Combined inhibition of PIM and PI3 kinases shows an enhanced efficacy in a number of solid tumour cell lines

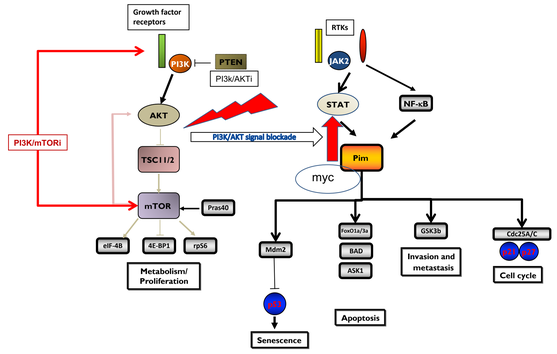
O'Neill, M[1], Blanco Aparicio, C. [2], Jiang S [3], Martinez S. [2], McKenzie A. [3], Page, M.[1], Pastor, J.[2]

1 Inflection Biosciences Ltd, Suite 15, Angelsea House, Carysfort Avenue, Blackrock, Co. Dublin, Ireland. 2 Experimental Therapeutics Programme, Spanish National Cancer Research Centre (CNIO). 3 Precos Ltd,, Loughborough LE12 9TE, UK. moneill@inflectionbio.com

2014 AACR POSTER A#4524

INFLECTION BIOSCIENCES

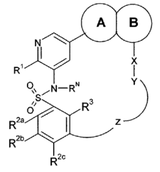
Rationale for Co-targeting PI3K/AKT, mTOR and PIM Pathways



- PIM kinases play an important role in a number of aspects of the regulation of cell cycle and proliferation. Over-expression of PIM kinases is associated with oncogenicity in a number of haematological and solid tumours.
- Recent evidence suggests that PIM expression is an important parallel pathway to the AKT/PI3K/mTOR pathway.
- Much of the work up to now with PIMs has focused on haematological malignancies. This study looked at the effect of combining PIM kinase inhibition with PI3K and PI3K/ mTOR inhibition. We have characterized these effects in a range of solid tumour cell lines.

Identification of dual PIM/PI3K and triple PIM/PI3K/mTOR inhibitors

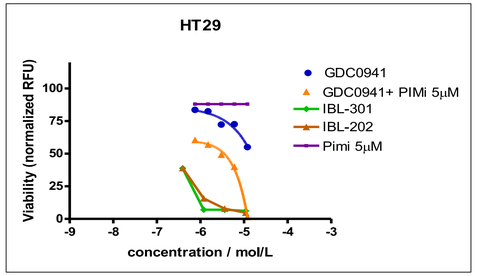
An SAR program was designed to balance the dual (PIM, PI3K) or triple (PIM, PI3K, mTOR) activities and to optimize the drug like properties of the compounds.
As a result of this exploration compounds IBL-202 (pan-PIM/PI3K) and IBL-301 (pan-PIM/PI3K/mTOR) were identified.

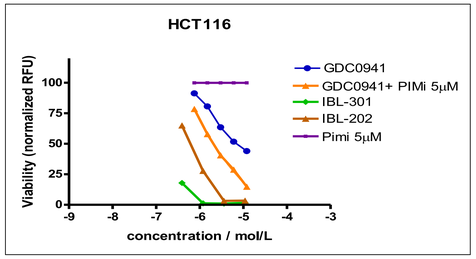


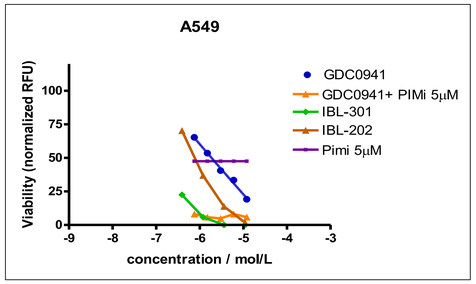
Molecule	PIM1 (IC50nM)	PIM2 (IC50nM)	PIM3 (IC50nM)	PI3Ka (IC50nM)	mTOR (IC50nM)
GDC-0941	100000	100000	1860	5.58	422
AZD-1208	0.4	1.9	5	n/a	n/a
IBL-PIMi	0.33	2.27	2.01	100000	10000
IBL-202	41.1	27.5	15	40.9	5690
IBL-301	22.9	16.8	3.66	3.02	135

Table 1. Activities of Inflection Biosciences Ltd and comparator compounds at target kinases
GDC-0941: PI3K inhibitor, AZD-1208: pan-PIM inhibitor

Combination of PIM and PI3 Kinase Inhibition in Solid Tumour Lines

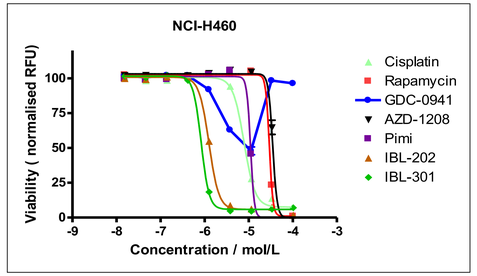


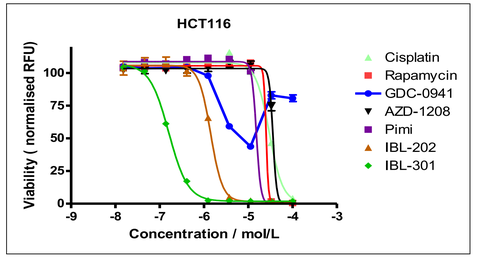


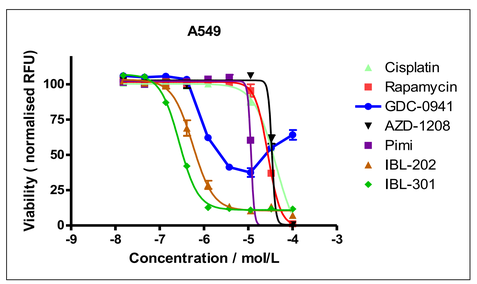


Antiproliferative activity of pan-PIM inhibitor (PIMi), GDC-0941 (PI3K), alone and in combination compared with IBL-202 and IBL-301 in colorectal and lung tumours lines.

Comparison of Efficacy of Single Agent PIM and PI3K inhibitors with Dual PIM/PI3K and Triple Acting PIM/PI3K/mTOR inhibitors (A)



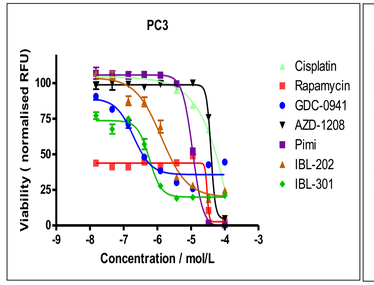


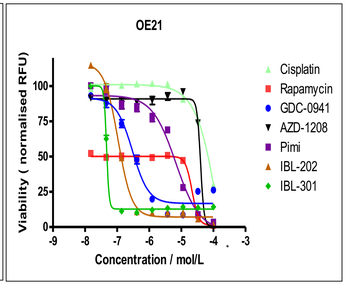


Antiproliferative activity in different cell lines of a pan-PIM inhibitor (AZD-1208 and PIMi), GDC-0941 (PI3K), rapamycin (mTORi) compared with IBL-202 and IBL-301 in colorectal and lung tumours lines.

METHOD: A panel of human cancer cell lines was selected to determine the anti-proliferative effect of PIM and PI3K inhibitors. Cells were seeded into 384-well plates 24h before compound addition and treated with test compounds for 72h. Cell viability was assessed by using CellTiter Blue® Cell Viability assays (Promega). Fluorescence is measured using a FlexStation II 384 microplate reader (Molecular Devices) and the data is graphed in GraphPad Prism 6.0 to generate IC_{50} values.

Comparison of Efficacy of Single Agent PIM and PI3K inhibitors with Dual PIM/PI3K and Triple Acting PIM/PI3K/mTOR inhibitors (B)





Antiproliferative activity in different cell lines of pan-PIM inhibitors (AZD-1208, PIMi), GDC-0941 (PI3K), compared with IBL-202 and IBL-301 in pancreatic and oesophageal tumour lines. Rapamycin mTOR inhibitor also included as positive control.

Data Summary

	IC50μM				
Cell Line ref	HCT116	A549	H460	OE21	PC3
Cancer type	Colon Cancer	Lung Cancer NSCLC	Lung Cancer Large Cell	Oesophageal Cancer	Prostate Cancer
Molecular Biology	mut K-Ras high myc high PIM3 mut PI3K	mut K-RAS	mut K-RAS mut PI3K		PTEN null
Cisplatin	30	33	7.9	59.68	45.54
AZD-1208	36	34	35	37.53	36.91
GDC-0941	-	-	-	0.27	0.15
Rapamycin	28	28	30	-	-
IBL-PIMi	15.45	11.50	11.07	6.79	11.20
IBL-202	1.42	0.57	1.26	0.12	0.12
IBL-301	0.17	0.29	0.85	0.05	0.05

Table 2. Summary of efficacy of compounds on cell viability in a range of solid tumour lines.

Conclusions

- Combined inhibition of PIM and PI3 kinases has a synergistic effect on cell proliferation in a range of solid tumour cell lines.
- This synergistic activity is evident with combinations of molecules that act as selective PIM and PI3 kinase inhibitors and with molecules specifically designed to combine both activities.
- Targeting PIM/PI3K or PIM/PI3K/mTOR activities in the same molecules appears to produce a more potent effect than targeting them with separate agents.
- Both IBL-202 (PIM/PI3K) and IBL-301 (PIM/PI3K/mTOR) showed more potent anti-proliferative activity than PIM and PI3K selective inhibitors alone. This effects has been shown previously to be correlated with higher induction of apoptosis and strong down-regulation of PIM, PI3K, mTOR pathways.
- Compounds from the PIM/PI3K series show excellent PK and have shown efficacy in vivo. Inflection is currently selecting its candidate for further development.