

## **Chromosome 6q deletions are common in Waldenström's Macroglobulinemia, and target regulatory genes for MYD88, CXCR4 and BCL2 signaling.**

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**Introduction:** Activating mutations in MYD88 mutations are highly prevalent (>90%) in Waldenström's Macroglobulinemia (WM), and trigger pro-survival NFκB signaling. Activating mutations in CXCR4 are also present in 30-40% of WM patients, trigger pro-survival AKT and ERK1/2 signaling, and are associated with both *in vitro* and clinical resistance to ibrutinib. Deletions involving the long arm of chromosome 6 (chr. 6q) are a common abnormality in WM, and include genes that modulate NFκB activity (TNFAIP3, HIVEP2), BCL2 family of proteins (BCLAF1), apoptosis (FOXO3), and BTK (IBTK), the target of ibrutinib. The impact of chr. 6q deletions on the expression of these critical survival determining genes remains unclear in WM.

**Patients and Methods:** Copy number alterations were measured in quadruplicate and gene expression in triplicate using TaqMan real-time polymerase chain reaction assays. DNA and RNA from CD19+ sorted bone marrow lymphoplasmacytic cells from 24 untreated WM patients and one patient with IgM and IgG-secreting lymphoplasmacytic lymphoma (LPL) were analyzed. Paired CD19-depleted peripheral-blood mononuclear cells (PBMCs) were used as germline controls. Paired CD19+ and CD19- PBMCs from 6 healthy donors were used to rule out possible B-cell specific findings. Based on location, function and existing literature, the genes IBTK, FOXO3, BCLAF1, TNFAIP3, HIVEP2 were selected for assessment. The WM patients included 18 males and 7 females, with a median age of 62 (range 35-91) years, median bone marrow involvement of 72% (range 20%-90%) and median serum IgM levels of 3510 (range 598-6910) mg/dl. All patients harbored MYD88 L265P mutations and 11 (44%) also carried somatic CXCR4<sup>WHIM</sup> activating mutations. The tumor/germline (T/G) copy number ratio was used to estimate the clonality of observed deletions. Monoallelic deletions affecting less than 20% of cells were considered to be beneath the threshold of detections for the PCR assays.

**Results:** CNA assays revealed absence of copy number changes in both B and non B-cell compartment for all the healthy donors. Somatic acquired fully clonal deletions in chr. 6q were observed in 8/25 (32%) patients. Fully clonal chr. 6q deletions spanned all studied genes in 7/8 (87.5%) patients; in one patient, FOXO3 remained fully intact. Subclonal deletions in chr. 6q were observed in 5 (20%), 6 (24%), 11 (44%), 7 (28%) and 5 (20%) of the 25 patients in IBTK, FOXO3, BCLAF1, TNFAIP3 and HIVEP2, respectively. The median T/G ratio of these deletions was 0.83 (range 0.64-0.89) corresponding to a monoallelic deletion affecting 32% of LPL cells. Unlike patients with fully clonal chr. 6q deletions whose deletions spanned all 5 studied genes, those patients with subclonal 6q deletions only had deletions that spanned all 5 study genes in 3/12 (25%) cases (Figure 1; p=0.02). Gene expression assays for all 25 patients showed significant decreases in the

transcriptional levels of IBTK, BCLAF1 and HIVEP2 that corresponded to the clonal deletions observed for their respective gene ( $p= 0.03, 0.01$  and  $0.01$ , respectively). In contrast, gene expression levels for TNFAIP3 and FOXO3 did not show alterations that corresponded to their respective CNA findings. CXCR4 mutations were absent in all 8 patients with fully clonal chr. 6q deletions. In contrast, CXCR4 mutations were present in 9/12 (75%) patients with subclonal chr. 6q deletions, and 2/5 (40%) patients with no chr. 6q deletions ( $p=0.001$ ).

**Conclusions:** Our findings demonstrate that gene loss of IBTK, FOXO3, BCLAF1, TNFAIP3 and HIVEP2 occurs in most patients with WM, including in patients with fully deleted chr. 6q, and those patients with subclonal chr. 6q deletions though gene loss patterns can differ. Despite clonal copy number losses in all 5 genes, gene expression was reduced for only IBTK, BCLAF1, and HIVEP2 suggesting that regulatory mechanisms may compensate for FOXO3 and TNFAIP3 gene loss. CXCR4 mutations were absent in chr. 6q fully deleted patients, but common in those with subclonal chr. 6q or no chr. 6q deletions. The findings provide valuable insights into WM pathogenesis, and may be relevant to understanding therapeutic outcome with agents that target MYD88, CXCR4 and BCL2.

Figure 1.

