The development of novel selective IRAK1 inhibitor for the treatment of Waldenstrom's Macroglobulinemia.

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MYD88 L265P mutation triggers multiple pro-growth and survival signaling through divergent pathways driven by IRAK4/IRAK1, BTK and HCK (Ngo et al, Nature 2011; Yang et al, Blood 2013; 2016). Ibrutinib targets both BTK and HCK, and has shown high levels of activity in WM patients carrying MYD88 mutations. Responses however are partial, and complete remissions are lacking. We therefore sought to clarify if persistent IRAK4/IRAK1 signaling was responsible for survival of malignant lymphoplasmacytic cells (LPC) in WM patients on ibrutinib. We observed by flow cytometric analysis that while BTK activation was suppressed in WM patients on ibrutinib for $\geq$6 months, both IRAK4 and IRAK 1 remained hyperactivated. To determine the importance of IRAK4 vs. IRAK1 for WM cell survival, we performed lentiviral transduction studies in MYD88 mutated BCWM.1 cells, and observed in an inducible model system that knockdown of IRAK1 produced higher levels of apoptosis versus IRAK4 (**Figure 1A**). In addition, ibrutinib showed more robust cell killing in BCWM.1 cells with inducible knockdown of IRAK1 vs. IRAK4. Given these findings, we have pursued a medical chemistry campaign to develop a potent and highly selective inhibitor of IRAK1 kinase activity, JH-X-119-01. JH-X-119-01 inhibited IRAK1 biochemically with an IC_{50} of 9.3 nM while exhibiting no inhibition of IRAK4 at concentrations up to 10 μM, and showed exceptional Kinome selectivity with off-target inhibition of only two kinases, YSK4 and MEK3 (**Figure 1B**). Mass spec labelling studies were used to confirm that JH-X-119-01 irreversibly labelled IRAK1 at cysteine 302. JH-X-119-01 showed antiproliferative effects on MYD88 mutated WM and ABC DLBCL cells. Importantly, the combination of JH-X-119-01 with Ibrutinib led to synergistic tumor cell killing in MYD88 mutated WM and ABC-DLBCL cells, and suppression of NF-κB activation (**Figure 1C**). *In vivo* PK studies revealed a favorable profile for JH-X-119-01 with a moderate half-life of 1.61 hours, a C_{max} of 9.95 μM, and a low clearance of 18.84 mL/min/kg when dosed IV. Our findings evidence the development of a novel, highly selective IRAK1 kinase inhibitor, JH-X-119-01, that shows specific inhibition of IRAK1 kinase activity and a reduction of tumor cell survival in

MYD88 mutated WM. Importantly, JH-X-119-01 shows synergistic tumor cell killing with ibrutinib in MYD88 mutated WM and ABC-DLBCL cells. This study provides a framework for the development of highly selective IRAK1 inhibitors for use alone, and in combination with ibrutinib in MYD88 mutated B-cell lymphomas.