

## **ULK complex blockade elicits NF- $\kappa$ B activation in GCB-DLBCL whilst augmenting cytotoxicity of Ibrutinib**

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Constitutive nuclear factor kappa B (NF- $\kappa$ B) activity is a hallmark feature in Diffuse Large B Cell Lymphomas (DLBCL) subtype Activated B Cell (ABC). NF- $\kappa$ B sustains activity in ABC due to chronic B Cell Receptor (BCR) signalling and NF- $\kappa$ B mutation(s). Germinal Centre B Cell Lymphomas (GCB) exhibits tonic BCR signalling promoting P3K-AKT-mTOR pathway activation. Albeit, a small subset of GCB patients harbour NF- $\kappa$ B addiction. BCR activity links to Bruton Tyrosine Kinase (BTK) in DLBCL. Ibrutinib is a BTK inhibitor and demonstrated preferential activity in ABC, not GCB. However, phase III Phoenix clinical trial[1] contradicted this. Autophagy elicits pro-survival and nutritional adaptation in malignant cells, whilst contributing to chemoresistance.

In this study, Oci-Ly1 cells (GCB) demonstrated partial to poor response to Ibrutinib (t=24hr, IC50: 14 $\mu$ M). Autophagy initiation complex inhibitors: MRT68921 (ULK1 complex, t=24hr, IC50: 2.5 $\mu$ M) and VPS34-IN-1 (VPS34, t=24hr, IC50: 5 $\mu$ M) enhanced the anti-tumour activity of Ibrutinib (1 $\mu$ M, t=24hr). Ibrutinib reduced the cellular viability of lymphoma cells by 28.7%, and MRT68921 by 35.5%. In comparison, Ibrutinib+ MRT68921 significantly decreased the cellular viability by 61.3%. Oci-Ly1 cells (basal) and Ibrutinib treatment demonstrated LC3B dependent autophagic flux. MRT68921 and combination treatment suppressed this. Cellular proliferation (t=8 and 24hrs) revealed Ibrutinib+ MRT68921 had considerably reduced to 74.1% (t=8hr) and 59.4% (t=24hr). Similar findings were observed using VSP34-IN-1. ULK complex blockade significantly reduced the colony forming capacity of lymphoma cells in comparison to VPS34-IN-1+ Ibrutinib (t=24hr). In both instances, apoptosis was induced via caspase 8, 9 and 3/7 (t=8hr). ULK blockade promoted the activation of NF- $\kappa$ B in a time dependent manner. After t=2hr IKB $\alpha$  activity declined, though a significant amount of phosphorylated p65 was identifiable in Oci-Ly1 cells. Oci-Ly3 cells (ABC) confirmed these findings. Endoplasmic reticulum sensor PERK modulated NF- $\kappa$ B activation. VPS34 blockade did not activate canonical/ non-canonical NF- $\kappa$ B, likely excluding a stress response.