Elucidating the role of PIM kinase and its therapeutic potential in NSCLC

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Introduction

PIM kinases are a family of three serine/threonine kinases: PIM1, PIM2 and PIM3 that have been shown to play a role in tumorigenesis. PIM1 is a downstream effector of oncoproteins ABL and JAK/STAT and regulator of BCL2/BAD and CXCR4. PIM activity is synergistic with the PI3K/Akt/mTOR pro-survival pathway and PIM2 has been shown to phosphorylate translational repressor 4E-BP1 and p70S6 independently of the PI3K pathway. Furthermore a synergism between PIM kinases and c-Myc has been reported. Here we investigate the expression of Pim-1/Pim-2/Pim-3 in NSCLC cell lines and patient matched normal/tissue samples. The effect of a novel combined inhibitor of PI3K/mTOR/PIM kinases (IBL-301) on cell signalling, cell viability is also examined.

Methods

•Pim-1/Pim-2/Pim-3 expression were examined by Western blot analysis in NSCLC cells lines.

•Additionally, the frequencies of Pim-1/Pim-2/Pim-3 expression in NSCLC patient tumour and matched normal adjacent samples (n=31) were investigated.

•The effectiveness of the novel inhibitor of PI3K/mTOR/PIM kinase, IBL-301, on cell signalling and cell viability were examined by Western blotting analysis and CellTitre Blue assay respectively.

Results: Expression of Pim kinase isoforms in NSCLC cell lines

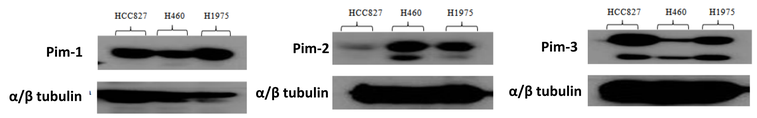


Figure 1: Expression of Pim isoforms in NSCLC cells lines.
Pim-1, Pim-2 and Pim-3 protein expression were measured in cell lysates of NSCLC cell lines (HCC827, H460 and H1975) by Western blotting analysis using antibodies specific for each isoform. All three forms of Pim protein were expressed in the cell lines tested.

Results: Expression of Pim kinase isoforms in lung cancer patients

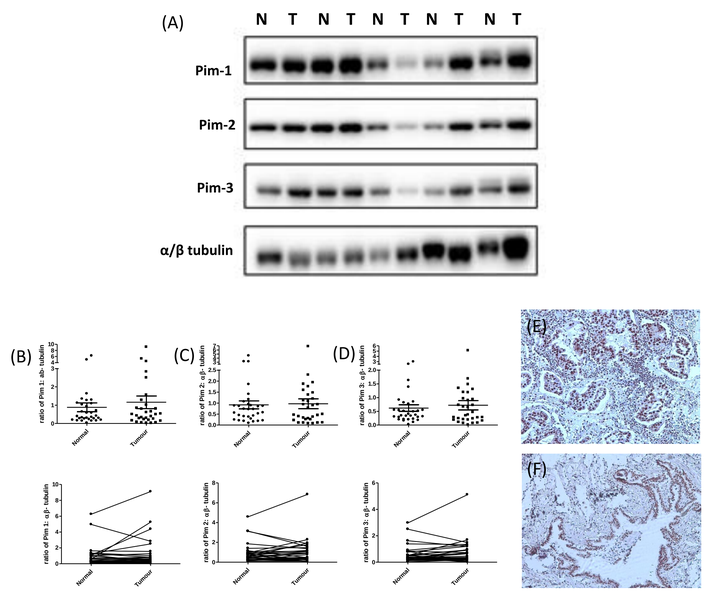


Figure 2: Expression of Pim kinase isoforms in NSCLC patient tumour tissue and matched normal tissue.
Pim-1, Pim-2 and Pim-3 expression was measured in total tissue lysates of matched tumour (T) and normal (N) tissue from NSCLC patients (n=31). All three Pim isoforms were detected in all patient samples and the expression pattern observed did not differ between the three kinase isoforms (see representative Western blot of 5 patient in (A)). As indicated by the scatter plots and paired scatter plots, the mean level of (B) Pim-1, (C) Pim-2 and (D) Pim-3 expression was similar between normal and tumour tissue samples. The localisation of Pim-1 protein expression was investigated by IHC in FFPE full-face tissue blocks in a subset of these patients. (E) Nuclear and cytoplasmic staining was observed in areas of carcinoma. (F) Additionally Pim-1 staining was noted in non-cancerous bronchiolar tissue and alveolar macrophages which would account the observed expression of Pim in normal lung tissue samples by Western blotting.

Results: Effect of IBL-301 on NSCLC cell viability

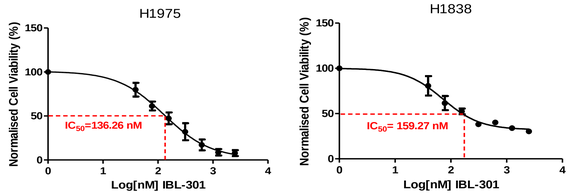


Figure 3: Novel PI3K/mTOR/PIM inhibitor and inhibits cell viability in NSCLC cell lines
IBL-301 was shown to have a dose response effect on H1975 and H1838 cell viability as measured by the CellTitre Blue assay, following 72 hour treatment. The determined IC50 doses were 136.26nM and 156.27nM, respectively (n=3).

Results: Alterations to PI3K/mTOR signalling by IBL-301 in NSCLC cell lines

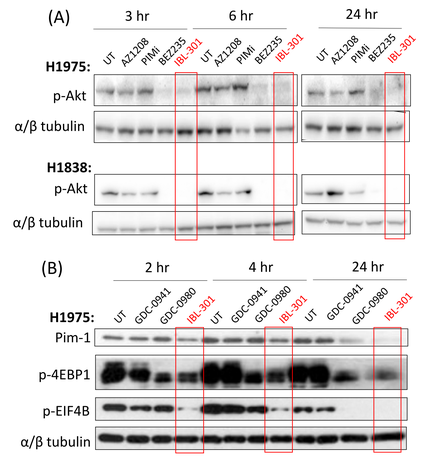


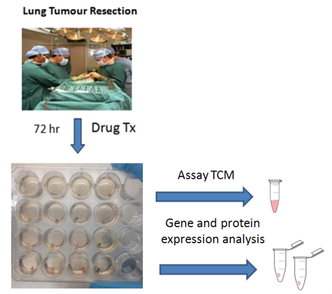
Figure 4: Novel PI3K/mTOR/PIM inhibitor decreases pAkt and downstream signalling
In Panel (A) H1975 and H1838 cells were treated with PIM kinase targeting inhibitors AZ1208 and PIMi at 2μM, and PI3K/mTOR inhibitor BEZ235 at 250nM, and PI3K/mTOR/PIM inhibitor IBL-301 at 250nM for the indicated time points. IBL-301 blocked phosphorylation of Akt at all time points in both cell lines (see red boxes). (B) Additional signalling work with H1975 demonstrated a decrease in phosphorylated 4EPB1 and EIF4B following treatment with IBL-301 (see red boxes).

Conclusion

This is the first study to investigate the expression of all 3 isoforms of Pim kinase in lung cancer specifically. All isoforms were abundantly expressed across cells lines and patient tumour samples. Observed Pim kinase expression in the immune cells of normal adjacent tissue may indicate a role in inflammation. These findings coupled with the promising *in vitro* data using the novel PI3K/mTOR/PIM-targeting inhibitor IBL-301 demonstrate the therapeutic potential of targeting PIM in NSCLC.

Future Directions

We are currently investigating the effect of novel compound IBL-301 and the clinically relevant PI3K/mTOR inhibitor BEZ235 in an *ex vivo* lung tumour model. In this model fresh tumour biopsies are cultured for 72 hours with the compounds at a chosen dose. Once we have sufficiently increased the sample study size, the biobanked tissue and tissue conditioned medium (TCM) will be used for down stream studies on alterations to gene and protein expression and the secretome.



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