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**Data Analytics of Treatment effects on Immune Response Pathways within the Tumour Microenvironment of Waldenström Macroglobulinaemia**

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Background

Bruton tyrosine kinase inhibitors (BTKi) represent a paradigm shift in Waldenström macroglobulinaemia (WM) management. BTK is involved in B-cell receptor & *MYD88* signalling, which are important pathways in WM pathogenesis. However, bidirectional communication between WM cells and their microenvironment is critical for tumour survival. The aim of this study was to assess the changes in the tumour immune microenvironment following treatment with BTKi or chemoimmunotherapy by a quantitative evaluation of gene expression.

Materials and methods

Immune gene expression was performed using the Oncomine Immune Response Research Assay (ThermoFisher™) on the Ion GeneStudio S5 System. Bone marrow samples were taken from matched patients with WM before and after treatment with BTKi or chemoimmunotherapy. All patients had the $MYD88^{L265P}$ mutation, confirmed by Multiplex Ligation-dependent Probe Amplification.

All samples which passed quality control (n=21) were compared. Principal Component Analysis showed substantial variance between samples extracted from formalin-fixed, paraffin-embedded (FFPE) bone marrow trephines and from bone marrow aspirates (BMA) irrespective of time point (pre or post treatment). These cohorts were therefore separated for subsequent analysis and selected for matched pairs (n=16). Statistical analyses of differential gene expression were performed using the Transcriptome Analysis Console software (ThermoFisher™); Ingenuity Pathway Analysis (IPA) (Qiagen) was used to analyse regulatory networks and pathways.

Results

Hierarchical clustering performed on 6 matched FFPE samples (n=3 patients) before and after treatment with BTKi showed differential gene expression (Figure 1), including upregulation of LEXM, involved in T cell differentiation and downregulation of B3GAT1, involved in NK cell activation. IPA identified NR3C1 and Tcf7 as likely upstream transcriptional regulators and CDKN2A as a likely negative regulator of gene changes caused by BTKi (Figure 2). Interestingly, findings from Ingenuity Knowledge Base imply *MYD88* as a potential regulator of Tcf7.

Separate hierarchical clustering on 4 matched BMA samples (n=2 patients) before and after treatment with BTKi showed upregulation of DGAT2 and downregulation of TNFRSF17 (B cell marker). IPA identified downregulation of TNFRSF17 and HLA-DOA expression within the dataset, predicted to be caused by inhibition of the upstream transcription regulator, NFKB2 (p-value 1.31E-04).

Finally, hierarchical clustering on 6 matched BMA samples (n=3 patients) before and after treatment with chemoimmunotherapy showed the most significant gene expression changes were upregulation of CDK1, involved in cell proliferation and downregulation of ZAP70, involved

in T cell receptor signalling. IPA identified genes from this dataset, which show a predicted activation and inhibition state within the network of upstream transcription regulators IRF1 and SP110, respectively.

Conclusion

This pilot study shows that BTKi and chemoimmunotherapy may have differential effects on the tumour microenvironment. Our results suggest multiple immune subsets (e.g. B cell, NK and T cell) within the microenvironment are likely altered by BTKi treatment. Of note, the BTKi treatment in the BMA cohort shows inhibition of NFKB and disrupts the previously identified pro-survival signalling activated by mutated MYD88.

The initial cohort of the study has been defined as part of the data pre-processing stage, in preparation for the deployment of multiple machine learning approaches. Nonetheless, these data provide a platform for a larger cohort study for future analyses.

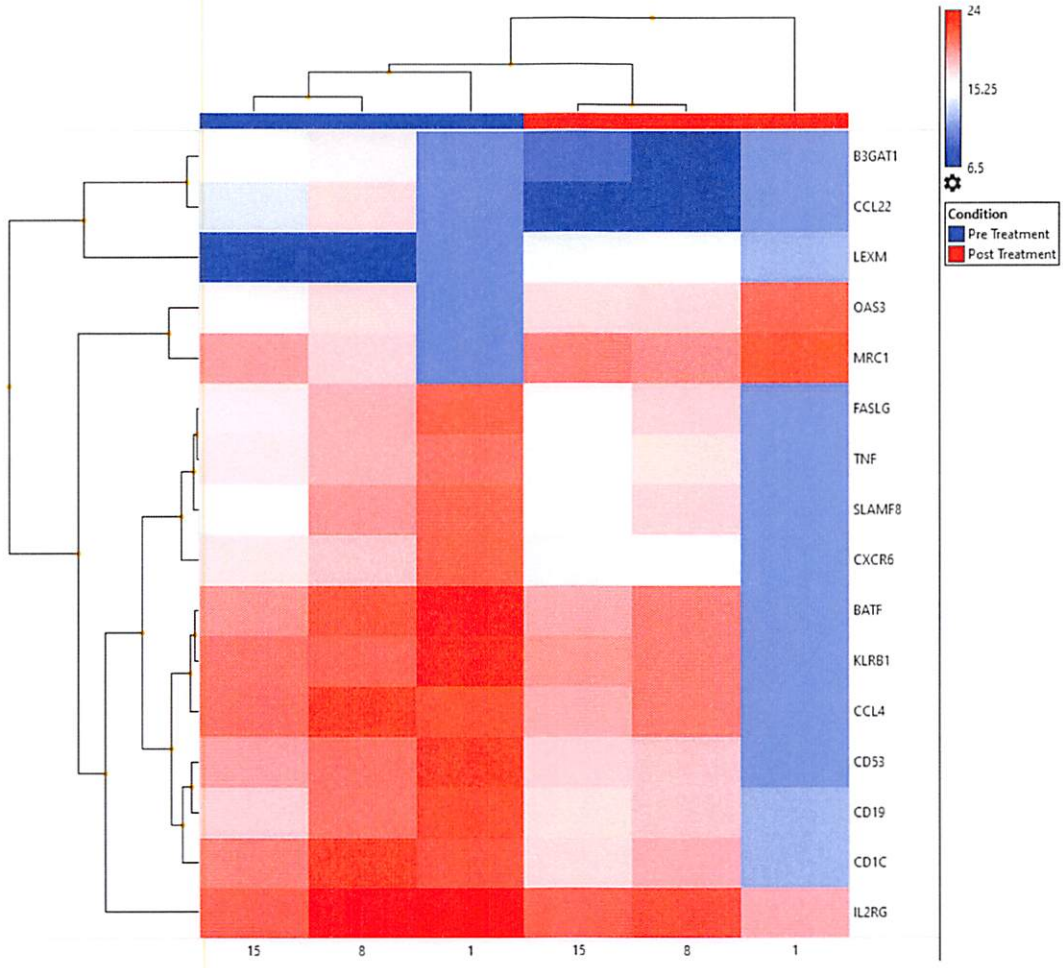


*Figure 1*

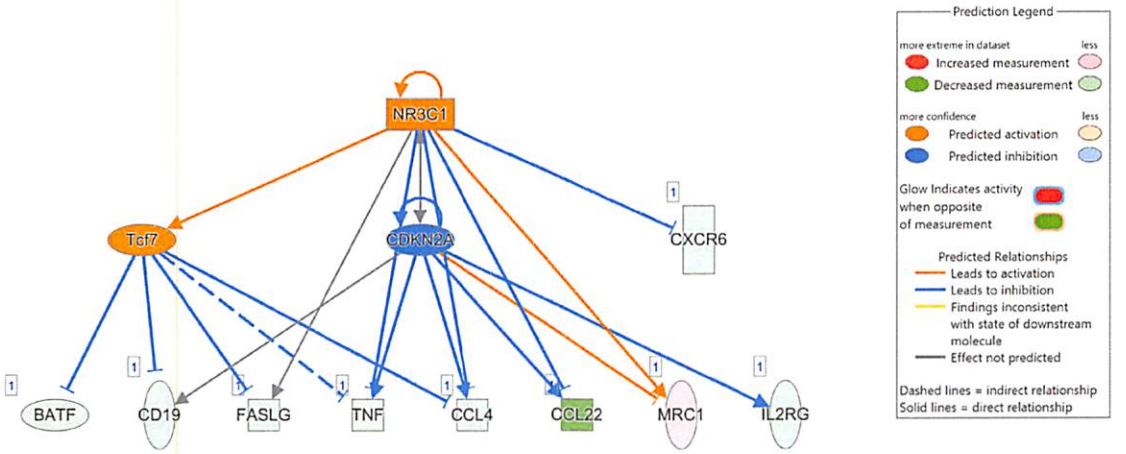


*Figure 2 - orange shows predicted activation, blue shows predicted inhibition.*