# Sequential analysis of TP53 variation using targeted Next Generation Sequencing (NGS) in patients with Waldenström Macroglobulinaemia

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## Background

Despite being the most commonly mutated gene in human cancers, investigation into the deletion or inactivation of the *TP53* gene in Waldenström Macroglobulinaemia (WM) has been limited, particularly in regards to changes over the course of the disease. Literature to date shows only approximately 8% of patients present with *TP53* variation, with no publications analysing *TP53* status in sequential samples.

## Aims

The aims of this study were to characterise *TP53* gene variation at clinically relevant time points using targeted NGS technology and to investigate associations with other clinical features and treatment status.

## Materials & Methods

Targeted NGS was performed on all coding exons plus some surrounding intronic regions of the *TP53* gene, using an Ion Torrent™ Personal Genome Machine™. Fifty-nine samples, taken at varying time points from 16 WM patients, were analysed. DNA was extracted from bone marrow, peripheral blood and plasma samples from patients followed up in the WM clinic at Royal Bournemouth Hospital. Constitutional DNA was extracted from 4 available saliva samples to confirm mutations were somatically acquired. Sanger sequencing was performed on 20 samples to evaluate the NGS findings. IBM® SPSS Statistics® software was used for statistical analysis.

## Results

*TP53* variants were detected in 4/16 patients (25%) at multiple time points, with 3/4 having a negative result for a prior time point. Variant allele frequency (VAF) ranged from 4.24-48.57%, with 2 exonic and 2 intronic variants detected. Saliva samples were available for 3/4 patients with variants and sequencing analysis confirmed that mutations were somatically acquired. Sanger sequencing was unable to validate all results likely due to low VAF. Three out of 4 variants were validated using different NGS technology at an external facility, with 1 failure due to insufficient DNA.

There was no association found between mutated *TP53* and OS, TTFT, *MYD88*$^{L265P}$ mutation status, IGHV gene mutational status or age. However, in patients who have had 3 lines of treatment or more, a *TP53* variant was more likely to be detected (Fisher's Exact test p=0.019) (Figure 1). Two out of the 4 patients with a *TP53* variant (1 intronic) have progressed whilst on Acalabrutinib and have started alternative therapies.

## Conclusion

The results from this novel sequential sample cohort show that *TP53* variation can develop and change over the disease course in WM patients. This implies that *TP53* gene testing should be performed both at diagnosis and at clinically relevant time points in order to direct

appropriate therapy for each patient. This study also provides data to support trial design using therapies targeting *TP53* independent pathways, earlier in the disease history, including frontline to avoid expansion of subclonal *TP53* disrupted clones. The use of targeted NGS for *TP53* testing improves upon existing techniques, offering a highly sensitive method to use in a routine clinical laboratory, detecting subclonal variants that otherwise would have been missed by Sanger sequencing.

**Future work**

Further cases will be required to extend and confirm these findings and *TP53* functional analysis is necessary to confirm the significance of the intronic variants detected.

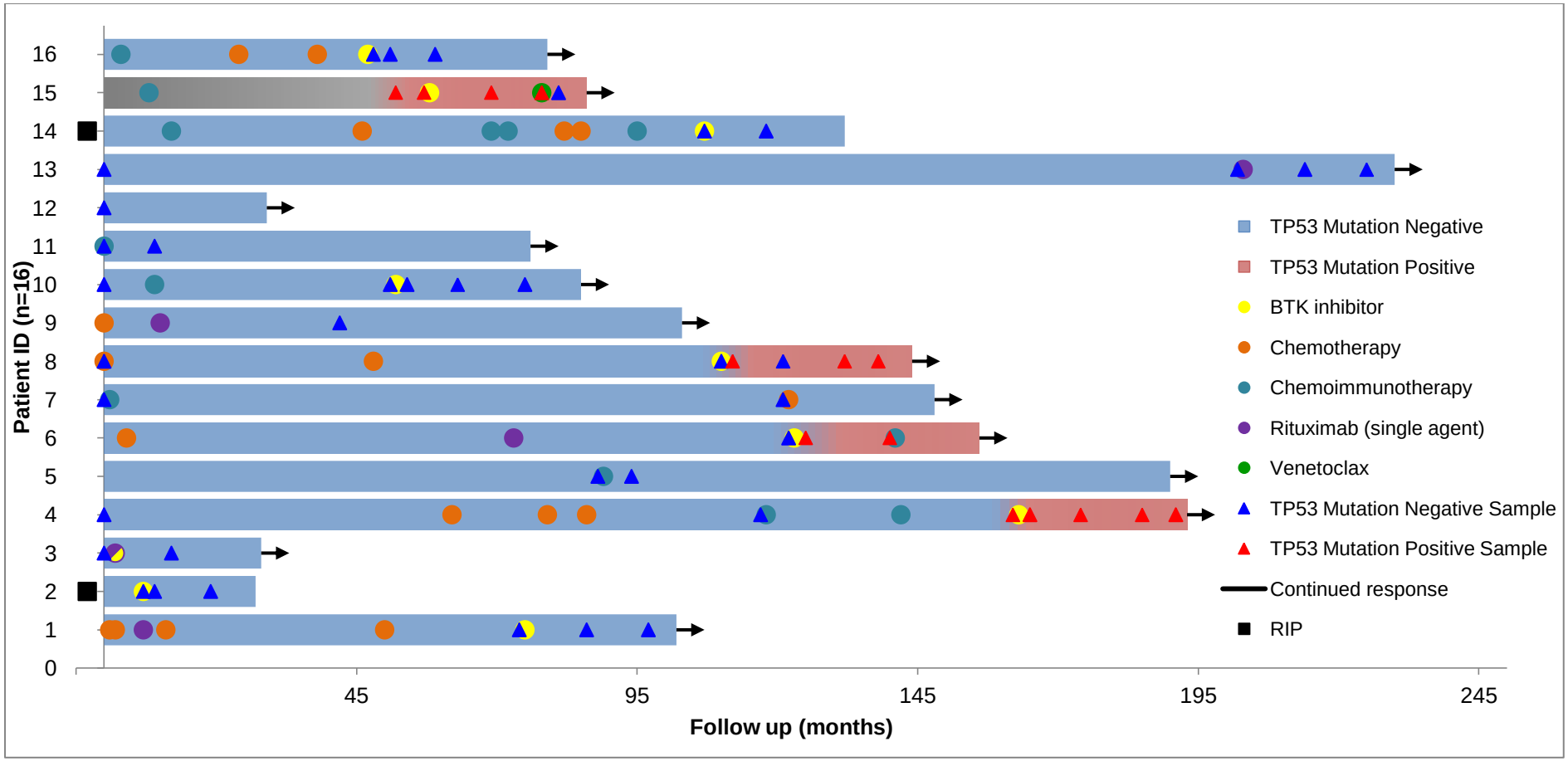


*Figure 1* – A swimmer's plot to show each patient's (n=16) disease course follow up, including treatment (circles) and sample time points tested (triangles). Samples tested were predominantly taken from bone marrow aspirate slides with some peripheral blood and plasma samples. Chemotherapy subtypes include alkylating agents (chlorambucil, cyclophosphamide and bendamustine), purine analogues (fludarabine) or combinations excluding Rituximab. Chemoimmunotherapy refers to all treatment combinations including Rituximab (FCR, DRC, RCHOP). The BTK inhibitor Acalabrutinib has been administered to 9 patients and Ibrutinib has been used in 1 patient (PID 3). Venetoclax has only been given to 1 patient post BTK inhibition (PID 15). PID 15 is highlighted grey where TP53 status is unknown prior to positive detection (due to a lack of diagnostic sample) but has been confirmed as somatic in nature.