

Development of a novel, second generation dual IRAK1/IRAK4 Proteolysis Targeting Chimera (DFCI-001-149) that is highly active in Waldenstrom's Macroglobulinemia Cells and shows synergistic activity with BTK-inhibitors.

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Background: Activating MYD88 mutations occur in approximately 95–97% of patients with Waldenström macroglobulinemia (WM) and promote malignant-cell survival through divergent HCK/BTK and IRAK1/4 signaling pathways. We previously developed JH-XIII-05-01, a first-generation dual IRAK1/4 PROTAC that combines degradation with kinase inhibition and shows synergistic activity with BTK-directed agents. However, JH-XIII-05-01 demonstrated low steady-state volume of distribution (V_{ss}, 0.81 L/kg) and limited tumor-tissue distribution, prompting a medicinal chemistry campaign to improve PK/PD properties.

Methods: JH-XIII-05-01 served as the benchmark for optimization of cereblon-recruiting dual IRAK1/4 degraders. Compounds were evaluated by immunoblotting, kinome profiling, matched global proteomics, and cell viability and apoptosis assays in MYD88-mutant lymphoma models, primary WM samples with paired CD19-negative cells, and TMD8 cells expressing WT BTK or clinically relevant BTK resistance mutations. Combination studies included covalent and non-covalent BTK inhibitors and BTK-directed degraders. In vitro ADME and mouse pharmacokinetic properties were also assessed.

Results: DFCI-001-149 demonstrated potent dual IRAK1/IRAK4 degradation and a kinome selectivity profile comparable to JH-XIII-05-01. DFCI-001-149 also showed synergistic activity with BTK-targeting agents, including zanubrutinib, pirtobrutinib, and BTK-directed degraders, supporting the feasibility of combining IRAK1/4 and BTK degradation. In TMD8 BTK WT cells, DFCI-001-149 was 2.3-fold more potent than JH-XIII-05-01 (EC₅₀, 3.9 versus 9.2 nM) and retained low-nanomolar antiproliferative activity across clinically relevant BTK resistance models tested, including T474I, M437R, V416L, A428D, C481S, and L528W, while maintaining strong pro-apoptotic activity in the evaluated mutant models. In TMD8 BTK A428D cells, JH-XIII-05-01 and DFCI-001-149 induced 66.5% and 75.2% apoptosis, respectively, at 0.5 μM after overnight treatment, compared with 18.4% for vehicle and 19.6% for the cereblon-inactive control. In TMD8 WT cells, DFCI-001-149 induced 58.6% apoptosis versus 19.2%, 19.9%, and 18.6% for the IRAK1- or IRAK4-directed degraders JNJ-1013, KT-474, and KTX-582, respectively, increasing to 71.8% when combined with zanubrutinib. In primary WM samples treated overnight at 0.5 μM, DFCI-001-149 showed greater apoptotic activity than JH-XIII-05-01 with minimal activity in paired CD19-negative nonmalignant cells. Matched proteomics showed preservation of a broader degradation signature, including PAK4, GAK, IKZF1/3, and CSNK1 family proteins. DFCI-001-

149 also reduced MYC and increased TP53, CDKN1A, and MDM2, consistent with a p53-associated stress response that may contribute to its enhanced antiproliferative and apoptotic activity. Relative to JH-XIII-05-01, DFCI-001-149 showed threefold higher aqueous solubility and 5- to 12-fold longer half-lives in human and mouse microsomes and hepatocytes. These improvements translated in vivo to an increase in Vss from 0.81 to 2.31 L/kg, 50% lower IV clearance, and extension of the subcutaneous half-life from 2.0–2.7 to 6.1–6.3 hours. Tumor pharmacodynamic studies assessing tissue exposure and IRAK1/IRAK4 degradation are ongoing.

Conclusions: DFCI-001-149 is a novel, second-generation IRAK1/IRAK4 PROTAC with enhanced potency, pro-apoptotic activity, and synergistic tumor-cell killing with BTK inhibitors, including BTK-directed PROTACs, thereby supporting the feasibility of combined PROTAC therapy for WM. The pronounced activity of DFCI-001-149 in TMD8 cells expressing clinically relevant BTK mutations supports its further investigation as a strategy to overcome resistance to BTK-directed therapy. DFCI-001-149 also showed improved metabolic stability, systemic persistence, and distribution, consistent with improved pharmacokinetic properties.

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