Investigation into the deletion or inactivation of the TP53 gene in Waldenström Macroglobulinaemia (WM) has been limited; the current literature shows a low proportion of patients presenting with TP53 variation at diagnosis (~8%) (Nguyen-Khac et al, 2013; Hunter et al, 2014; Poulain et al, 2017).

In chronic lymphocytic leukaemia (CLL), small TP53 mutated subclones can expand over time under selective therapy pressures, creating chemorrefactoriness and a more aggressive clinical phenotype (Landau et al, 2013). Conventional chemo-immunotherapy is still indicated for most WM patients (Leblond et al, 2016) but recently, Poulain et al (2017) highlighted TP53 as a potential prognostic tool, indicating that TP53 independent therapy, would be the treatment of choice for WM patients harbouring TP53 variation. Gustine et al (2018) have also shown that TP53 mutations are associated with mutated MYD88 and CXCR4, and confer an adverse outcome in WM.

This study aimed to evaluate temporal TP53 gene variation at clinically relevant time points and investigate associations with clinical features and treatment. Fourteen WM patients who had sequential sample time points (n = 58), at clinical presentation and subsequent intervals were analysed. All patients were analysed for TP53 variation using targeted next generation sequencing (NGS) on genomic DNA extracted from bone marrow, peripheral blood and saliva. Patient characteristics and clinical information, including therapy and MYD88L265P/CXCR4S338X status, was also assessed (Table 1).

Four TP53 variants were identified in 4 patients at subsequent time points. Of the variants found, 2 were exonic and 2 were intronic, with a 4.25–48.57% range of variant allele frequency (VAF) (Table S1). Exonic variants impacted the DNA-binding domain, one was classed as pathogenic (Fig 1A) and one was probably pathogenic according to the TP53 database (Fig 1B). Intronic variants were detected in intron 9; one was considered to probably be benign due to limited evidence (Fig 1C) and one showed evidence of a common (Minor Allele Frequency = 2%) non-pathogenic constitutional variant (Fig 1D) (Soussi et al, 2014). This was confirmed in the constitutional DNA of this patient. The other three variants were confirmed as somatic; negative at diagnosis, observed post-conventional chemotherapy and present in at least 2 subsequent time points (Fig 1E).

Unlike Gustine et al (2018), two of the four patients with a TP53 variant were negative for MYD88L265P and both these patients have become refractory to therapy with Bruton tyrosine kinase inhibitor (BTKi). One harbours a deleterious TP53 variant, which was detected prior to BTKi therapy, and is now responding to therapy with venetoclax (a BCL2 inhibitor); the other is the constitutional variant and is now stable post-chemoimmunotherapy. The other 2 patients with a TP53 variant are currently stable on BTKi therapy.

TP53 variants were also detected in cell-free DNA (cfDNA) extracted from plasma samples in 3 of the 4 patients. One variant was undetectable, probably due to receiving additional therapy at the sample time point. Sanger sequencing was performed on 20 samples and, as expected, only the constitutional variant was confirmed, based on the VAF of >10%. In addition, samples of the 4 TP53 positive cases were also sent for external validation and 3/4 variants were validated using alternative NGS technology; the constitutional variant could not be validated due to insufficient DNA.

Univariate analysis of all patients found no association between mutated TP53 and overall survival, time to first treatment, MYD88L265P or CXCR4S338X mutation status, IGHV gene mutational status or age. However, when analysed in those patients on BTKi therapy, a positive TP53 and negative MYD88L265P was significantly associated with progression (P = 0.040). Multivariate analysis showed a similar trend towards significance; however, due to cohort size, this did not reach significance (P = 0.062).

In current clinical practice, TP53 testing is not regularly performed and WM patients received chemoimmunotherapy and BTKi regardless of TP53 status. However, here 2 patients with a TP53 variant (1 exonic, 1 intronic) have progressed...
whilst on BTKi therapy, both these patients are also MYD88L265P negative and ibrutinib, the first generation BTKi, was shown to be insufficient in MYD88L265P negative patients (Yang et al, 2013). Whilst TP53 may not be the contributing factor to therapy resistance, it may be an indicator of genomic instability. This suggests that additional mutation

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Fig 1. (A–D) The development of TP53 variation by variant allele frequency (VAF), over the time course of the disease, for each patient where a TP53 variant was detected. Arrows show therapy details relative to TP53 detection. The blue line at 10% VAF indicates the Sanger sequencing detection limit. Patient numbers correlate with the following swimmer’s plot. (E) A swimmer’s plot to show each patient’s (n = 14) disease course follow up, including treatment (circles) and sample time points tested (triangles). Chemotherapy subtypes include alkylating agents (chlorambucil, cyclophosphamide and bendamustine), purine analogues (fludarabine) or combinations excluding rituximab. Chemoimmunotherapy refers to all treatment combinations including rituximab. Venetoclax has only been given to 1 patient post-BTK inhibition (patient 13). BR, bendamustine, rituximab; BTKi, Bruton tyrosine kinase inhibitor; Chl, chlorambucil; DCR, dexamethasone, cyclophosphamide, rituximab; FCR, fludarabine, cyclophosphamide, rituximab; RCHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone. [Colour figure can be viewed at wileyonlinelibrary.com]
testing may be required for BTK and PLCG2, both of which have been shown to drive resistance to BTKi therapy in WM (Xu et al., 2017).

This pilot study used targeted NGS to test for TP53 variation in a sequential cohort not previously undertaken in WM patients. Despite the small cohort size, a variant was detected in 4/14 (29%) patients. The development of TP53 variation over time indicates that TP53 gene testing should be performed at presentation and prior to each line of therapy to guide therapy choice and identify genetically high-risk patients. This could be performed as part of a panel to include the pertinent genes involved in WM, such as MYD88, CXCR4 and BTK. This study has also shown that targeted NGS has a greater potential for use in a clinical laboratory, overcoming the limitations of Sanger sequencing and the successful detection of these variants in cfDNA taken from peripheral blood, shows that liquid biopsies can be used to monitor patients for TP53 variation. This would enable regular monitoring of patient disease whilst eliminating the need for repetitive invasive bone marrow testing. Further longitudinal studies of these patients in a larger multicentre setting are required to further elucidate the incidence and significance of acquired TP53 variation.

Competing interests
The authors have no competing interests.

References


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