Novel PROTACs Demonstrate Dual Kinase Inhibition and Degradation of IRAK1 and IRAK4, and Are Highly Active in MYD88 Mutated WM and ABC DLBCL Cells

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Background: Approximately 95-97% of WM patients carry $MYD88^{Mut}$ (Blood 123(11): 1637-46, Blood 121(11): 2051-8, Leukemia 27(8): 1722-8, Blood 121(22): 4504-11, Blood 2013; 121(13): 2522-28). Our previous studies also showed that $MYD88^{Mut}$ is the primary driver of pro-survival signaling in WM, enabling multiple signaling cascades (Blood 122(7):1222-32, Blood 127(25):3237-52, Blood Adv. 14;4(1):141-153, Blood Cancer J. 10(1):12, Blood Adv. 6(11):3332-3338, Blood 3;131(18):2047-2059). The identification of BTK as a downstream pro-survival molecule of $MYD88^{Mut}$ supported development of BTK-inhibitors and FDA/EMA approval of ibrutinib and zanubrutinib for WM. While BTK-inhibitors are highly active in WM with overall response rates of 80-90%, complete responses are rare, and the median progression-free survival is 4-6 years (N Engl J Med. 372(15):1430-40, Clin Oncol. 36(27): 2755-2761, Cancer Sci. 113(6):2085-2096, Blood 140 (Suppl 1): 557-560). Intrinsic resistance to BTK-inhibition is related to alternative NFKB pro-survival signaling by IRAK1/4 (Blood. 122(7):1222-32, Blood 129(1):2519-2525). IRAK4 triggers IRAK1 in response to $MYD88^{Mut}$ and both trigger NFKB pro-survival signaling. We previously developed a highly potent and selective IRAK1 inhibitor (JHX-119-01) that showed synergistic killing of $MYD88^{Mut}$ lymphoma cells with ibrutinib (ACS Med Chem Lett. 2020; 11(11):2238-2243). However, recent studies in our lab suggest pro-survival signaling is dependent upon the scaffolding functions of IRAK1/4. We therefore converted our selective IRAK1 inhibitor into a dual inhibitor and degrader of IRAK1/4.

Methods: To target both IRAK1/4, we converted our IRAK1 selective inhibitor into a dual IRAK1/4 inhibitor. We leveraged our new dual inhibitor, along with various E3 ligase-targeting ligands in PROTAC molecules. Compounds were tested using a Cell Viability Assay in WM, $MYD88^{Mut}$ and $MYD88^{Wt}$ lymphoma cell lines. Potent compounds were selected for further validation by measuring apoptotic and cytotoxic effects on WM and MYD88-mutated lymphoma cell lines, and primary human peripheral blood mononuclear cells (PBMCs). The kinase inhibition profile of lead compounds was then assessed using KINOMEscan assay against a panel of 468 kinases at 1 uM.

Results: We identified novel PROTACs that exhibited potent inhibition of IRAK1/4 kinase activity and marked degradation of IRAK1/4 in $MYD88^{Mut}$ WM and ABC DLBCL cell lines. Enhanced cell killing due to degradation of IRAK1/4 versus inhibition alone was confirmed. JH-XIII-05-1 emerged as the lead compound and demonstrated remarkably low IC_{50} values (10-50 nM) in proliferation assays of BCWM.1 and TMD-8 cells. JH-XIII-05-1 elicited higher levels of apoptosis in WM and ABC DLBCL cells than parent inhibitor while sparing healthy donor B- and T-cells. JH-XIII-05-1 showed potent synergy with ibrutinib and Venetoclax. Murine PK studies showed that JH-XIII-05-1 had an excellent PK profile.

Conclusions: In these studies, we identified a novel, dual IRAK1/4 PROTAC that demonstrates potent and selective kinase inhibition and degradation of IRAK1/4. JH-XIII-05-1 showed enhanced apoptosis of MYD88 mutated WM and ABC DLBCL cells over inhibitor alone and spared healthy donor B- and T-cells. JH-XIII-05-1 showed potent synergistic killing in combination with ibrutinib or Venetoclax in both BCWM.1 and TMD8 cells. Our studies therefore provide a framework for the advancement of dual IRAK1/4 PROTACs for the treatment of MYD88 mutated lymphomas.