PI3K/AKT Pathway Is Activated by MYD88 L265P and Use of PI3Kδ Inhibitor Induces Robust Tumor Cell Killing in Waldenstrom's Macroglobulinemia

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Background

Waldenstrom's macroglobulinemia (WM) is a distinct B-cell lymphoma resulting from the accumulation, predominantly in the bone marrow, of clonally related IgM secreting lymphoplasmacytic cells (LPCs). MYD88 L265P is a somatic mutation present in more than 90% of Waldenstrom's Macroglobulinemia (WM) patients. The MYD88 L265P mutant was reported to promote malignant cells growth and survival in ABC type Diffuse Large B-cell Lymphoma (DLBCL) (Ngo et al, *Nature* 2011) and WM (Yang et al, *Blood* 2013). The MYD88 L265P mutant assembled a signaling complex that simultaneously triggers IRAK1 and BTK, leading to downstream NF-kB activation in supporting of WM cells growth and survival (Treon et al, *NEJM* 2012; Yang et al, *Blood* 2013). In addition to IRAK1 and BTK, the downstream signaling pathways remain to be fully clarified.

Methods

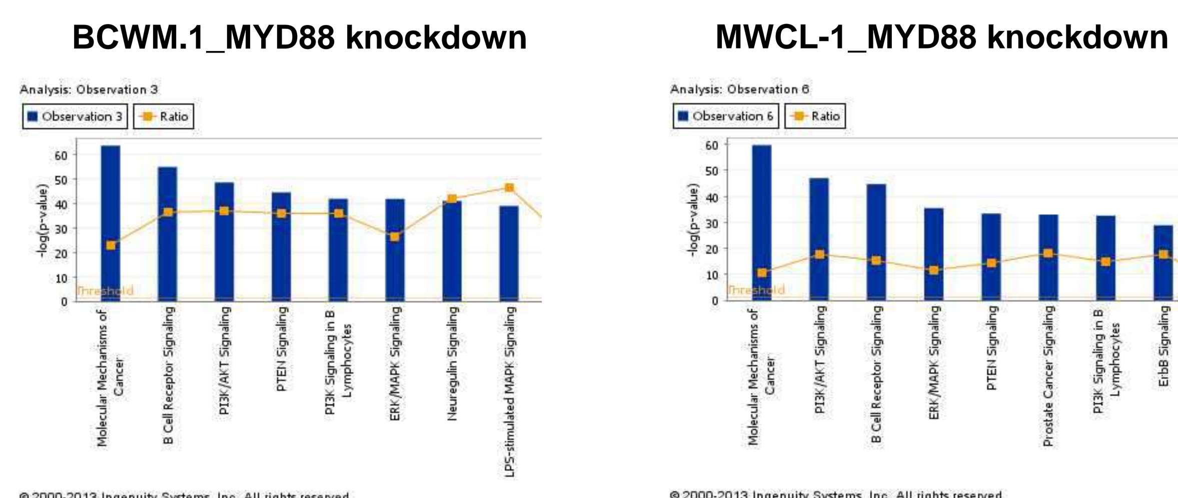
To further clarify the downstream signaling associated with MYD88 L265P in WM cells, we employed Phospho Explorer Antibody Arrays in MYD88 L265P expressing BCWM.1 and MCWL-1 WM cells following lentiviral mediated knockdown of MYD88, or over-expression of MYD88 L265P; or the use of MYD88 homodimerization inhibitor that block MYD88 signaling.

Arrays were scanned by Axon GenePix Microarray Scanner and data analyzed by Ingenuity Pathway Analysis. Western blot analysis was performed using total and phospho-specific antibodies in WM cells. CellTiter-Glo® Luminescent cell viability assay (Promega) was used to assess cell survival following treatment with the PI3K-delta inhibitor, CAL-101 (Idelalisib, GS-1101) (Selleck Chemicals).

Results

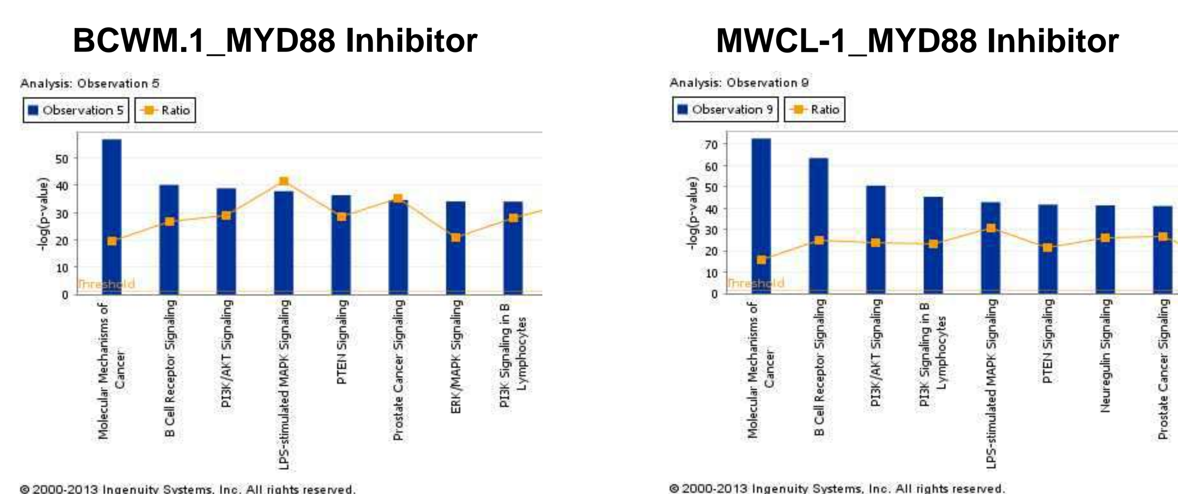
Modulation of MYD88 affected downstream signaling proteins that involved in canonical BCR, PI3K / AKT and ERK / MAPK signaling in MYD88-L265P expressing WM cells.

BCWM.1_MYD88 knockdown | MWCL-1_MYD88 knockdown



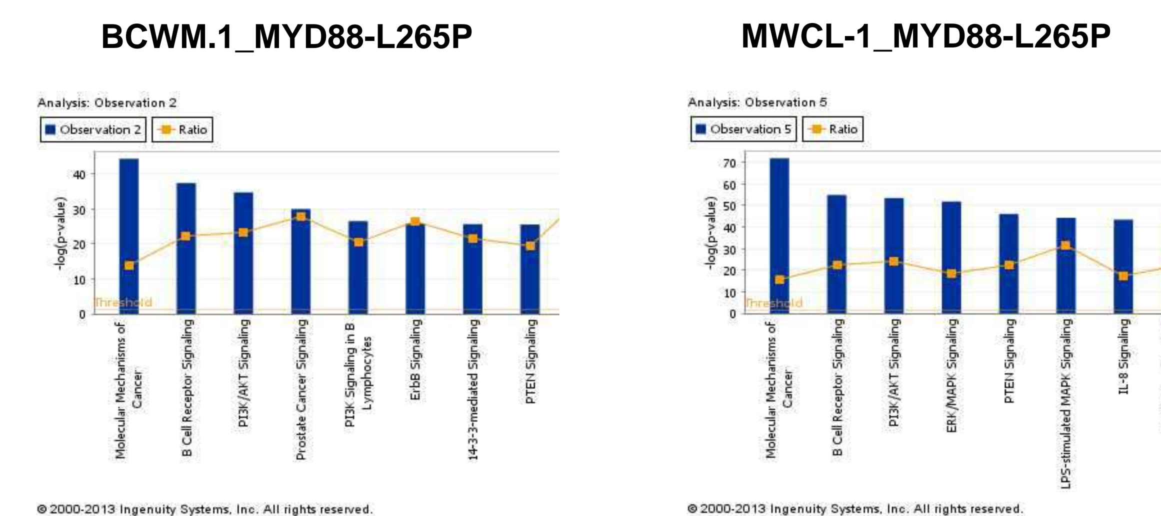
Top canonical pathways in Inguity Pathway Analysis that affected by MYD88 knockdown in WM cells

BCWM.1_MYD88 Inhibitor | MWCL-1_MYD88 Inhibitor



Top canonical pathways in Inguity Pathway Analysis that affected by MYD88 homodimerization inhibitor in WM cells

BCWM.1_MYD88-L265P | MWCL-1_MYD88-L265P



Top canonical pathways in Inguity Pathway Analysis that affected by MYD88-L265P overexpression in WM cells

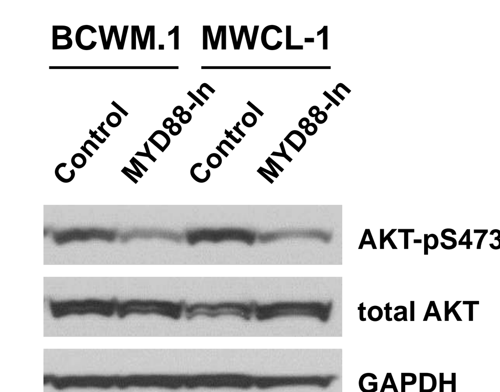
Table1. Signaling proteins that demonstrated the greatest changes in phosphorylation following MYD88 modulations

Canonical Signaling	MYD88 knockdown	MYD88-L265P overexpression	MYD88 inhibitor
B Cell Receptor Signaling	PLCG1(–)	BTK(+)	PLCG1(–)
PI3K / AKT Signaling	AKT1(–), TP53(–)	FOXO1(–), FOXO3(–), TP53(+)	mTOR(–)
ERK / MAPK Signaling	RELA(–)	IKBKG(+), MAP3K5(+), MAPKAPK2(+)	RELA(–), CHUK(–), MAP3K5(–), NFKB1(–)

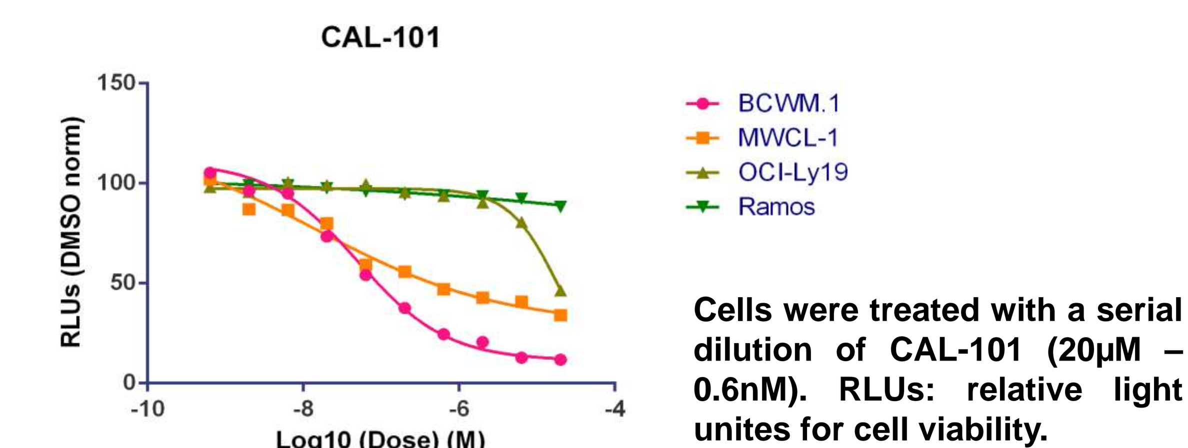
(+): up-regulation of phosphorylation.
(–): down-regulation of phosphorylation.

The PI3K downstream signaling protein ATK is highly activated, and its phosphorylation is significantly decreased by the application of a MYD88 homodimerization inhibitor in WM cells.

Western blot analysis confirmed the reduction of ATK phosphorylation (downstream of PI3K) in MYD88-L265P expressing WM cells following treatment of cells with an inhibitor of MYD88 homodimerization (MYD88-In).

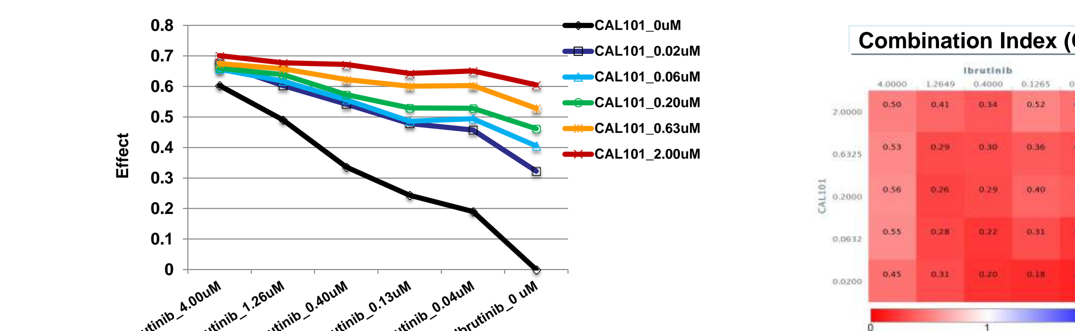


Cell viability analysis demonstrated robust tumor cell killing following treatment with the PI3Kδ inhibitor CAL-101 (Idelalisib, GS-1101) in MYD88 expressing WM cells.



Cells were treated with a serial dilution of CAL-101 (20μM – 0.6nM). RLUs: relative light unites for cell viability.

CAL-101 shows synergistic tumor cell killing in combination with the BTK inhibitor Ibrutinib.



The combination effect curves shows robust tumor cell killing when combine CAL-101 with Ibrutinib in WM cell line BCWM.1

Combination Index (CI) < 1 : Synergism

Conclusion

In addition to activation of NF-kB through IRAK and BTK signaling, MYD88 L265P also promotes enhanced survival of WM cells also by activation of PI3K/AKT signaling. Inhibition of PI3K is associated with robust killing of WM cells. The combination of PI3Kδ inhibitor, CAL-101, with the BTK inhibitor, Ibrutinib resulted in synergistic WM tumor cell killing. These studies provide the framework for the investigation of PI3Kδ inhibitors, alone and in combination with Ibrutinib in WM.