## W36: TP53 mutations are associated with mutated MYD88 and CXCR4, and confer an adverse outcome in Waldenström macroglobulinemia.

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**Introduction:** Whole genome sequencing has identified highly recurrent somatic mutations in Waldenström macroglobulinemia (WM). Activating somatic mutations in MYD88 and CXCR4 are present in 90-95% and 30-40% of WM patients, respectively, and impact disease presentation, treatment outcome, and overall survival (Treon *et al*, NEJM 2012; Hunter *et al*, Blood 2014; Treon *et al*, Blood 2014; Treon *et al*, NEJM 2015). In contrast, the impact of somatic mutations in the tumor suppressor gene TP53 are less well understood. A recent study observed TP53 mutations or deletions in 7% of WM patients, and were associated with shorter overall survival (Poulain *et al*, CCR 2017). We sought to further characterize the clinical implications as well as the clonal architecture of TP53 mutations in WM.

**Methods:** We searched our database for WM patients with a TP53 mutation identified by a clinical next generation sequencing (NGS) assay using unsorted bone marrow samples (Kluk *et al*, J Mol Diagn 2016). To validate the findings, CD19+ cells from BM aspirates were isolated, and DNA was extracted and used for mutational analysis. CD19-depleted peripheral blood mononuclear cells were used as normal paired samples. All samples were screened for MYD88, CXCR4, and TP53 mutations by Sanger sequencing, and zygosity was determined by establishing the ratio of mutant versus wild-type (WT) allele expression. TP53 copy number was determined using TaqMan Copy Number Assays (Applied Biosystems, Grand Island, NY, USA).

**Results:** Thirteen WM patients (13/265; 4.9%) had a TP53 mutation identified by a clinical NGS assay. Sanger sequencing identified somatic TP53 mutations within the WM clones in six patients, including one patient with two somatic mutations (WM1). Three patients had a TP53 mutation identified in both CD19+ and CD19- tissues, and 4 patients were WT. The clinical and

genetic characteristics of WM patients with validated somatic TP53 mutations are shown in **Table 1.** All mutations were localized to the DNA-binding domain, and biallelic inactivation of TP53 was identified in four patients (67%). Three patients had a homozygous TP53 mutation determined by Sanger sequencing; one patient (WM1) had both a homozygous and heterozygous mutation. Copy number analysis was performed for five patients (all except WM3), and included the 4 patients with a homozygous TP53 mutation. TP53 deletion was only detected in one patient (WM6) with mutated TP53, suggesting an involvement of acquired uniparental disomy in TP53 loss of heterozygosity in WM. All six patients had both a CXCR4 mutation (4 nonsense, 2 frameshift) and the MYD88 L265P mutation, of which 4 patients (67%) had homozygous mutated MYD88. At the time of the TP53 mutation was detected, three patients were untreated, two patients were refractory to their most recent therapy, and one patient was relapsing. Three patients were treated with ibrutinib, including two as salvage therapy, and all obtained a major response. After a median follow-up of 18 months, 2 patients (33%) have died due to progressive disease; both patients had biallelic inactivation of TP53.

**Conclusion:** Somatic TP53 mutations are uncommon but can confer an aggressive disease course in WM. TP53 mutations occur concurrently with both MYD88 and CXCR4 mutations, and ibrutinib showed activity in patients carrying all three mutations. Our findings highlight the need to understand the cell-specific origin of mutations detected by clinical NGS assays.

**Table 1.** Clinical characteristics of WM patients with validated somatic TP53 mutations.

| | WM1 | WM2 | WM3 | WM4 | WM5 | WM6 |
|---|---|---|---|---|---|---|
| **Age** | 71 | 63 | 58 | 39 | 63 | 66 |
| **Bone marrow (%)** | 90 | 35 | 50 | 80 | 80 | 90 |
| **Serum IgM (mg/dl)** | 2,476 | 7,005 | 1,429 | 10,020 | 1,130 | 2,539 |
| **Hemoglobin (g/dl)** | 8.3 | 11.0 | 14.6 | 5.0 | 10.0 | 8.1 |
| **Treatment status** | Relapsed | Untreated | Refractory | Untreated | Untreated | Refractory |
| **Prior therapies** | CDR, BDR | N/A | BDR | N/A | N/A | BDR |
| **Treatment (response)** | Benda-R (NR), ibrutinib (PR) | Ibrutinib (PR) | None | BDR (NR), venetoclax (MR), ibrutinib (PR) | IDR (PR) | None |
| **MYD88 L265P** | Mutated; hom | Mutated; hom | Mutated; het | Mutated; hom | Mutated; het | Mutated; hom |
| **CXCR4 WHIM** | Mutated (S338X) | Mutated (S338X) | Mutated (K331fs) | Mutated (S339fs) | Mutated (S338X) | Mutated (S338X) |
| **Survival** | Dead; 15.4 months | Alive; 23.1 months | Alive; 10.4 months | Alive; 31.2 months | Alive; 20.8 months | Dead; 2 weeks |

CDR: cyclophosphamide, dexamethasone, rituximab; BDR: bortezomib, dexamethasone, rituximab; hom: homozygous; het: heterozygous; fs: frameshift; Benda-R: bendamustine, rituximab; IDR: ixazomib, dexamethasone, rituximab; NR: no response; MR: minor response; PR: partial response