# Novel BiFunctional HCK/BTK PROTACs for MYD88 Mutated WM and ABC DLBCL

John M Hatcher, PhD[1], Shirong Liu, MD, PhD[1], Amanda Kofides[1], Alexa G Canning, BA[1], Jinhua Wang, PhD[2], Xia Liu, MD[1], Nickolas Tsakmaklis, BA[1], Maria Luisa Guerrera, MD[1], Christopher J Patterson[1], Zachary R Hunter, PhD[1], Shayna R Sarosiek[1], Jorge J. Castillo, MD[1], Sara J. Buhrlage, PhD[2] and Steven P. Treon, MD, PhD, FRCP[1]

[1]Dana-Farber Cancer Institute, Bing Center for Waldenström Macroglobulinemia, Boston, MA

[2]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA

**Background**: Activating mutations in MYD88 are prevalent in many B-cell malignancies, including Waldenström Macroglobulinemia (95-97%), primary CNS lymphoma (70-80%), ABC DLBCL (40%), marginal zone lymphoma (5-10%), and CLL (5-15%). Mutated MYD88 transcriptionally upregulates the SRC family member HCK, which serves as a master signal for triggering multiple pro-survival cascades in mutated MYD88 lymphoma cells including BTK/NFKB, SYK, and ERK (Blood 127:3237-52; Blood Adv 4:141-153; Blood Adv 6:3332-38). Proteolysis targeting chimeras (PROTACs) represent a novel approach for blocking signaling and provide an advantage over kinase inhibitors with greater selectivity and sustained target inhibition. Therefore, we endeavored to develop potent and selective HCK/BTK targeting PROTACS for the treatment of MYD88 driven lymphomas.

**Methods**: We developed and characterized several PROTACs using different scaffolds and E3 ligase-targeting warheads. Compounds were tested using ATP-based Cell Titer-Glo Luminescent Cell Viability Assay in TMD8 and BCWM.1 cells to identify potent compounds. Compounds with IC50 scores lower than the dual HCK/BTK inhibitor KIN-8194 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). Western blot analysis was used to determine degradation of HCK and BTK in TMD8 and BCWM.1 cells. Kinome selectivity was assessed using the KINOMEscan assay against a panel of 468 kinases at 1 uM. Lead compounds were subjected to a battery of in vitro ADME profiling and in vivo mouse PK studies. Compounds with ideal profiles were assessed in a mouse xenograft model using TMD8 cells.

**Results**: We identified several novel PROTACs with IC50's < 1nM on HCK and BTK and marked degradation of HCK and BTK at dose levels as low as 100 nM in $MYD88^{Mut}$ WM and ABC DLBCL cell lines. Importantly, DFCI-002-6 maintains potent degradation of both BTK and HCK in mutant TMD8 C481S cells at a dose of 1 μM. Enhanced cell killing due to degradation of HCK and BTK versus inhibition alone was confirmed using analogues that do not bind cereblon. We observed suppression of downstream pro-survival NFKB and ERK signaling in response to the dual HCK/BTK PROTACs. Moreover, several HCK/BTK PROTACs demonstrated remarkable low IC50 values (1-100 nM) in proliferation assays of MYD88 mutated BCWM.1 and TMD-8 cells. The dual HCK/BTK

PROTACs elicited higher levels of apoptosis in BCWM.1 WM and TMD-8 ABC DLBCL cells than KIN-8194 and spared healthy donor B- and T-cells. Murine PK studies showed that DFCI-002-6 was highly bioavailable with F=40%, and CMax of 700-2700 nM at oral doses of 10-60 mg/kg. PD studies with DFCI-002-6 in a mice TMD8 xenograft model showed nearly complete arrest of tumor growth following oral doses of 15mg/kg and 30mg/kg. PK/PD studies confirmed potent degradation of both HCK and BTK (Fig. 1).

**Conclusions**: In these studies, we demonstrated the development and characterization of novel, dual HCK/BTK PROTACs with selective kinase inhibition and protein degradation of HCK, BTK and the BTK C481S mutant. DFCI-002-6 showed enhanced apoptosis of MYD88 mutated WM and ABC DLBCL cells over KIN-8194 and spared healthy donor B- and T-cells. DFCI-002-6 shows excellent oral PK including high levels of bioavailability.

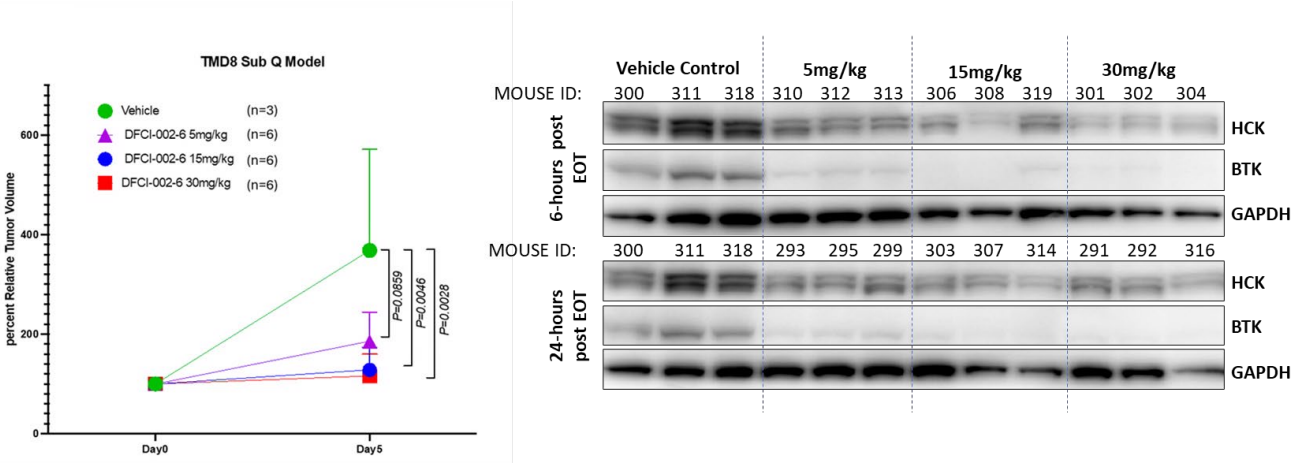


Figure 1. Results from 5-day PD study using a TMD8 Mouse Xenograft Model