BTK participates in MYD88 signaling in malignant cells expressing the L265P mutation in Waldenstrom's Macroglobulinemia, and shows robust tumor cell killing with the BTK-inhibitor PCI-32765 in combination with MYD88 pathway inhibitors

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Background

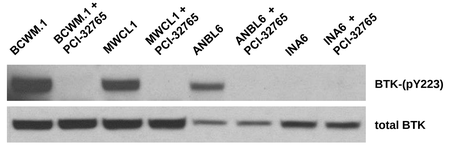
Waldenstrom's macroglobulinemia (WM) is a distinct B-cell lymphoma resulting from the accumulation, predominantly in the bone marrow, of clonally related IgM secreting lymphoplasmacytic cells (LPCs). Bruton's tyrosine kinase (BTK) promotes B-cell receptor signaling along with B-cell expansion and survival through NF-κB and MAPK. MYD88 L265P is a widely expressed somatic mutation in tumor cells from WM patients. MYD88 L265P promotes enhanced tumor cell survival through IRAK 1/4 mediated NF-κB and MAPK signaling. We therefore sought to clarify the role of BTK signaling in MYD88 L265P expressing WM cells, and the impact of BTK and MYD88/IRAK inhibition on WM cell signaling and survival.

Patients and Methods

Western blot analysis was performed using total and phospho-specific antibodies in MYD88 L265P expressing WM cells, BCWM.1 and MCWL-1 following MYD88 knockdown by lentiviral transduction, and/or use of MYD88 or IRAK signal inhibitors. Cells were also treated with the BTK inhibitor PCI-32765, in the presence or absence of MYD88 homodimerization or IRAK1/4 inhibitors. Annexin V / PI staining was used to assess cell survival, and synergism assessed with CalcuSyn software.

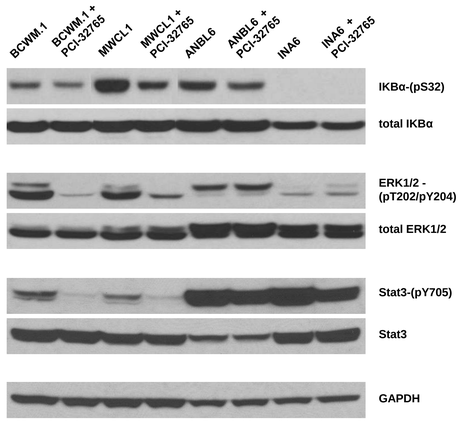
Results

BTK was highly expressed and phosphorylated in MYD88-L265P expressing WM cells and PCI-32765 significantly blocked the BTK activation.



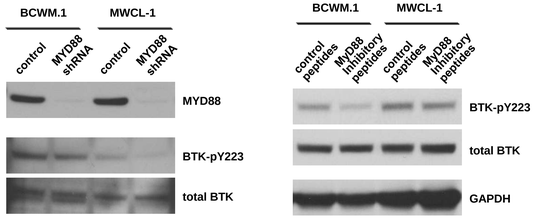
Increased phosphorylation of BTK was confirmed by western blotting with phospho-specific antibody in Waldenstrom's Macroglobulinemia (WM) cell lines, BCWM.1 and MWCL-1, compared to Multiple myeloma cell lines, ANBL6 and INA6. Antibody against total BTK was used as loading control. PCI-32765 significantly blocked the BTK phosphorylation in WM cells.

PCI32765 significantly reduced downstream NF-kB, MAPK and STAT3 signaling in WM cells.



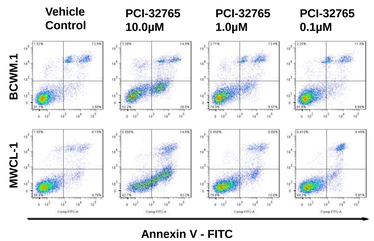
In addition to significantly blocked the BTK activation, PCI-32765 also blocked the downstream NF-kB, MAPK, Stat3 signaling by significantly reduced the phosphorylation of IKBα, ERK1/2 and Stat3 proteins in WM cell lines, BCWM.1 and MWCL-1, compared to multiple myeloma cell lines, ANBL6 and INA6. Antibodies against corresponding total proteins and GAPDH were used as loading controls.

Knockdown of MYD88 by lentiviral transduction, and/or use of a MYD88 inhibitor leads to decreased BTK phosphorylation.



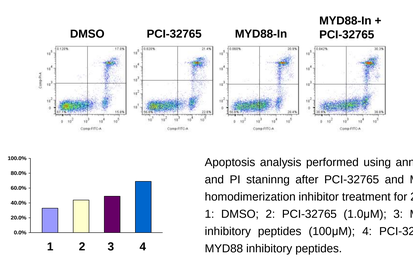
MYD88 knockdown was confirmed by western blot in BCWM.1 and MWCL-1 cells. The knockdown of MYD88 reduced BTK phosphorylation compared with controls. MYD88 homodimerization inhibitory peptides significantly reduced BTK phosphorylation compared with control peptides. Antibodies against total BTK and/or GAPDH were used as loading control..

Treatment with PCI-32765 induces apoptosis of MYD88 L265P expressing WM cells



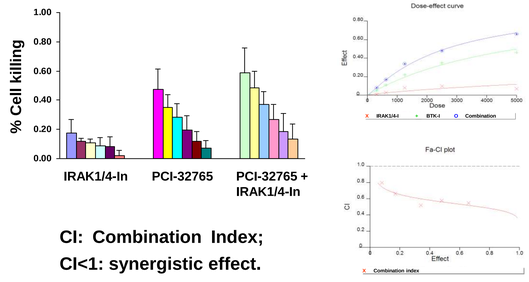
Apoptosis analysis performed using Annexin V and PI staining after PCI-32765 treatment for 24hrs.

PCI-32765 shows robust tumor cell killing in combination with a MYD88 pathway inhibitor in primary WM patients bone marrow tumor cells.



Apoptosis analysis performed using annexin V and PI staninng after PCI-32765 and MYD88 homodimerization inhibitor treatment for 24hrs. 1: DMSO; 2: PCI-32765 (1.0μM); 3: MYD88 inhibitory peptides (100μM); 4: PCI-32765 + MYD88 inhibitory peptides.

PCI-32765 shows synergistic tumor cell killing in combination with an IRAK 1/4 kinase inhibitor.



CI: Combination Index;
CI<1: synergistic effect.

Conclusion

BTK activation is facilitated by MYD88 pathway signaling in MYD88 L265P expressing WM cells, and participates in MYD88 downstream signaling. Inhibition of BTK by PCI-32765 leads to robust tumor killing of MYD88 L265P expressing WM cells, which is potentiated by MYD88 pathway inhibitors. These studies provide the framework for the investigation of BTK inhibitors in WM, as single agents and in combination with MYD88 pathway inhibitors.

MYD88 and BTK interaction and downstream signaling pathways.