

CRT Licensing Opportunity



Unique PIP5 Kinase Inhibitor Programme

- Novel cancer target for therapeutic intervention
- Potent and selective PIP5K inhibitors with efficacy in cellular models and good *in vivo* PK
- Inhibition of PIP5K leads to an anti-proliferative response in cancer cells
- Potential for treating tumours with excessive AKT and PI3K activation

SMALL MOLECULES | Lead Optimisation

October 2009

Therapeutic Rationale

Phosphatidylinositol 4-phosphate 5-kinase (PIP5K) phosphorylates the inositol component of the membrane anchored phosphatidylinositol lipid PI4P at the D-5 position to generate the signalling phospholipid PI(4,5)P₂ or PIP₂. PIP₂ is a precursor of 3 second messengers: DAG, IP₃ and PIP₃, and therefore plays a crucial role in cell signalling (Figure 1). PIP₂ has many cellular effects including the regulation of cell proliferation, survival, actin remodelling & cytoskeletal changes (cell migration) and membrane trafficking. In cancer cells flux through the PI cycle is up-regulated 100 fold compared to normal cells.

Potent and selective PIP5K inhibitors have been generated showing efficacy in cell based assays. PIP5K inhibitors have therapeutic potential in cancers characterised by excessive flux through the PI cycle, including those with excessive AKT and PI3K activation.

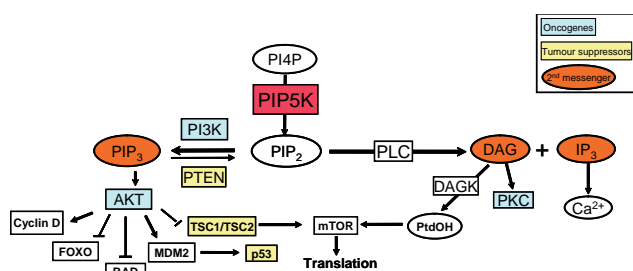


Figure 1: Cellular roles of PIP₂

Target Validation

Increased PIPK activity has been demonstrated in human tumour biopsies (Weber *et al.*, 2005), while a 90% reduction in PIP₂ levels has been shown to suppress breast cancer metastasis (DeWald *et al.*, 2005). Increased expression of specific PIP5K isoforms has been shown in the majority of cancer cell lines tested (CRT unpub data), and over expression of PIP5K isoforms has been shown to protect cancer cells from apoptosis (Mejillano *et al.*, 2001 and Halstead *et al.*, 2006). siRNA data has demonstrated a reduction in migration and cell viability and an increase in apoptosis in cancer cell lines, and dominant negative constructs have been shown to block PLD stimulated adhesion of leukemia cells (CRT unpub data; Halstead *et al.*, 2006; Kisseleva *et al.*, 2005; Powner *et al.*, 2005 and Aza-Blanc *et al.*, 2003).

In summary, the target validation data indicates that PIP5K is a very exciting therapeutic target. Small molecule inhibitors would be expected to down regulate PIP₂ production in tumours, resulting in an anti-proliferative and pro-apoptotic response.

First in Class Potent and Cell Active PIP5K Inhibitors

The Cancer Research Technology Discovery Laboratories identified five preliminary PIP5K inhibitor hit series and two series were prioritised for lead optimisation. Current data demonstrates that representatives of both series inhibit PIP5K with nM potency, have good ADMET properties and impressive

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selectivity over PIP4K and a panel of other kinases (Table below).

	Series 1	Series 2
Biochemical IC ₅₀ (nM)	200	70
Mol Wt	<400	<400
Mechanism of Action	ATP and substrate competitive	ATP and substrate competitive
Selectivity over a panel of 40 kinases including PIP5K	Yes	Yes

Compounds have been tested in a cell membrane assay and shown to selectively inhibit PIP5K (Figure 2). Cellular PIP₂ levels have been shown to be significantly reduced following compound addition, thus confirming the rationale for targeting PIP5K and the use of PIP₂ as a biomarker. Activity in a phenotypic assay confirms the rationale that these compounds will be effective against tumours with excessive AKT activation.

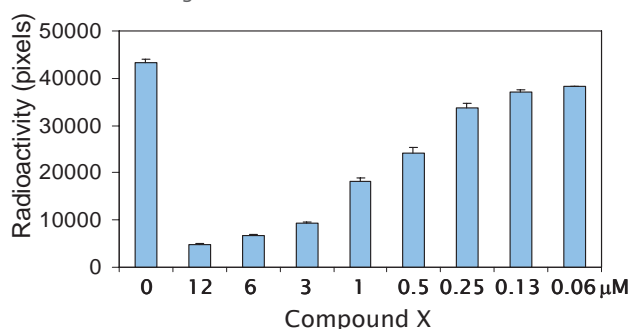


Figure 2: PIP5K inhibitor (Compound X, Series 2) decreases *in vitro* PIP₂ labelling of cell membranes

Intellectual Property

CRT has filed a patent to protect the biochemical assay. CRT are preparing to file a patent on the two lead series.

Commercial Opportunity

CRT are currently looking for a suitable commercial partner to pursue development of this programme either by collaboration or licensing.

Further Work

Medicinal chemistry efforts are ongoing to improve potency whilst maintaining selectivity and ADMET properties. Further development of the cellular biomarker and phenotypic assays and the performance of a proof of concept *in vivo* study are underway.

References

- Halstead *et al.*, 2006. *Curr. Biol.* **16**:1850-1856
DeWald *et al.*, 2005. **65**:713-717
Kisseleva *et al.*, 2005. *Mol. Cell Biol.* **10**:3956-3966
Powner *et al.*, 2005 *J. Cell Sci.* **118**:2975-2986
Weber 2005. *Advan. Enzyme Regul.* **45**:37-51
Aza-Blanc *et al.*, 2003. *Mol. Cell* **12**:627-637
Mejillano *et al.*, 2001. *J.B.C.* **276**:1865-1872

Cancer Research Technology

CRT is an oncology-focused development and commercialisation company. The CRT Discovery Laboratories (DL) focuses on early stage small molecule and biological drug discovery. DL bridges the gap between academia and industry by working in collaboration with the originating academic laboratories and enabling their discoveries to be turned into projects that are readily recognisable and valued by the pharmaceutical industry. CRT accesses projects from a wide variety of sources, not only Cancer Research UK (CRT's parent charity), but also from other sources including several European institutes, including the Netherlands Cancer Institute (NKI) in Amsterdam. The PIP5K project was initiated in the laboratory of Nullin Divecha at the NKI. Nullin Divecha has subsequently moved to the Paterson Institute, Manchester.

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