectively. Hemoglobin levels improved by 40.5%, 16.2% and 21.6% among MYD88Mut/WTCXCR4WT, MYD88Mut/WTCXCR4Mut, and MYD88Mut/Mut patients, respectively. Major responses (PR or better) were observed in 100%, 50%, and 89% of MYD88Mut/WTCXCR4WT, MYD88Mut/WTCXCR4Mut, and MYD88Mut/Mut patients, respectively. VGPR occurred in 50% of MYD88Mut/WTCXCR4WT patients, and 16.7% and 22.2% of MYD88Mut/WTCXCR4Mut, and MYD88Mut/Mut patients, respectively. Progression-free survival was also impacted by mutated MYD88 zygosity and CXCR4 mutation status (Figure 1). Median PFS was not reached for patients with MYD88Mut/WTCXCR4WT and MYD88Mut/Mut, and was 25.5 months for those with a MYD88Mut/WTCXCR4Mut mutation status (Log-rank Chi-square: 14.74, p=0.0006).

Conclusions: MYD88 homozygosity is strongly associated with activating mutations in CXCR4, and appears to confer a better outcome for WM patients with CXCR4 mutations on ibrutinib therapy.

Whole genome sequencing first identified acquired UPDs at 3p22 in WM patients that confer MYD88 homozygosity.

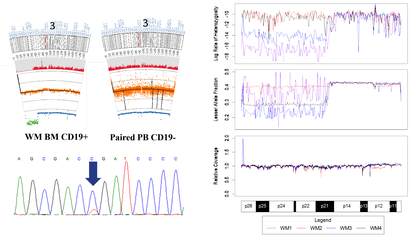
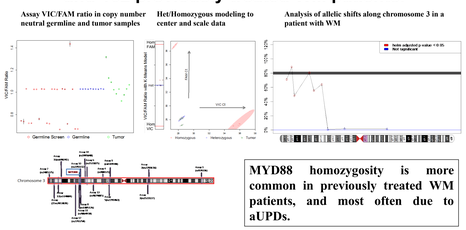


Figure 1. Revelation of an acquired uniparental disomy (aUPD) at chromosome 3p encompassing MYD88 as determined by WGS. Chromosome wide data demonstrating copy number neutral loss of heterozygosity with corresponding allele imbalance for patients WM1 and WM3 indicating presence of an aUPD throughout the tumor clone. For patients WM2 and WM4, an aUPD was present in a subpopulation of tumor cells as demonstrated by sustained allele imbalance. Confirmatory Sanger sequencing of the MYD88 mutation mirrored allele imbalances observed by WGS. Chromosome wide data and confirmatory Sanger sequencing was performed for MYD88 L265 using CD19-depleted PB mononuclear cells for normal tissue comparisons. Treon et al NEJM, 2012; Hunter et al, Blood 2014.

TaqMan Copy Number and SNP Assays were subsequently used to investigate increased MYD88^{Mutant} Allele Frequency in 138 untreated and 102 previously treated WM patients.



MYD88 homozygosity is more common in previously treated WM patients, and most often due to aUPDs.

Figure 2. Determination of MYD88 mutant allele frequency in WM. Sanger sequencing for MYD88, copy number (CNA) and aUPD analysis was performed on bone marrow CD19-selected cells. Mutated MYD88 homozygosity was determined by establishing the ratio of mutant (Mut) versus wild-type (WT) allele expression by Sanger sequencing. CNA status was determined using TaqMan real-time PCR assays. For patients determined to have unaltered copy number, the presence of aUPD was determined by analyzing the tumor/germline allele balance in up to 14 germline heterozygous single nucleotide polymorphisms using established TaqMan genotyping assays. Among untreated patients, 18/138 (13.0%) were homozygous for mutated MYD88. Among previously treated patients, 24/102 (23.5%) were homozygous for mutated MYD88 (p=0.039 vs. untreated patients). Among 21 evaluated patients with homozygous MYD88 expression, 16 demonstrated an aUPD, and 5 CNA (3 amplification of mutant MYD88, 2 deletion of wild-type MYD88). Tsakmaklis et al, 9th International Workshop on WM, Amsterdam 2016.

MYD88 homozygosity is more common in previously treated patients with CXCR4 mutations.

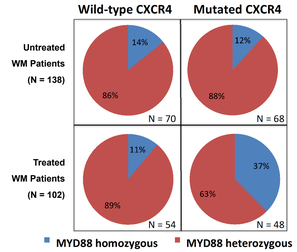
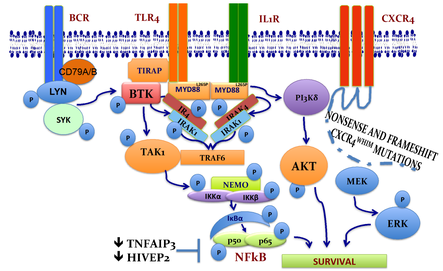


Figure 3. MYD88 homozygosity in untreated and previously treated WM patients and relationship to CXCR4 mutation status. CXCR4 mutations were more prevalent among previously treated patients with homozygous vs. heterozygous MYD88 (p=0.001).

Activating mutations in MYD88 trigger BTK mediated survival while CXCR4 mutations trigger AKT/ERK related ibrutinib resistance in WM.



Yang et al, Blood 2013; Blood 2016; Cao et al, Leukemia 2013; BJH 2014.

Responses to ibrutinib were impacted by MYD88 (L265P and non-L265P) and CXCR4 mutation status in a study of 63 previously treated patients (NCT01614821).

	MYD88^{MUT} CXCR4^{WT}	MYD88^{MUT} CXCR4^{WHIM}	MYD88^{WT} CXCR4^{WT}	p-value
N=	36	21	5	
Overall RR	100%	85.7%	60%	<0.01
Major RR	91.7%	61.9%	0%	<0.01

* 2 patients subsequently found to have other MYD88 mutations not picked up by AS-PCR

Treon et al, N Engl J Med. 2015; 372(15):1430-40; NEJM 2015; Letter, August 6, 2015.

Table 1. Baseline characteristics of patients.

	Age	BM Involvement %	IgM	IgA	IgG	Hb	Serum B_2M	Prior Therapies	Ratio of Relapsed / Refractory
MYD88$^{Mut/WT}$ CXCR4^{WT} (N=18)	64	80	4045	26	349	9.7	5.5	2	0.50
MYD88$^{Mut/WT}$ CXCR4^{Mut} (N=6)	67	60	3505	25	369	10.8	5.2	2	0.66
MYD88$^{Mut/Mut}$ (N=9)	61	70	3935	33	528	12.1*	3.0*	1	0.66

* $p<0.05$ vs. MYD88$^{Mut/WT}$CXCR4^{WT}; not significant for all other comparisons.

Impact of Mutated MYD88 zygosity and CXCR4 mutation status was assessed on ibrutinib response and progression free survival in 33 previously treated WM patients treated on a clinical trial (NCT01614821).

- Impact of MYD88 zygosity evaluated for 33/63 patients included in prospective study who had DNA available for MYD88 zygosity genotyping.
- 9/33 (27.3%) found to be homozygous for mutated MYD88.
- aUPD in 6; CNA in 2 (del MYD88 WT; add MYD88 Mut); 1 Unknown.
- CXCR4 mutations were highly prevalent among mutated MYD88 homozygous (8/9; 89%) vs. heterozygous (7/24; 29.2%) patients (p=0.014).

Mutated MYD88 zygosity impacts Major and VGPR responses in WM patients treated with ibrutinib.

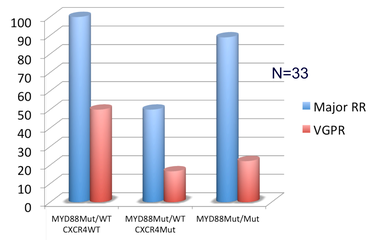
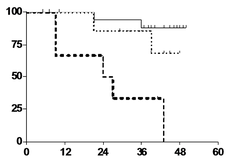


Figure 4. Response to ibrutinib among 33 previously treated MYD88 mutated WM patients who were genotyped for mutated MYD88 zygosity and CXCR4 mutation status. Major responders include all patients who achieved at least a partial response (≥50% reduction sIgM). VGPR denotes very good partial response (≥90% reduction sIgM).

Mutated MYD88 zygosity impacts PFS in WM patients treated with ibrutinib.



Median PFS was not reached for patients with MYD88$^{Mut/WT}$CXCR4^{WT} and MYD88$^{Mut/Mut}$, and was 25.5 months for those with a MYD88$^{Mut/WT}$CXCR4^{Mut} mutation status (Log-rank Chi-square: 14.74, p=0.0006).

Conclusions

- Mutated MYD88 homozygosity is more common in previously treated vs. untreated WM patients.
- Acquired UPDs more often contribute to mutated homozygosity, though copy number alterations can also occur, i.e. addition of mutated MYD88 or deletion of wild-type MYD88.
- Mutated MYD88 homozygosity is associated with mutated CXCR4.
- The presence of homozygous MYD88 confers better major and VGPR responses and PFS outcome vs. heterozygous MYD88 despite presence of concurrent CXCR4 mutations.

Acknowledgements

Supported by Gifts and Grants from

- Peter Bing M.D.
- International WM Foundation
- Leukemia and Lymphoma Society
- Bauman Family Foundation
- Kerry Robertson Family Foundation
- Orzag Family Foundation
- NIH Spore Grant Development Award
- Clinical trial was supported by Pharmacyclics Inc., Janssen Pharmaceuticals Inc.