Targeting WM with MYD88 and IRAK inhibitors

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Background: We recently identified a broadly expressed somatic mutation (MYD88-L265P) which was present in 90% of WM patients through whole genome sequencing of lymphoplasmacytic cells (LPCs) from WM patients (*Treon SP. et. al. NEJM, 2012*). This mutation has been reported to confer oncogenic activity in cell lines derived from patients with DLBCL ABC subtype lymphomas by triggering IRAK, NF-κB and JAK-STAT signaling (Ngo et al, Nature 2011). Our preliminary data demonstrated that the MYD88-L265P promoted WM-LPC survival by overexpression or knockdown (Yang G., et. al. ASH, 2011). We therefore sought to develop agents which either disrupt MYD88 homodimerization, a prerequisite to IRAK signaling, or by direct inhibition of IRAK 1/4 phosphorylation in WM.

Patients and Methods: Primary WM-LPCs were isolated from bone marrow biopsy specimens from WM patients, and normal $CD19^+$ B-cells were isolated from the peripheral blood of healthy donors. Western blot and phos-flow studies using phospho-specific antibodies were performed for signaling proteins downstream of MYD88. Cell killing was evaluated by Annexin V/PI staining or AlamarBlue® assay after treatments with inhibitors of MYD88 homodimerization (IMGENEX) or IRAK 1/4 kinases (EMD). MYD88 homodimerization inhibitory compounds (Sigma-Tau) or IRAK4 kinase inhibitors (synthesized) were screened by high-throughput (HTP) cell viability assay using JANUS automated workstation and EnVision multilabel detection system with ATPLite® as well as by lentiviral NFkB-reporter assay.

Results: The mutational status of MYD88 was identified by Sanger sequencing, which confirmed the presence of the L265P mutation in L265P carrying primary WM patient LPCs, BCWM.1 and MWCL-1 cells; and absent in healthy donor $CD19^+$ B-cells, Ramos, MM1S, RPMI-8226 and U266 cells. Phosphorylation of the MYD88 downstream signaling proteins IRAK1, IκBα, NFκβ-p65 and STAT3 was greater in L265P expressing BCWM.1 and MWCL-1 cells, and WM-LPCs, versus wild type MYD88 expressing Ramos, MM1S, RPMI-8226, U266 and healthy donor $CD19^+$ B-cells. Inhibition of MYD88 homodimerization and IRAK 1/4 phosphorylation led to decreased phosphorylation of these proteins 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 reduced IL-6 and IgM secretion accompanied treatment of WM cells with inhibitors of MYD88 homodimerization or IRAK 1/4 kinases. By HTP screening of 16 IRAK4 kinase inhibitors, JH1-017-01 and its analogs JH1-023-01 and JH1-025-01 demonstrated preferable cell killing to MYD88-L265P expression cells. In addition, JH1-017-01 showed significant inhibition of the NFkB activation by both phos-flow and NFkB luciferase reporter assays. Among the 23 MYD88 homodimerization inhibitors, ST5571AA1 and ST5613AA1 showed robust cell killing and significant inhibition of the NFkB activation in WM cells.

Conclusion: 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 WM. The HTP screening provided us with several potential drug candidates that targeting MYD88/IRAK4 signaling pathway in WM.