

Targeting compensatory signaling associated with acquired kinase-dead BTK mutations in MYD88 mutated lymphoma cells

Shirong Liu, MD, PhD^{1,2,3,*}, Xia Liu, MD^{1,*}, Amanda Kofides, BA¹, John M Hatcher, PhD^{1,2,3}, Yuntong Liu BA¹, Hao Sun MD, PhD^{1,2,3}, Nickolas Tsakmaklis, BA¹, Alberto Guijosa, MD¹, Jinhua Wang, PhD⁴, Maria Luisa Guerrero, MD^{1,2,3}, Christopher J Patterson¹, Zachary R Hunter, PhD^{1,2,3}, Shayna R Sarosiek, MD^{1,2,3}, Jorge J. Castillo, MD^{1,2,3}, Prafulla Gokhale, PhD,⁵ Sara J. Buhrlage, PhD^{3,4}, and Steven P. Treon, MD, PhD^{1,2,3}

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

²Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA

³Department of Medicine, Harvard Medical School, Boston, MA, USA

⁴Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA

⁵Experimental Therapeutics Core and Belfer Center for Applied Cancer Science, Dana-Farber Cancer Institute, Boston, Massachusetts 02215, United States

*Co-first authors

Abstract

Activating MYD88 mutations drive pro-survival signaling through pathways that include HCK-directed BTK signaling (Yang et al., Blood, 2016). BTK inhibitors are active in many MYD88-mutated malignancies, but resistance commonly arises through acquired BTK mutations affecting covalent inhibitors (BTK^{Cys481Ser}, BTK^{Cys481Arg}, BTK^{Cys481Tyr}, BTK^{Ala428Asp}, and BTK^{Leu528Trp}), non-covalent inhibitors (BTK^{Val416Leu}, BTK^{Ala428Asp}, BTK^{Thr474Ile}, BTK^{Leu528Trp}, and BTK^{Met437Arg}), and BTK-directed proteolysis-targeting chimeras (PROTAC) (BTK^{Ala428Asp}). Previous studies demonstrated increased HCK

signaling and dependence in kinase-impaired BTK-mutant cells, suggesting that SRC-family kinases compensate for loss of canonical BTK signaling (Dhami et al., Sci. Signal., 2022). Whether clinically relevant BTK resistance mutations similarly engage compensatory signaling pathways remains incompletely defined.

We therefore expressed mutant BTK^{Cys481Ser}, BTK^{Val416Leu}, BTK^{Ala428Asp}, BTK^{Met437Arg}, BTK^{Thr474Ile}, and BTK^{Leu528Trp} in MYD88^{L265P}-mutated BCWM.1 and TMD8 lymphoma cells and characterized mutation-associated pro-survival signaling by Western blot analysis. In BCWM.1 and TMD8 cells expressing the kinase-impaired BTK mutants BTK^{Ala428Asp} and BTK^{Leu528Trp}, loss of BTK^{Tyr223} phosphorylation was observed, indicating reduced dependence on canonical BTK kinase activation. Expression of HCK and/or its homolog LYN was increased in selected cells expressing kinase-impaired BTK mutants. To clarify the role of HCK, we performed HCK knockdown studies in BTK^{Ala428Asp}-expressing cells, which resulted in increased apoptosis. Similar findings were previously reported for BTK^{Cys481Phe} and BTK^{Cys481Tyr} (Dhami et al., Sci. Signal., 2022).

Given the dependence of kinase-impaired BTK mutants on SRC-family signaling, we assessed the activity of the novel HCK/LYN/BTK PROTAC, DFCI-002-06 (Hatcher et al., J. Med. Chem., 2026), in BCWM.1 and TMD8 cells expressing kinase-impaired BTK mutants. DFCI-002-06 suppressed BTK^{Tyr551} phosphorylation, induced degradation of HCK, LYN, and BTK, and inhibited downstream PLC γ 2 and ERK signaling. These signaling effects were accompanied by potent antiproliferative and pro-apoptotic activity *in vitro* and significant tumor-growth inhibition in BTK^{Ala428Asp}- and BTK^{Leu528Trp}-expressing TMD8 xenograft models.

These findings demonstrate that acquired resistance to BTK-targeting agents is associated with mutation-specific compensatory signaling involving SRC-family kinases. Simultaneous degradation of HCK, LYN, and BTK by DFCI-002-06 suppressed compensatory signaling and produced potent antitumor activity in cells expressing kinase-impaired BTK mutants. These results identify HCK and LYN signaling as therapeutically actionable vulnerabilities and support multi-node degradation as a strategy for BTK-directed therapy-resistant MYD88-mutated B-cell malignancies.

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First Name: Shirong

Last Name: Liu

Email: SHIRONG_LIU@DFCI.HARVARD.EDU

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Conference: IWWM13