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 aberration in WM, and include genes that modulate NFκB activity (TNFAIP3, HIVEP2, IBTK), 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

CNAs were measured in quadruplicate and gene expression in triplicate using TaqMan real-time polymerase chain reaction (RT-PCR) assays. DNA and RNA from CD19+ sorted bone marrow lymphoplasmacytoid cells from 24 untreated WM patients and 1 patient with IgM and IgG-secreting lymphoplasmacytoid lymphoma (LPL) were analyzed (Patient characteristics are listed in Table 1). 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.

TABLE 1: PATIENT CHARACTERISTICS

<table>
<thead>
<tr>
<th>Gender</th>
<th>n (%)</th>
<th>MYD88 L265P, n (%)</th>
<th>Mutated</th>
<th>%</th>
<th>Male</th>
<th>18 (72%)</th>
<th>Wild-type</th>
<th>25 (100%)</th>
<th>0 (0%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>7 (28%)</td>
<td>Wild-type</td>
<td>0 (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years), median (range)</td>
<td>62 (35-91)</td>
<td>62 (35-91)</td>
<td>62 (35-91)</td>
<td></td>
<td>11 (44%)</td>
<td></td>
<td>11 (44%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% BM Involvement, median (range)</td>
<td>72% (20%-90%)</td>
<td>72% (20%-90%)</td>
<td>72% (20%-90%)</td>
<td></td>
<td>Mutated</td>
<td>3 (12%)</td>
<td>11 (44%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum IgM Levels mg/dL, median (range)</td>
<td>3510 (598-6910)</td>
<td>3510 (598-6910)</td>
<td>3510 (598-6910)</td>
<td></td>
<td>Wild-type</td>
<td>15 (56%)</td>
<td>15 (56%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The median tumor/germline ratio of the subclonal deletions was 0.83 (range 0.64-0.89) corresponding to a monomorphic deletion affecting 34% of LPL cells. Clonality of the deletions was strongly associated with CXCR4 mutations status.

RESULTS: CNA PATTERNS CORRESPOND TO CXCR4 MUTATION STATUS

IBTK FOXO3 BCLAF1 TNFAIP3 HIVEP2

Subclonal Deletions Clonal Deletions

The median tumor/germline ratio of the subclonal deletions was 0.83 (range 0.64-0.89) corresponding to a monomorphic deletion affecting 34% of LPL cells. Clonality of the deletions was strongly associated with CXCR4 mutations status.

CONCLUSIONS

- Gene loss of IBTK, FOXO3, BCLAF1, TNFAIP3 and/or HIVEP2 occurs in most patients with WM;
- Gene loss patterns differ between patients with fully clonal deleted chr 6q and those with subclonal chr 6q deletions;
- Despite clonal genomic losses in all 5 genes, the expression levels were significantly reduced only for IBTK, BCLAF1, and HIVEP2, suggesting the possibility of their haplosufficiency in WM while regulatory mechanisms may compensate for FOXO3 and TNFAIP3 gene loss;
- CXCR4 mutations were absent in chr 6q fully clonal deleted patients, but common in those with subclonal chr 6q or no chr 6q deletions;
- The present findings provide valuable insights into WM pathogenesis, and may be relevant to understanding therapeutic outcome with agents that target MYD88, CXCR4, BCL2 and BTK.