bjh short report

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

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Summary

Little is known about *TP53* mutations in Waldenström Macroglobulinaemia (WM). We evaluated 265 WM patients for *TP53* mutations by next-generation sequencing, and validated the findings by Sanger sequencing. *TP53* mutations were identified and validated in 6 (2·6%) patients that impacted the DNA-binding domain. All six were *MYD88*- and *CXCR4*-mutated. Ibrutinib showed activity in patients carrying all three mutations. With a median follow-up of 18 months, 2 (33%) with biallelic *TP53* inactivation died of progressive disease. *TP53* mutations are rare in WM, and associate with *MYD88* and *CXCR4* mutations. WM patients with *TP53* mutations show response to ibrutinib.

Keywords: Waldenström macroglobulinaemia, *TP53*, *CXCR4*, *MYD88*, ibrutinib.

Whole genome sequencing has identified highly recurrent somatic mutations in Waldenström macroglobulinaemia (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*, 2012, 2014, 2015; Hunter *et al*, 2014). In contrast, the impact of somatic mutations in the tumour 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. Herein, we sought to further characterize the clinical implications as well as the clonal architecture of *TP53* mutations in WM.

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 1604× (range 701–3707×), and 91·1% of amplicons had >200× coverage, consistent with the reported performance of this clinical NGS assay (Kluk *et al*, 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, USA).

Thirteen WM patients (13/265; 4·9%) had a *TP53* mutation identified by the clinical NGS assay (Table I). 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 patients were WT (4/265; 1·5%). No recurrent variants were identified.

The clinical and genetic characteristics of WM patients with validated somatic *TP53* mutations are shown in Table II. All

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Table I. *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

BM, bone marrow; PB, peripheral blood; WT, wild type.

mutations were localized to the DNA-binding domain. Biallelic inactivation of *TP53* was identified in four patients (67%). Three patients had a homozygous *TP53* mutations 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*.

Clinically, patients with somatic *TP53* mutations exhibited an aggressive disease course. At the time the *TP53* mutation was detected, the median BM involvement and haemoglobin level was 80% and 92 g/l, respectively. The median serum IgM level was 25·08 g/l, and two patients had symptomatic hyperviscosity. 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 ibrutinib for nearly 1 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*.

The finding of mutated *CXCR4* in all six patients with somatic *TP53* mutations contrasts with those by Poulain *et al* (2017), who observed no relationship between *TP53* and *CXCR4* mutations in WM patients. The concurrent finding of *CXCR4* and *TP53* mutations may indicate an underlying genomic instability. We previously reported a similar observation in ibrutinib-resistant WM patients wherein *BTK* C481S mutations were acquired mainly in patients with *CXCR4* mutations (Xu *et al*, 2017). The presence of *CD79B* mutations also appears to be nearly exclusive to *CXCR4*-mutated patients, and was associated with histological transformation to diffuse large B-cell lymphoma in a small series (Alonso *et al*, 2016). These results may indicate an increased susceptibility of patients harbouring *CXCR4* mutations to acquire adverse genetic mutations. *CXCR4* mutations are primarily subclonal in WM patients, suggesting their acquisition after mutated *MYD88*, although this may occur early in WM pathogenesis given their detection in patients with IgM MGUS (Xu *et al*, 2016). We were unable to address in this study the temporal acquisition of mutations in *TP53* relative to *CXCR4*, or whether *TP53* mutations occurred within *CXCR4*-mutated cells. Prospective longitudinal studies using single cell tumour sequencing will be needed to clarify these points.

Few studies have addressed the relationship between somatic *TP53* mutations and 17p deletion [del(17p)] in WM patients. We observed five patients (83%) with a somatic *TP53* mutation and no concurrent del(17p). Previous studies have also shown 40–50% of WM patients have *TP53* mutations without an accompanying deletion (Hunter *et al*, 2014; Poulain *et al*, 2017). The rate of del(17p)was not available for our cohort, but has been reported in approximately 8% of WM patients (Nguyen-Khac *et al*, 2013). Given the low mitotic index of tumour cells and lack of disease-defining abnormalities, cytogenetic analysis is not routinely recommended in patients with WM (Castillo *et al*, 2016).

An important observation from this study is the association between *MYD88*, *CXCR4* and *TP53* mutations. While there is no current evidence that management should be tailored for *TP53*-mutated WM patients, *MYD88* and *CXCR4* mutation status can be useful for treatment selection. Ibrutinib is the

Table II. 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 (g/l)	24·76	70·05	14·29	100·2	11·3	25·39
Hemoglobin level (g/l)	83	110	146	50	100	81
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

BDR, bortezomib, dexamethasone, rituximab; Benda-R, bendamustine, rituximab; CDR, cyclophosphamide, dexamethasone, rituximab; fs, frameshift; het, heterozygous; hom, homozygous; IDR, ixazomib, dexamethasone, rituximab; MR, minor response; N/A, not available; NR, no response; PR, partial response.

first agent approved by the U.S. Food and Drug Administration and the European Medicines Agency for the treatment of WM. Patients with *MYD88* WT showed an absence of major responses to ibrutinib versus patients with mutated *MYD88*. Moreover, the presence of *CXCR4* mutations adversely impacted response rates and kinetics as well as progression-free survival, although higher major response rates were observed for *CXCR4*-mutated patients with homozygous versus heterozygous mutated *MYD88* (Treon *et al*, 2015, 2016). The three *TP53*-mutated patients treated with ibrutinib in this study had both a *CXCR4* mutation and carried homozygous mutated *MYD88*, and all attained a major response. Furthermore, *in vitro* studies have shown that ibrutinib can induce apoptosis in WM cells independent of *TP53* mutation status (Poulain *et al*, 2017). Ibrutinib may therefore represent an optimal therapeutic approach for bypassing the defective TP53 (p53) pathway and overcoming chemoresistance in *TP53*-mutated WM patients.

Clinical NGS assays are increasingly being performed for patients with haematological malignancies to identify mutations that impact treatment selection and prognosis. These assays often provide improved sensitivity over Sanger sequencing, and may permit detection of mutations with low variant allele frequency (VAF), as evidenced by the four discordant patients in this study. In addition, clinical NGS assays typically use unsorted samples, which is ideally suited for clinical laboratories because cell sorting can be time consuming and cost-additive. Three untreated WM patients with *TP53* mutations in the present study detected by the clinical NGS assay were determined to have such mutations in both $CD19^+$ and $CD19^-$ sorted samples by Sanger sequencing, and may represent germline variants of *TP53*. Alternatively, variants in *TP53* have been identified in the age-related condition clonal haematopoiesis of indeterminate potential (CHIP) (Steensma *et al*, 2015). A CHIP-associated mutation in *DNMT3A* was also identified in one patient by the clinical NGS assay. The incidence and clinical implications associated with CHIP in WM patients remains to be delineated. However, the presence of CHIP in the background of previously treated lymphoma patients has been shown to increase the risk of therapy-related myeloid neoplasms (TMN) following autologous stem cell transplantation (Gibson *et al*, 2017). Prospective studies assessing the risk for TMN after cytotoxic chemotherapy in the context of CHIP status in WM patients would be illuminating, and may permit the identification of patients in whom such therapy should be avoided. Nonetheless, these findings highlight the need to understand the cell-specific origin of mutations identified by clinical NGS assays.

In summary, 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.

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Author contributions

JG, ZRH, SPT, and LX conceived and designed the experiments, and wrote the manuscript. JG, ZRH, SPT, LX, GGC performed data analysis. NT, MGD, AK, LX performed the sequencing studies. JJG, XL, MM, MLG, GY prepared samples. KM, TD, JG, CJP, JJC, SPT provided patient care, obtained consent and were responsible for sample collection.

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