Acquisition of BTK C481S Produces Resistance to Ibrutinib in MYD88 Mutated WM and ABC DLBCL Cells that is Accompanied by ERK1/2 Hyperactivation, and is Targeted by the Addition of the ERK1/2 Inhibitor Ulixertinib.

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Background

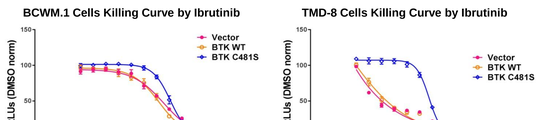
Activating Toll / interleukin-1 receptor homology (TIR) domain mutations in MYD88 trigger BTK (Yang et al, Blood 2013; Wilson et al, Nat. Med. 2015) and HCK (Yang et al, Blood 2016) activation in Waldenström's macroglobulinemia (WM) and ABC-DLBCL, which activate multiple downstream pro-survival signaling cascades that include NFkB, AKT, and ERK1/2. Ibrutinib targets BTK and HCK, and shows high levels of activity in MYD88 mutated WM and ABC DLBCL. Resistance to Ibrutinib has been observed in CLL, MCL, and in WM (Xu et al, submitted) due to acquisition of mutations that impact the binding of Ibrutinib to BTK at Cysteine 481, which include the C481S mutation.

Methods

We transduced MYD88 L265P mutated BCWM.1 and MWCL-1 WM, and TMD-8 and HBL-1 ABC DLBCL cell lines with either lentiviral vector alone, or lentiviral vectors expressing BTK wild-type (BTK WT) or BTK with C481S (BTK C481S) mutation. Drug effects on signaling pathways downstream of BTK were evaluated by western blots. Cell viability under drug treatment was determined using CellTiter-Glo® Luminescent Cell Viability Assay. Apoptosis analysis was done by annexin V / PI staining. Combination indices for synergism evaluations were calculated using CalcuSyn software.

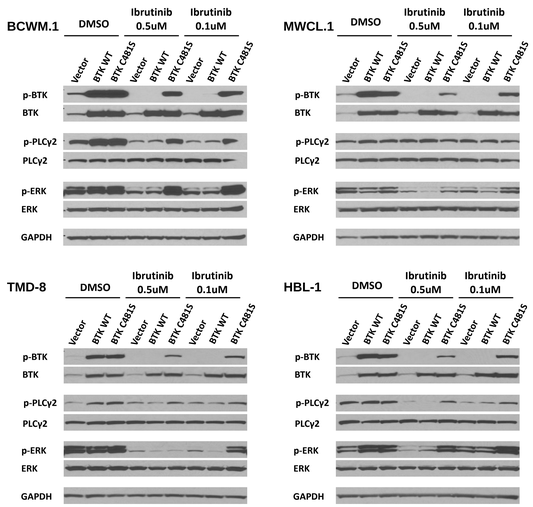
Results

Transduction with BTK C481S led to a 1~3 log fold increase in EC50 for Ibrutinib versus vector only or BTK WT transduced cells.



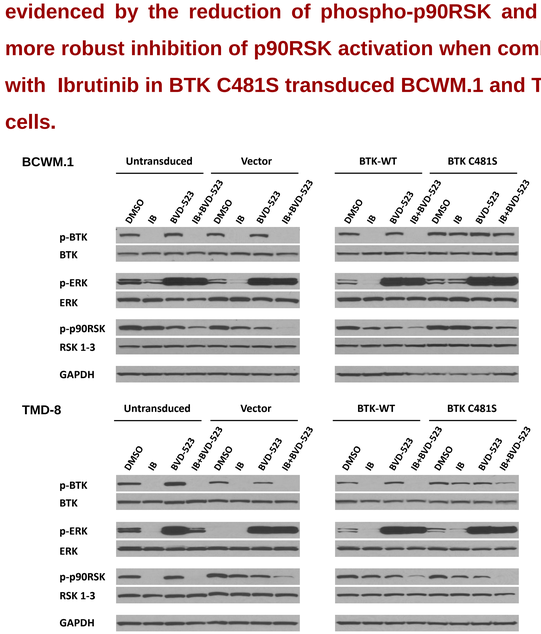
MYD88 mutated BCWM.1 and TMD-8 cells first transduced with control vector or vector expressing wild-type BTK (BTK-WT) or BTK with mutated Ibrutinib binding site (BTK C481S), then treated with Ibrutinib. BTK C481S mutation causes Ibrutinib resistance in both BCWM.1 and TMD-8 cells.

BTK C481S expressing cells displayed persistent activation of BTK and hyperphosphorylation of PLCγ2 and ERK1/2 versus vector only or BTK WT cells following Ibrutinib treatment.



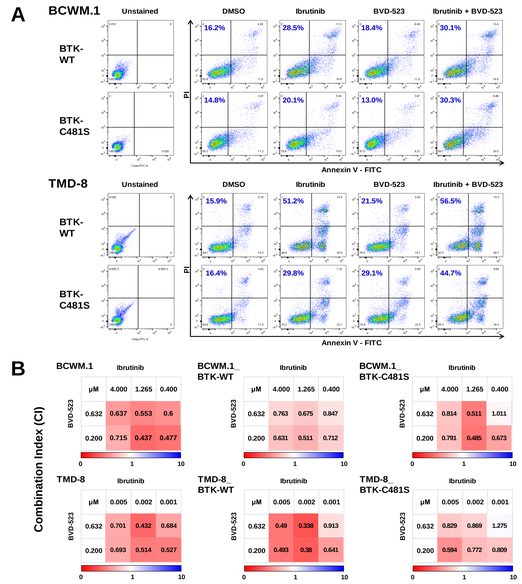
Persistent hyperphosphorylation of BTK and PLCγ2 which is immediately downstream of BTK, as well as ERK1/2 was only seen in all four BTK C481S mutant transduced versions of MYD88 mutated cell lines following Ibrutinib treatment (0.5, 0.1 μM) for 1~2 hours. The corresponding total proteins and GAPDH were used as protein loading controls.

Ulixertinib blocks the persistent activation of ERK1/2 as evidenced by the reduction of phospho-p90RSK and even more robust inhibition of p90RSK activation when combined with Ibrutinib in BTK C481S transduced BCWM.1 and TMD-8 cells.



Ulixertinib (BVD-523, VRT752271), a highly selective ERK1/2 inhibitor that is currently under clinical investigation blocks ERK1/2 activity, but not the phosphorylation of ERK1/2. Phospho-p90RSK, which is immediately downstream of ERK1/2 and is used to gauge the ERK1/2 activity, reduces significantly in the presence of Ulixertinib (0.5 μM), and decreases even more when Ibrutinib (IB, 0.1 μM) is added in combination within 2 hours treatment.

The combination of Ulixertinib with Ibrutinib produced higher levels of tumor cell killing than either agent alone, and showed synergistic interactions at pharmacologically relevant concentrations.



(A). The combination of Ulixertinib (2μM) with Ibrutinib (1μM) induced higher levels of apoptosis than either agent alone in BTK C481S expressing BCWM.1 or TMD-8 cells. The percentage of apoptotic cells were indicated.

(B). The combination of Ulixertinib with Ibrutinib showed synergistic interactions in both BCWM.1 and TMD-8 cells with combination index (CI) values below 1.0 at pharmacologically relevant concentrations.

Conclusion

The acquisition of BTK C481S produces resistance to Ibrutinib in MYD88 mutated WM and ABC DLBCL cells that is accompanied by ERK1/2 hyperactivation. The addition of the ERK1/2 inhibitor Ulixertinib to Ibrutinib at produced synergistic tumor cell killing in BTK C481S Ibrutinib resistant cells. The findings provide rationale for the investigation of ERK1/2 inhibitors in Ibrutinib resistant MYD88 driven WM and ABC DLBCL disease mediated by BTK mutations.