BTK participates in MYD88 signaling in malignant cells expressing the L265P mutation in Waldenstrom’s Macroglobulinemia, and shows robust tumor cell killing with the BTK-inhibitor PCI-32765 in combination with MYD88 pathway inhibitors


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**Background**

Waldenstrom’s macroglobulinemia (WM) is a distinct B-cell lymphoma resulting from the accumulation, predominantly in the bone marrow, of clonally related IgM secreting lymphoplasmacytic cells (LPCs). Bruton’s tyrosine kinase (BTK) promotes B-cell receptor signaling along with B-cell expansion and survival through NF-kB and MAPK. MYD88 L265P is a widely expressed somatic mutation in tumor cells from WM patients. MYD88 L265P promotes enhanced tumor cell survival through IRAK 1/4 mediated NF-kB and MAPK signaling. We therefore sought to clarify the role of BTK signaling in MYD88 L265P expressing WM cells, and the impact of BTK and MYD88/IRAK inhibition on WM cell signaling and survival.

**Patients and Methods**

Western blot analysis was performed using total and phospho-specific antibodies in MYD88 L265P expressing WM cells, BCWM.1 and MCWL-1 following MYD88 knockdown by lentiviral transduction, and/or use of MYD88 or IRAK signal inhibitors. Cells were also treated with the BTK inhibitor PCI-32765, in the presence or absence of MYD88 homodimerization or IRAK1/4 inhibitors. Annexin V / PI staining was used to assess cell survival, and synergism assessed with CalcuSyn software.

**Results**

BTK was highly expressed and phosphorylated in MYD88-L265P expressing WM cells and PCI-32765 significantly blocked the BTK activation.

Knockdown of MYD88 by lentiviral transduction, and/or use of a MYD88 inhibitor leads to decreased BTK phosphorylation.

**Conclusion**

BTK activation is facilitated by MYD88 pathway signaling in MYD88 L265P expressing WM cells, and participates in MYD88 downstream signaling. Inhibition of BTK by PCI-32765 leads to robust tumor killing of MYD88 L265P expressing WM cells, which is potentiated by MYD88 pathway inhibitors. These studies provide the framework for the investigation of BTK inhibitors in WM, as single agents and in combination with MYD88 pathway inhibitors.