Scaffold Roles of IRAK1 and IRAK4 in Driving ERK1/2 Pro-Survival Signaling: A Rationale for Developing IRAK1/4 Degraders for the Treatment of Waldenström's Macroglobulinemia and MYD88-Mutated Lymphomas

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Background: Somatic MYD88 L265P mutations are present in 95–97% of Waldenström's macroglobulinemia (WM) patients (NEJM 367:826-33), activating multiple pro-survival pathways, including BTK and IRAK1/IRAK4. Although ibrutinib, a BTK inhibitor, shows high response rates, complete responses are uncommon, suggesting that alternative pathways such as ERK activation (Blood 110:4417–4426; Leukemia 29:169-76) contribute to resistance by bypassing BTK signaling. IRAK1 and IRAK4, key components of MYD88 signaling, may promote survival independently of BTK, making them compelling therapeutic targets. However, the relative importance of their kinase versus scaffold functions in this context remains unclear. This distinction is critical for developing effective therapeutics, such as degraders, that target both scaffold and kinase activities.

Methods: Lentiviral shRNA transduction was used to knock down IRAK1 or IRAK4, and kinase-dead constructs (Nature 470:115–119) were re-introduced to distinguish between their scaffold and kinase functions. Western blotting was employed to evaluate total and phosphorylated signaling proteins; and AlamarBlue® assays were used to assess cell survival following IRAK1 or IRAK4 knockdown.

Results: The roles of IRAK1 and IRAK4 in supporting WM cell survival were investigated. Effective knockdown of IRAK1 and IRAK4 was confirmed by Western blot, and knockdown of either protein significantly reduced tumor cell survival in MYD88-mutated BCWM.1 and TMD8 cells compared to controls (**Figure 1**). Further analysis of downstream signaling revealed an increase in ERK activation (p-ERK) following knockdown of either IRAK1 or IRAK4 (**Figure 2A**). Remarkably, reintroduction of kinase-dead or wild-type IRAK1 or IRAK4 similarly reduced p-ERK levels (**Figure 2B, C**), suggesting that the scaffold functions of IRAK1 and IRAK4, rather than their kinase activities, are essential for the activation of ERK signaling.

Conclusions: MYD88 L265P-mutated cells depend on both IRAK1 and IRAK4 for survival, with their scaffold roles driving ERK-mediated pro-survival signaling. These findings strongly support the development of bifunctional IRAK degraders (PROTACs) that disrupt both the kinase function and scaffold-mediated survival signals, representing a novel therapeutic approach for MYD88-mutated B-cell malignancies.

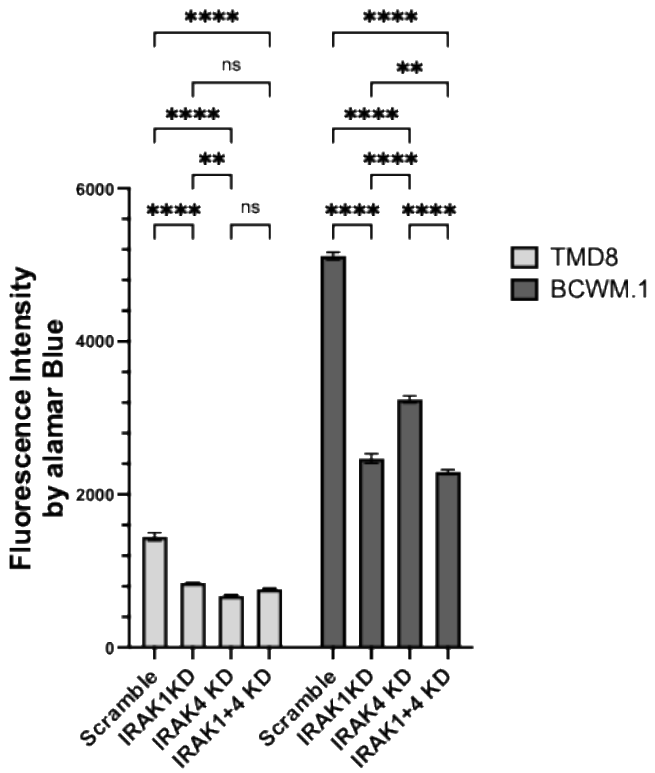

Figure 1. IRAK1 and IRAK4 are crucial for cell survival in MYD88-mutated BCWM.1 and TMD8 cells.

Cell proliferation was assessed using the AlamarBlue assay. Bar graphs show the number of viable cells 72 hours after seeding TMD8 and BCWM.1 cells with knockdown of IRAK1, IRAK4, or both.

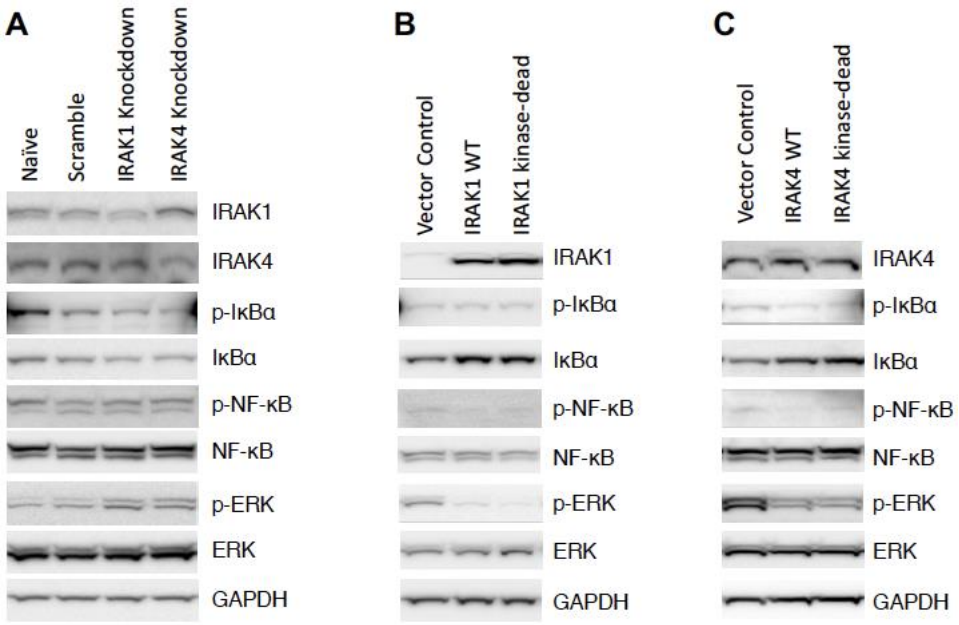

Figure 2. **Scaffold role of IRAK1 and IRAK4 in ERK activation.** The impact of IRAK1 and IRAK4 on IκB-NF-κB and ERK signaling was assessed by Western blot in TMD8 cells. (A) Knockdown of IRAK1 or IRAK4. (B) IRAK1 knockdown followed by rescue with wild-type (WT) or kinase-dead IRAK1. (C) IRAK4 knockdown followed by rescue with wild-type (WT) or kinase-dead IRAK4.