

## **Inhibition Of The Jak/Stat Pathway In Waldenström Macroglobulinemia**

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Waldenström macroglobulinemia (WM) is a B-cell malignancy that is characterized by the production of a monoclonal IgM protein. We have previously shown that IL-6 significantly upregulates IgM secretion by WM cells and that IL-6 secretion is regulated by CCL5 (Rantes). We have also shown that IL-6 mediated IgM secretion in WM requires phosphorylation of Stat1 and Stat3. Because IL-6 induced signaling involves the Jak/Stat pathway, we tested whether the use of a Jak/Stat inhibitor, TG101348, would result in down regulation of CCL5, IL-6 and IgM production and inhibit cell proliferation and viability in WM.

Using IgM producing cell lines as well CD19+ malignant cells from bone marrow specimens from WM patients, we found that CCL5 secretion was decreased when cells were treated with a Jak inhibitor. In contrast, suppression of IL-6 production in the presence of a Jak inhibitor was modest and supported our previous data showing that IL-6 secretion is mediated by GLI (a member of the Hedgehog pathway) rather than the Jak/Stat pathway. Our previous work had shown that IL-6 mediated IgM secretion was dependent on the Jak/Stat pathway and we found that Stat activation was suppressed by the use of a Jak/Stat inhibitor. Similarly, we also found that IgM production was inhibited. Finally, we measured the effect of TG101348 on cell proliferation and survival. We found that cell proliferation as determined by tritiated thymidine uptake was inhibited in a dose dependent fashion. Inhibition of cell viability as measured by Annexin V/propidium iodide staining, however, required higher concentrations of the inhibitor. The effects of TG101348 on cell proliferation and survival were then confirmed in primary malignant cells from patients with Waldenstrom macroglobulinemia.

These data confirm the role of Jak/Stat signaling in the CCL5-IL-6-IgM axis in WM. We found that a Jak/Stat inhibitor TG101348 generally suppressed the signaling and growth of WM cells but that pathways that were known to be Jak/Stat dependent required significantly lower doses to be completely inhibited.