Clonal architecture of CXCR4 WHIM-like mutations in Waldenström Macroglobulinaemia

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$CXCR4^{WHIM}$ somatic mutations are distinctive to Waldenström Macroglobulinaemia (WM), and impact disease presentation and treatment outcome. The clonal architecture of $CXCR4^{WHIM}$ mutations remains to be delineated. We developed highly sensitive allele-specific polymerase chain reaction (AS-PCR) assays for detecting the most common $CXCR4^{WHIM}$ mutations (CXCR4 (S338X C>A and C>G)) in WM. The AS-PCR assays detected CXCR4 (S338X) mutations in WM and IgM monoclonal gammopathy of unknown significance (MGUS) patients not revealed by Sanger sequencing. By combined AS-PCR and Sanger sequencing, $CXCR4^{WHIM}$ mutations were identified in 44/102 (43%), 21/62 (34%), 2/12 (17%) and 1/20 (5%) untreated WM, previously treated WM, IgM MGUS and marginal zone lymphoma patients, respectively, but no chronic lymphocytic leukemia, multiple myeloma, non-IgM MGUS patients or healthy donors. Cancer cell fraction analysis in WM and IgM MGUS patients showed CXCR4 (S338X) mutations were primarily subclonal, with highly variable clonal distribution (median 35.1%, range 1.2-97.5%). Combined AS-PCR and Sanger sequencing revealed multiple $CXCR4^{WHIM}$ mutations in many individual WM patients, including homozygous and compound heterozygous mutations validated by cloning/sequencing and deep RNA sequencing analysis. The findings show that $CXCR4^{WHIM}$ mutations are

more common in WM than previously revealed, and are primarily subclonal, supporting their acquisition after $MYD88^{L265P}$ in WM oncogenesis. The presence of multiple $CXCR4^{WHIM}$ mutations within individual WM patients may be indicative of targeted CXCR4 genomic instability. Recent studies identified BTK Cys481 mutations in WM patients with relapsed diseases after ibrutinib treatment. Interestingly, the patients positive for $CXCR4^{WHIM}$ were also positive for the Cys481 mutations in the study cohort. The strong correlation between $CXCR4^{WHIM}$ and the Cys481 mutations may suggest a contribution of $CXCR4^{WHIM}$ to the risk of ibrutinib resistance.