597 Disruption of MYD88 Pathway Signaling Leads to Loss of Constitutive IRAK1, NF-κβ and JAK/STAT Signaling and Induces Apoptosis of Cells Expressing the MYD88 L265P Mutation in Waldenstrom's Macroglobulinemia

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Introduction: Through whole genome sequencing of bone marrow lymphoplasmacytic cells (LPCs) from WM patients, we recently identified a broadly expressed somatic variant (MYD88 L265P) which was present in 90% of WM patients, as well as in BCWM.1 and MWCL-1 WM cells. The presence of this variant has been reported to confer oncogenic activity in cell lines derived from patients with DLBCL ABC subtype lymphomas by induction of IRAK 1/4 kinase activity, and downstream signaling via NF-κβ (Ngo et al, Nature 2011, 470:115-119). We therefore sought to delineate the activity of agents which either disrupt MYD88 homodimerization, a prerequisite to IRAK 1/4 auto-phosphorylation, or by direct inhibition of IRAK 1/4 kinase activity in WM.

Patients and Methods: Primary WM LPCs were isolated from bone marrow biopsy specimens from WM patients, and $CD19^+$ B-cells were isolated from the peripheral blood of healthy donors. The mutational status of MYD88 was identified by Sanger sequencing, which confirmed the presence of the L265P mutation in BCWM.1 and MWCL-1 cells, and absent in Ramos and MM1S cells. Western blot and phospho-flow studies using phospho-specific antibodies were performed for signaling proteins downstream of MYD88, cell killing evaluated by Annexin V/PI staining and AlamarBlue, and IgM and IL-6 release determined after treatment with and without inhibitors of MYD88 homodimerization (IMG-2005-5, IMGENEX, San Diego CA), and IRAK 1/4 kinase function (407601, EMD, Gibbstown, USA).

Results: Phosphorylation of the MYD88 downstream signaling proteins IRAK1, IκBα, NFκβ-p65 and STAT3 was greater in MYD88 L265P expressing BCWM.1 and MWCL-1 cells, and primary WM patient LPC, versus MYD88 wild type expressing Ramos, MM1S, and healthy donor $CD19^+$ B-cells. Inhibition of MYD88 homodimerization and IRAK 1/4 kinase activity led to decreased phosphorylation of IRAK1, IκBα, NFκβ-p65 and STAT3 in BCWM.1 and MWCL-1 cells. Importantly, both inhibitors induced robust apoptosis of BCWM.1 and MWCL-1 cells, and primary WM LPCs bearing the MYD88 L265P mutation in comparison to Ramos, MM1S, or healthy donor $CD19^+$ B-cells. Activation of caspase-3 and PARP, decreased BCL-2 expression, and decreased release of IL-6 and IgM accompanied treatment of WM cells following treatment with inhibitors of MYD88 homodimerization and/or IRAK 1/4 kinase activity.

Conclusion: Disruption of MYD88 pathway signaling leads to loss of constitutive IRAK1, NF-κβ and JAK/STAT activation and induces apoptosis of cells expressing the MYD88 L265P mutation in WM. The results of this study provide novel insights into the pathogenesis of WM, and provide a framework for the clinical investigation of inhibitors of the MYD88 pathway in WM.

Disclosures: No relevant conflicts of interest to declare.