The WHIM-like CXCR4\textsuperscript{S338X} somatic mutation activates AKT and ERK, and promotes resistance to ibrutinib and other agents used in the treatment of Waldenstrom’s Macroglobulinemia.

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**Background:** Whole genome sequencing (WGS) has revealed CXCR4 mutations as the second most prevalent somatic mutations in Waldenstrom’s Macroglobulinemia, which affect 30% of WM patients. The impact of CXCR4 somatic mutations remains to be clarified in WM.

**Methods:** CXCR4\textsuperscript{WT} and CXCR4\textsuperscript{S338X} cDNAs were subcloned into plenti-IRES-GFP vector, and transduced using an optimized lentiviral based strategy into BCWM.1 and MWCL-1 WM cells. GFP positive cells were sorted and used for functional studies. CXCR4 internalization was studied by comparing CXCR4 surface expression before and after SDF-1α stimulation. The expression of phosphorylated AKT, ERK1/2, and BTK was determined by phosphoflow, and confirmed by western blot. Cell survival studies were accessed by Annexin V staining and immunoblotting using antibodies for cleaved PARP and cleaved caspase 3. Bone marrow core biopsies from WM patients whose aspirates were used to sort for CD19\textsuperscript{+} cells and Sanger sequencing for the C-terminal domain were stained for phospho-AKT and phospho-ERK before and after ibrutinib therapy.

**Results:** Following SDF-1α stimulation, CXCR4\textsuperscript{S338X} WM cells exhibited decreased receptor internalization; enhanced and sustained AKT and ERK signaling; decreased PARP and caspase 3 cleavage; and decreased Annexin V staining versus CXCR4 wild-type (WT) cells. CXCR4\textsuperscript{S338X} related signaling and survival effects were blocked by the CXCR4 inhibitor AMD3100. SDF-1α treated CXCR4\textsuperscript{S338X} WM cells showed sustained AKT and ERK activation and decreased apoptotic changes versus CXCR4\textsuperscript{WT} cells following ibrutinib treatment, findings which were also reversed by AMD3100. AKT or ERK antagonists restored ibrutinib-triggered apoptotic changes in SDF-1α treated CXCR4\textsuperscript{S338X} WM cells demonstrating their role SDF-1α mediated ibrutinib-resistance. Enhanced bone marrow pAKT staining was also evident in CXCR4\textsuperscript{WHIM} versus CXCR4\textsuperscript{WT} WM patients, and remained active despite ibrutinib therapy in CXCR4\textsuperscript{WHIM} patients. Lastly, CXCR4\textsuperscript{S338X} WM cells showed varying levels of resistance to other WM relevant therapeutics including bendamustine, fludarabine, bortezomib and idelalisib in the presence of SDF-1α.

**Conclusion:** our findings show that the most common CXCR4 WHIM-like somatic mutation in WM (CXCR4\textsuperscript{S338X}) confers decreased SDF-1α triggered CXCR4 receptor internalization, enhanced AKT and ERK activation, and resistance to ibrutinib triggered apoptosis in WM cells. Use of inhibitors targeting CXCR4 or AKT/ERK can restore the sensitivity of CXCR4\textsuperscript{S338X} expressing WM cells to ibrutinib as well as other WM relevant agents, thereby providing a framework for the investigation of these combinations in WM.