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Characterization of a novel dual HCK/BTK PROTAC (DFCI-002-06) that demonstrates potent *in vitro* activity and shows robust degradation of HCK and BTK in xenografted MYD88-mutated tumors in murine models.

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Background

Activating mutations in MYD88 are prevalent in various 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 upregulates the SRC family member HCK, which serves as a master signal for multiple pro-survival cascades in mutated MYD88 lymphoma cells, including BTK pathways (Blood 127:3237-52; Blood Adv 4:141-153; Blood Adv 6:3332-38). Previously, we developed KIN-8194, a novel HCK kinase inhibitor that blocks both HCK and BTK and is active *in vitro* and *in vivo* in MYD88-mutated xenograft lymphoma models, including $BTK^{Cys481Ser}$ ibrutinib-resistant models (Blood 138:1966-79, Leukemia 38:1570-1580). Proteolysis targeting chimeras (PROTACs) represent a novel approach for blocking kinase-based signaling, offering greater selectivity and sustained target inhibition over traditional kinase inhibitors. We evaluated our newly developed highly potent, selective, and bioavailable HCK/BTK-targeting PROTACs for treating MYD88-driven lymphomas.

Methods

A series of potent HCK/BTK PROTACs were developed. Biological efficacy screenings using the Cell Titer-Glo Luminescent Cell Viability Assay identified two compounds with IC_{50} scores lower than KIN-8194. These were selected for further validation through flow cytometry-based quantitative assays, which verified apoptotic and cytotoxic effects on WM and MYD88-mutated lymphoma cell lines, as well as primary human bone marrow mononuclear cells (BMMCs), utilizing Annexin V and Propidium iodide (PI) staining. A pharmacodynamics (PD) study was completed on one of the most potent PROTACs using a MYD88-mutated TMD8 cell-xenografted murine model.

Results

We identified two novel PROTACs (DFCI-002-05 and DFCI-002-06) that exhibited potent degradation of HCK and BTK by western blot (**Figure 1A**). Both PROTACs demonstrated low IC_{50} values (1-100 nM) in proliferation assays of MYD88-mutated BCWM.1 WM and TMD-8 ABC DLBCL cells (**Figure 1B**). Importantly, the dual HCK/BTK PROTACs induced high levels of apoptosis in MYD88-mutated BCWM.1 WM, HBL-1, and TMD8 ABC DLBCL cells (**Figure 1C**). Additionally, treatment of cultured bone marrow cells from a newly diagnosed WM patient with mutated MYD88 showed profound cell death and apoptosis induction in $CD19^+$ cells by the PROTACs, especially DFCI-002-06, compared with Zanubrutinib and KIN-8194 (**Figure 1D**).

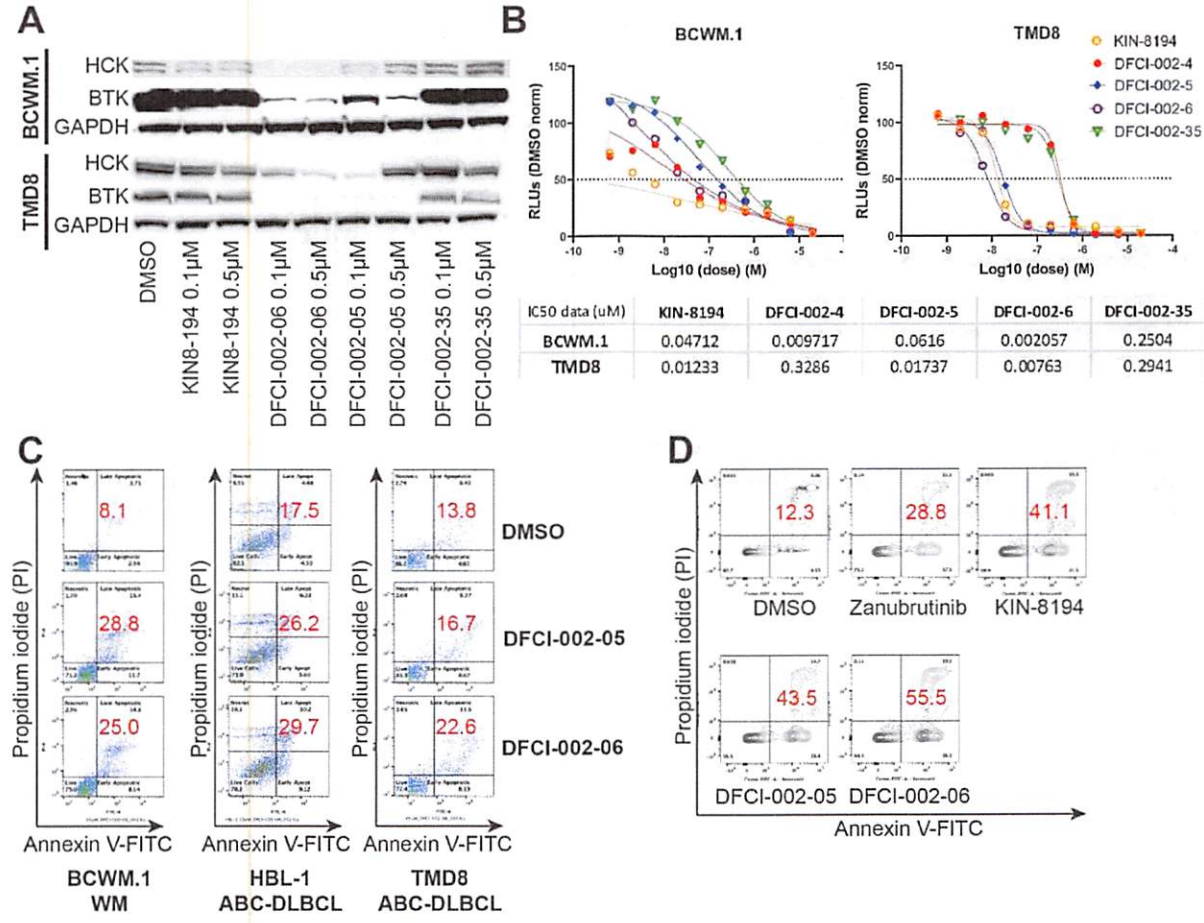
We then conducted a PD study using a MYD88-mutated TMD8 cell-xenograft murine model. DFCI-002-06 was administered orally once daily for five days at doses of 5 mg/kg, 15 mg/kg, and 30 mg/kg (**Figure 2A**). We measured the HCK and BTK levels in the tumor and found significant degradation of HCK and BTK in the tumor tissue (**Figure 2B**). Consistent with this, treatment with DFCI-002-06 at the given dosages achieved significant suppression in tumor growth (**Figure 2C**).

Conclusions

We characterized novel dual HCK/BTK PROTACs that demonstrate potent protein degradation of HCK and BTK, and induction of cell death and apoptosis in MYD88-mutated WM and ABC DLBCL cells, as well as in the $CD19^+$ population of WM patient bone marrow cells. The PD study of PROTAC DFCI-002-06 demonstrated robust degradation of HCK and BTK in xenografted MYD88-mutated tumors in a murine model and exhibited potent tumor suppression. Our studies provide an advancement in dual HCK/BTK PROTACs for the treatment of MYD88-mutated lymphomas.

Figures:

Figure 1



IC50 data (uM)	KIN-8194	DFCI-002-4	DFCI-002-5	DFCI-002-6	DFCI-002-35
BCWM.1	0.04712	0.009717	0.0616	0.002057	0.2504
TMD8	0.01233	0.3286	0.01737	0.00763	0.2941

Figure 1. A. Degradation of HCK and BTK by PROTACs DFCI-002-05 and DFCI-002-06 compared with KIN-8194, evaluated in BCWM.1 WM and TMD8 ABC-DLBCL cell lines by western blot. B. ATP-based Cell Titer-Glo Luminescent Cell Viability Assay assessment of candidate PROTACs (DFCI-002s) compared to HCK/BTK inhibitor KIN-8194. C. Evaluation of cell death and apoptosis induction by the PROTACs in MYD88-mutated cell lines at the concentration of 0.5µM. D. Evaluation of cell death and apoptosis induction by the PROTACs in the $CD19^{+}$ cell population of bone marrow cells from an untreated, newly diagnosed WM patient.

Figure 2

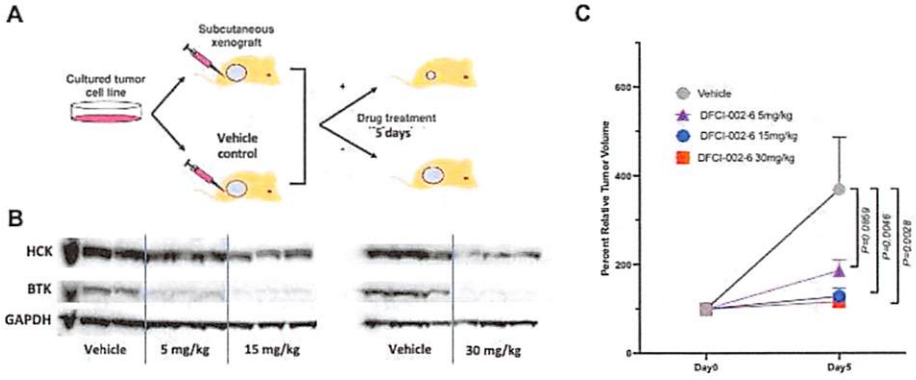


Figure 2. PD study of HCK degrader DFCI-002-06 in a TMD8 cell-xenograft murine model. A. Design of the study. B. Verification of HCK and BTK degradation in the tumor tissue post treatment. C. Tumor growth curve.