Targeting IRAK1 / IRAK4 Signaling in Waldenström's Macroglobulinemia

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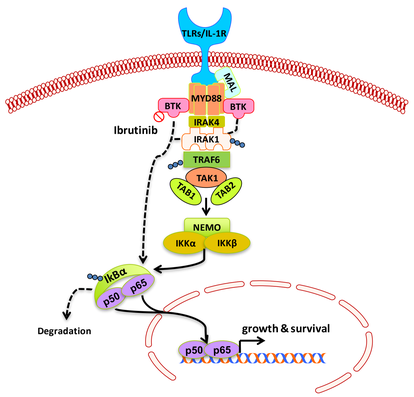
Background

MYD88 L265P somatic mutations are highly prevalent in Waldenström's macroglobulinemia (WM) (*NEJM* 367(9): 826-33). MYD88 L265P activates multiple downstream signaling pathways including BTK and IRAK4/IRAK1 that support malignant cell growth and survival (*Blood* 122(7): 1222-32; *Nature* 470(7332): 115-9). Ibrutinib targets BTK, and shows high overall and major clinical response rates, though no complete responses are observed indicating alternative survival signaling.



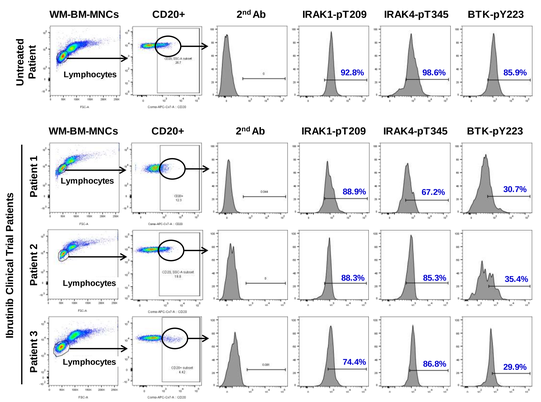
Methods

PhosFlow analysis of IRAK1, IRAK4, and BTK was performed in primary WM cells taken from untreated WM patients, and those on ibrutinib therapy.

IRAK1 or IRAK4 knockdown experiments were performed using lentiviral shRNA transduction. Immunoprecipitation, western blot and phospho-flow studies were used to detect protein expression and phosphorylation in WM cells. Cell survival following IRAK4 or IRAK1 knockdown, ibrutinib and/or IRAK4/IRAK1 inhibitor (EMD Millipore) treatment was assessed by Annexin V staining, AlamarBlue® Cell Viability Assay or *CellTiter-Glo*® Luminescent Cell Viability Assays.

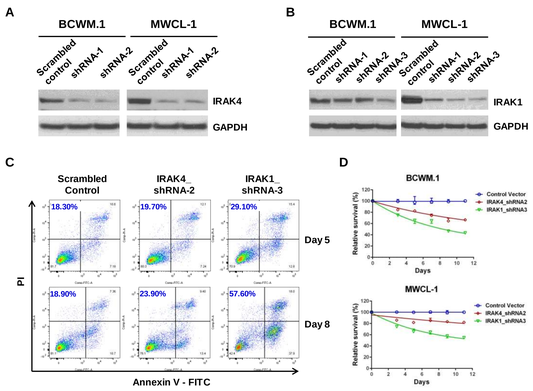
Results

Bone marrow lymphoplasmacytic cells taken from WM patients following more than 6 months of continued ibrutinib treatment indicates IRAK1 remain activated.



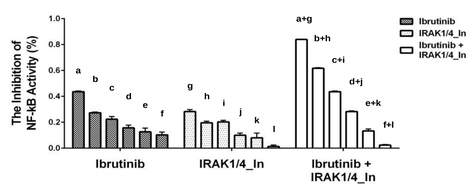
Phospho-flow analysis indicates IRAK1 was still hyper-phosphorylated in primary lymphoplasmacytic cells in WM patient following 6 cycles of ibrutinib therapy.

More pronounced apoptosis, as well as sustained reduction in tumor cell growth occurred following knockdown of IRAK1 versus IRAK4



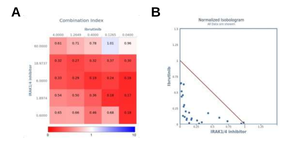
The knockdown of efficiency for IRAK4 (A) or IRAK1 (B) was confirmed by western blot in MYD88-L265P expressing BCWM.1 and MWCL-1 cell lines. Apoptosis analysis by flow cytometry with annexin V / PI staining (C) and cell viability analysis by AlamarBlue® assay (D) indicate more robust cell death in IRAK1 knockdown cells versus IRAK4 knockdown cells.

Treatment of MYD88 mutated WM cells with ibrutinib and a toolbox IRAK4/IRAK1 inhibitor resulted in more robust reductions in NF-kB signaling

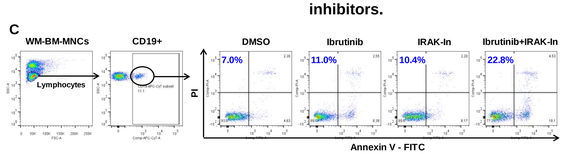


NF-kB Luciferase Reporter Assay was performed in BCWM.1 cells. The NF-kB activity was measured following the treatment with ibrutinib, IRAK1/4 inhibitor alone or in combination. The drug doses were used as: a, 5.00; b, 1.58; c, 0.50; d, 0.16; e, 0.05; f, 0.016 (μM); g, 20.00; h, 6.30; I, 2.00; j, 0.63; k, 0.20; l, 0.06 (μM)

Treatment of MYD88 mutated WM cells with ibrutinib and IRAK4/IRAK1 inhibitor resulted in synergistic tumor cell killing in WM cell lines and at least additive tumor cell killing in the presence of microenvironment supporting cells in WM patient bone marrow mononuclear cells.



The synergism was determined by combination index (CI<1, A) and isobologram (below the oblique red line, B) analysis on the tumor cell killing in WM cells with ibrutinib and IRAK1/4 inhibitors.



The apoptosis of primary WM patient bone marrow tumor cells was determined by Annexin V / PI staining following treatment with ibrutinib (0.5μM), IRAK1/4 inhibitor (5.0μM) alone or in combination in whole bone marrow mononuclear cells for 18 hrs.

Conclusion

MYD88 L265P mutated WM cells show greater dependence on IRAK1 versus IRAK4 directed signaling. IRAK1/IRAK4 signaling may contribute to persistent WM cell survival following ibrutinib treatment. Combined BTK and IRAK inhibition leads to augmented blockade of NFKB signaling and enhanced WM cell killing. The studies provide a framework for the development and investigation of IRAK inhibitors, alone and in combination with ibrutinib in WM patients.