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**Introduction**

Ibrutinib is a Bruton tyrosine kinase (BTK) inhibitor highly active in Waldenström’s Macroglobulinemia (WM) patients. Despite that, disease progression can occur due to acquired mutations in BTK at the binding site of ibrutinib (Cys481), or in PLCG2, the protein downstream BTK (Xu, Blood 2017). However, not all ibrutinib resistant patients harbor these alterations.

The aim of this study was to identify alternative molecular mechanisms that can drive ibrutinib resistance.

**Methods**

- Five previously treated WM patients who progressed on ibrutinib (IBR1-IBR5):
  - IBR1-IBR3: Tumor DNA samples at diagnosis, relapse, and germline DNA
  - IBR4-IBR5: Relapse and germline samples
- Tumor DNA: CD19-selected bone marrow mononuclear cells
- Germline DNA: Non-CD19 cells from peripheral blood
- Data were analyzed following the Broad Institute’s GATK Best Practice Guidelines
- Small variants \(\rightarrow\) Strelka and MuTect2
- Copy number alterations \(\rightarrow\) Control-FREESC

**Copy number alterations**

CNA analysis identified del6q in all 5 patients, becoming homozygous in two of them at progression. Another patient demonstrated a subclonal homozygous deletion at baseline that increased at the time of disease progression. We also observed del8p in 4/5 patients at ibrutinib progression with the remaining patient having a microdeletion as well. No other recurrent CNA were detected (Figure 1).

**Small variants**

Regarding small variants, progression samples showed a high proportion of acquired vs. persistent mutations (median 87% vs. 13%), the former being more subclonal (MAF=8.4% vs. 13.9%). Among variants acquired at progression, BTKSTOP1808 (n=2), a ubiquitin ligase whose substrates are CXC4R, LYN or SYK; RNF19P (n=2), another ubiquitin ligase involved in STAT1-mediated transcriptional activity; FCRCLSTOP (n=1), a protein that modulates the innate immune signaling in B-cells; BIRC2STOP (n=1), a regulator of alternative NF-κB and MAPK signaling; and negative regulators of TLR signaling including TOLLIMOD (n=1), and DOK2STOP (n=1). In one patient, a truncating SYKSTOP at baseline was not detectable during progression (Figure 2).

**Conclusions**

Our whole exome sequencing study provides new important insights into clonal evolution associated with ibrutinib resistance in WM patients. Deletions on chromosomes 6q and 8p can accompany disease progression, a notable finding since these regions encompass many key regulators of BTK, MYD88/NF-κB, and apoptotic signaling. Moreover, we have also identified recurring mutations in ubiquitin ligases, innate immune signaling and TLR/MYD88 pathway regulators in ibrutinib resistant WM patients.

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