TP53 mutations are associated with mutated *MYD88* and *CXCR4*, and confer an adverse outcome in Waldenström macroglobulinemia

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Background

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; Blood 2014; NEJM 2015; BJH 2018; Hunter *et al*, Blood 2014). In contrast, the impact of somatic mutations in the tumor suppressor gene *TP53* are less well understood. Poulain *et al* (2017) observed *TP53* mutations or deletions in 7% of WM patients, which were associated with shorter overall survival. We sought to further characterize the clinical implications as well as the clonal architecture of *TP53* mutations in WM.

Patients and Methods

We searched our database for WM patients with a *TP53* mutation identified by a routine clinical next-generation sequencing (NGS) assay using unsorted bone marrow (BM) samples. The median average coverage of the samples was 1604X (range 701-3707X), and 91.1% of amplicons had >200X coverage, consistent with the reported performance of this clinical NGS assay (Kluk *et al*, J Molec 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 (PB) 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).

Results

Thirteen WM patients (13/265; 4.9%) had a *TP53* mutations identified by the clinical NGS assay (**Table 1**). Sanger sequencing identified somatic *TP53* mutations within the WM clones in six patients (6/265; 2.3%), including both untreated (3/116; 2.6%) and previously treated patients (3/149; 2.0%). One patient had two somatic *TP53* mutations identified. Three patients (3/265; 1.1%) had a *TP53* mutation identified in both CD19+ and CD19- tissues, and 4 were wild-type (4/265; 1.5%). No recurrent variants were identified.

Results

Table 1. *TP53* mutations identified by the clinical next-generation sequencing assay.

Patient	Nucleotide change	Amino acid change	Variant allele fraction (%)	Total number of reads	Sanger sequencing	
					CD19+ BM	CD19- PB
WM1	574 C>T; 916 C>T	Q192; R306	15.0; 39.8	878; 379	Present	WT
WM2	833 C>G	P278R	4.9	509	Present	WT
WM3	584 T>C	I195T	11.1	878	Present	WT
WM4	488 A>G	Y163C	8.5	118	Present	WT
WM5	586 C>T	R196	56.1	239	Present	WT
WM6	722 C>T	S241F	46.6	476	Present	WT
WM7	289 G>C	V97L	41.2	182	Present	Present
WM8	847_847insGGG	282_283insG	32.3	690	Present	Present
WM9	704 A>G	N235S	44.4	563	Present	Present
WM10	659 T>C	Y220C	31.2	955	WT	WT
WM11	701 A>G	Y234C	8.5	791	WT	WT
WM12	745 A>G	R249G	3.2	568	WT	WT
WM13	843 C>A	D281E	5.1	431	WT	WT

The clinical and genetic characteristics of WM patients with validated somatic *TP53* mutations are shown in **Table 2.** All mutations were localized to the DNA-binding domain. 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 *TP53* 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*.

Table 2. 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 involvement (%)	90	35	50	80	80	90
Serum IgM (mg/dl)	2,476	7,005	1,429	10,020	1,130	2,539
Hemoglobin level (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; homozygous	Mutated; homozygous	Mutated; heterozygous	Mutated; homozygous	Mutated; heterozygous	Mutated; homozygous
***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

Abbreviations: BDR, bendamustine, dexamethasone, rituximab; Benda-R, bendamustine, rituximab; CDR, cyclophosphamide, dexamethasone, rituximab; CR, complete response; IDR, ibrutinib, dexamethasone, rituximab; MR, minor response; NR, no response; PR, partial response

Results

Patients with somatic *TP53* mutations exhibited an aggressive disease course. Three patients were untreated, 2 patients were refractory to their most recent therapy (bortezomib, dexamethasone, rituximab [BDR]), and one patient was relapsing. Two patients went on to receive frontline therapy with ibrutinib, and ixazomib, dexamethasone, and rituximab (IDR), respectively; both patients obtained a partial response. Patient WM1 was refractory to bendamustine and rituximab, and then responded to nearly one year before relapsing. Patient WM3 was refractory to both BDR and venetoclax, and was subsequently salvaged with ibrutinib therapy. Patient WM6 died after being refractory to frontline therapy with BDR. 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 in WM patients, and ibrutinib showed activity in patients carrying all three mutations. Prospective evaluation of ibrutinib and other novel therapies accounting for *TP53* mutation status are needed in WM.