Mutated MYD88 activates the BCR component Syk and provides a rationale therapeutic target in Waldenstrom’s Macroglobulinemia

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**Background**

Mutations in the Toll receptor (TLR) pathway are highly prevalent in Waldenstrom’s Macroglobulinemia (WM) and ABC DLBCL, wherein mutated MYD88 triggers NF-κB pro-survival signaling through BTK/IRAK. Activation of B-cell receptor (BCR) signaling can also be triggered by activating mutations in CD79A/B in ABC DLBCL, though are rare in WM (5-8%). Despite these findings, there is evidence for BCR activation in WM (Argyropoulos et al, Leukemia 2016). We hypothesized that crosstalk between TLR and BCR might account for aberrant BCR signaling in WM and ABC DLBCL.

**Methods**

Phospho-flow analysis of MYD88, Syk (pY525-pY526) and other BCR signaling components was performed in MYD88 mutated WM cells compared to wild-type MYD88 expressing control cells. Knockdown or overexpression of MYD88 by lentiviral transduction in MYD88 mutated BCWM.1 cells. Western blot and phospho-flow studies were used to detect protein expression and phosphorylation in WM cells. Co-Immunoprecipitation assay was used to detect the involvement of Syk in the Myddosome complex. Cell survival following treatment was assessed by Annexin V staining or CellTiter-Glo® Luminescent Cell Viability Assays.

**Results**

MYD88 mutated WM and ABC-DLBCL cell lines express higher levels of phospho-Syk.

Knockdown of MYD88 reduces phospho-Syk while the over-expression of MYD88 L265P enhances phospho-Syk expression in MYD88 mutated BCWM.1 WM cells.

The knockdown of MYD88 in MYD88 mutated WM cells reduced the phosphorylation of Syk (A). The transduction of MYD88 L265P increases Syk phosphorylation compared to vector only or MYD88 WT in MYD88 mutated WM cell line (BCWM.1) as well as increases the phosphorylation of its downstream STAT3 (B). While this increased phosphorylation of STAT3 through Syk activation by the overexpression of mutated MYD88 were reduced by a Syk inhibitor, R406 in WM cell line, BCWM.1.

Syk is involved in the Myddosome complex in MYD88 mutated WM cells.

**Conclusion**

Our findings demonstrate that in addition to activation of the TLR pathway, mutated MYD88 activates the BCR component Syk. These findings provide the rationale for combined therapeutics targeting the TLR and BCR pathways in MYD88 mutated WM and possibly other mutated MYD88 driven B-cell malignancies including ABC DLBCL.