933: Targeting PIM kinase in NSCLC

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PIM Kinase

- PIM kinase expression is regulated by JAK-STAT and NF-KB1 pathways.
- Promotes pro-survival signaling through regulation of BCL2 family.
- Synergistic activity of PIM1 & c-MYC in tumours.
- Positive regulation of cell cycle progression through regulation of p21/p27.
- Increased cap-dependent protein synthesis through phosphorylation and thus inhibition of translation repressor 4E-BP1.
- Once expressed PIM kinases are active; regulation occurs at the transcriptional and translational levels.
- PIM kinase and PI3K/AKT converge on regulation of TORC1/2/4E-BP1 pathway and cap dependent translation.
- Resistance to PI3K inhibition could be due to parallel signalling through the PIM kinase pathway.

PIM Regulation of Tumour Progression

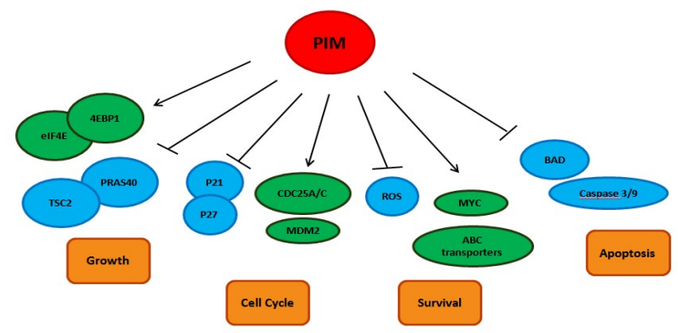


Figure 1 PIM substrates regulate cellular processes that are critical for tumour progression and therapeutic resistance, making PIM an ideal target for cancer therapy.

PIM proteins belong to a family of serine/threonine kinases composed of 3 isoforms, PIM1, PIM2 and PIM3, that play a key role in cell cycle regulation, have potent anti-apoptotic activity and play a role in the homing and migration of metastatic cells. Furthermore, PIM kinases have also been shown to be activated in response to Akt pathway inhibition, indicating a role in adaptive responses to inhibition of this pathway potentially leading to treatment resistance. Thus, there is a strong rationale for combining PIM kinase inhibition with inhibition of the Akt pathway (i.e., inhibitors of EGFR, PI3K, Akt and mTOR). PIM kinase has been recognised as a therapeutic target particularly in haematological malignancies however the role of PIM kinases in solid tumours and NSCLC in particular are less well characterised. This study is the first to elucidate the expression of all 3 PIM isoforms in NSCLC cell lines and patient tumours as well as to examine the effect of Inflection Bioscience Ltd novel dual PI3K/PIM kinase (IBL-202) [redacted] targeted therapies *in-vitro* and *in-vivo*.

PIM 1/2/3 protein expression was quantified by western blot analysis in a panel of NSCLC cell lines (with different mutation profiles) and 40 matched normal/tumour tissues from NSCLC patients (20 adenocarcinoma and 20 squamous cell carcinoma). Immunohistochemical analysis is ongoing in NSCLC patient TMAs and will be correlated to patient clinicopathological characteristics and survival data. The effectiveness of IBL-202 [redacted] on proliferation and apoptosis in NSCLC cell lines were examined by BrdU and Annexin V/PI FACS analysis, respectively. A head-to-head *in-vivo* study was completed with GSK-2126458 (PI3K-mTOR inhibitor) versus [redacted] in a mouse model.

PIM1/2/3 Expression in NSCLC Cell Lines

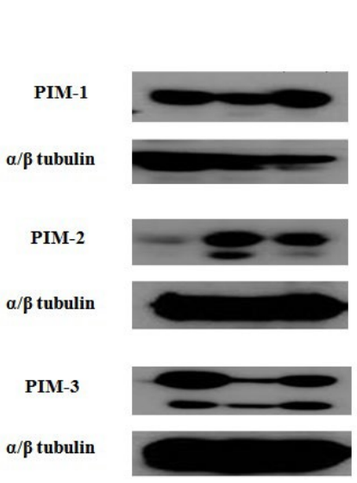


Figure 2 PIM1, PIM2 and PIM3 expression in a panel of NSCLC cell lines.
Protein was isolated from a panel of NSCLC cell lines with different mutation profiles and PIM1, PIM2 and PIM3 protein expression were quantified by Western blot analysis. All three isoforms are expressed in the cell lines. Initial data indicates there is a compensatory mechanism of expression between the isoforms.

PIM1/2/3 Expression NSCLC Normal/Tumour Patient Tissues

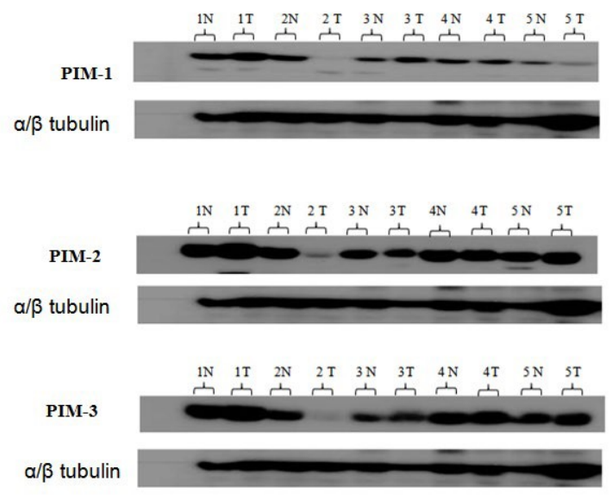


Figure 3 PIM1, PIM2 & PIM3 expression in matched normal (N) and tumour (T) tissues from NSCLC patients.
Protein was isolated from matched normal/tumour tissue from 40 resected NSCLC patients (20 adenocarcinoma & 20 squamous cell carcinoma) and PIM1, PIM2 and PIM3 protein expression were quantified by Western blot analysis.

Overlapping Signalling Mechansims of Akt & PIM kinase

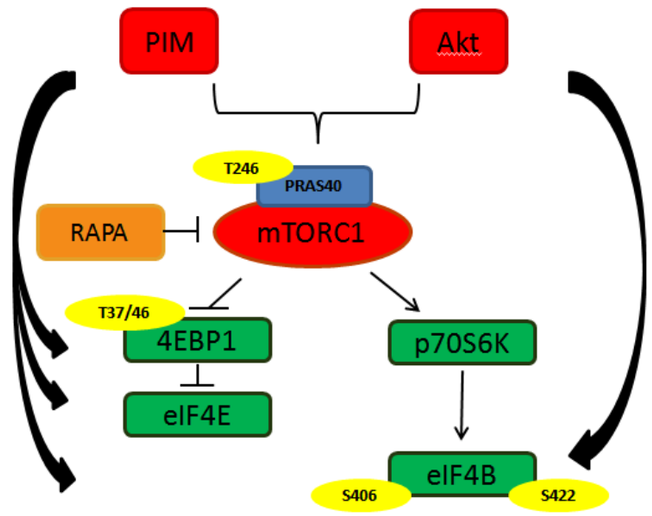


Figure 4 Akt & PIM kinases control translation & cell growth through distinct & overlapping mechanisms.
Compensatory signaling via PIM kinase is a bypass mechanism of resistance to PI3K/AKT inhibition hence there is a rationale for simultaneously targeting both pathways.

Effect of PI3K, PIM kinase, PI3K/PIM kinase [redacted] inhibitors on Proliferation in Cancer Cell Lines

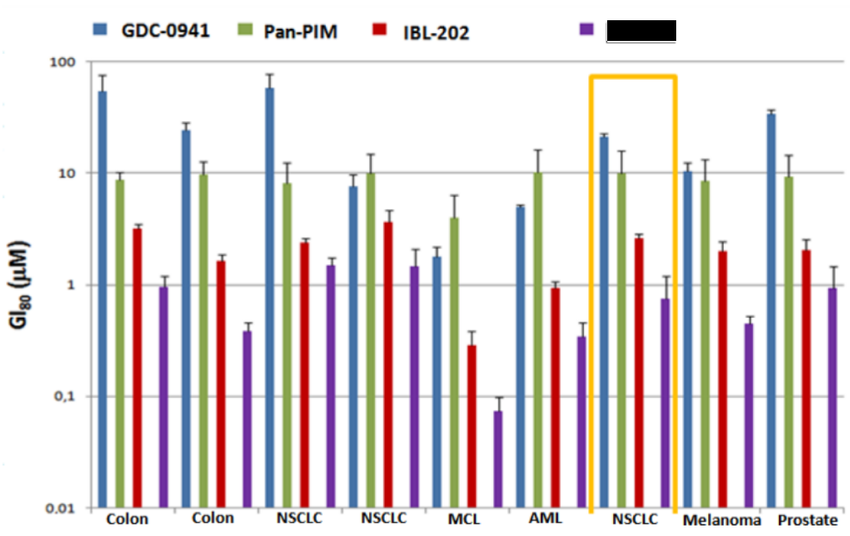
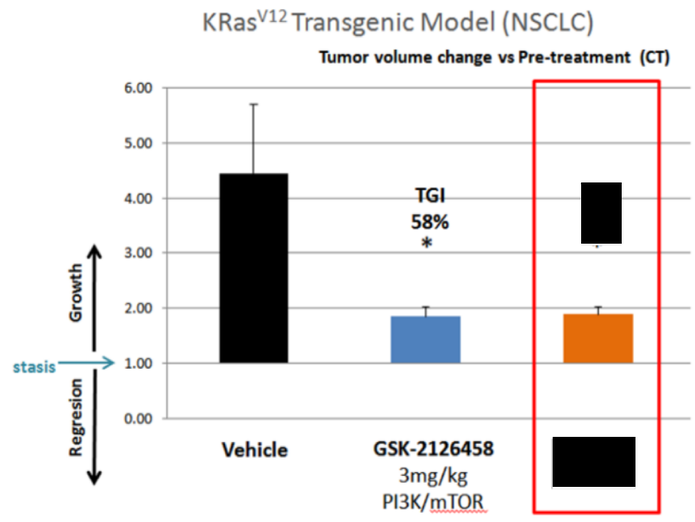


Figure 5 Comparison of the effect of GDC-0941, pan-PIM, IBL-202 [redacted] inhibitors on proliferation in a panel of cancer cell lines.
GDC-0941 and pan-PIM have similar efficacy . Dual PI3K/PIM [redacted] (IBL-201 [redacted]) inhibition drives significant efficacy benefit in all NSCLC cell lines.

In-vivo Efficacy of GSK-2126458 (PI3K/mTOR inhibitor) [redacted]



The K-*Ras* mutation was induced by i.p.tamoxifen in one month old mice and six and a half months later when lung tumors developed, mice were treated orally with **GSK-2126458 3mg/kg [redacted], 5 days a week for 3 weeks** Computed tomography (CT) scans of K-*Ras*$^{+/LSLG12Vgeo}$; RERT $^{ert/ert}$ conditional mice before and after treatment was done . Tumor volumes of five mice in each treatment group are shown as percentage of relative change to pretreatment tumor volumes. Values are means ± s.e.m.

Figure 6 *In-vivo* Efficacy of GSK-2126458 (PI3K/mTOR inhibitor) [redacted].
[redacted] achieved comparable efficacy with the GSK-2126458 (near MTD) compound in a KRAS^{V12} transgenic mouse model.

CONCLUSIONS

- [redacted] All three isoforms of PIM kinase are expressed in a panel of NSCLC cell lines.
- PIM kinase is expressed in ~ 90% of NSCLC tumour tissues across all stages of the disease.
- PIM kinase is a promising new therapeutic target for the treatment of NSCLC patients.
- Dual PI3K/PIM kinase (IBL-202) [redacted] targeted therapies have demonstrated pro-apoptotic and anti-proliferative activity *in-vitro* and *in-vivo* and should be considered in the treatment of NSCLC patients.

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