

# CRT Licensing Opportunity



## Small Molecule Inhibitors of Autotaxin

- Autotaxin is overexpressed in a variety of human cancers
- Programme in Lead Optimisation with two potent Autotaxin inhibitor series
- Assay screening cascade with validated biomarker assays in place
- Two *in vivo* models in development

SMALL MOLECULES | Lead Optimisation

JUNE 2011

## Therapeutic Rationale

Autotaxin (ATX) is an extracellular phospholipase that cleaves choline from lysophosphatidylcholine (LPC), the most abundant phospholipid in plasma, forming lysophosphatidic acid (LPA). ATX and/or LPA receptors are overexpressed in various cancers, including breast, glioblastoma, ovarian and prostate cancer, and LPA is present at high levels in the ascites fluid of ovarian and pancreatic cancer patients.

LPA is a bioactive phospholipid that stimulates the proliferation, migration and survival of many cell types. LPA acts through several G protein-coupled receptors ( $LPA_{R1-6}$ ), which couple to multiple signalling pathways, including those initiated by Ras and Rho GTPases. LPA signalling has been implicated in a wide range of biological processes, ranging from vascular development to inflammation and tumour progression.

The oncogenic potential of the ATX/LPA receptor axis has become evident from several studies in mice. Overexpression of ATX or  $LPA_{R1-3}$  in transgenic mouse models promotes breast tumour initiation, progression and metastasis (1). LPA enhances the tumourigenesis and metastasis of ovarian cancer cells *in vivo* (2). ATX inhibition using analogues of cyclic phosphatidic acid reduces ATX-mediated invasion of melanoma cells *in vitro* and *in vivo* (3, 4). Dual inhibitors of ATX and  $LPA_R$  inhibit tumour growth and angiogenesis in a lung cancer xenograft model (5) and MDA-MB-231 tumour growth and cause tumour regression *in vivo* in an orthotopic breast tumour model (6). ATX inhibition in mice results in a rapid decrease in plasma LPA levels (7). Collectively, these data reinforce the view that ATX is an attractive target for therapeutic intervention in a number of oncology indications.

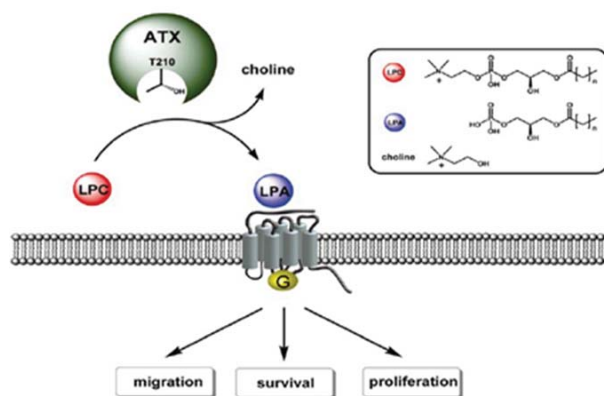


Figure 1: ATX/LPA receptor signalling.

## Potent Autotaxin Inhibitors

There are currently no small molecule inhibitors of ATX on the market or in the clinic. The Discovery Laboratories of Cancer Research Technology have recently screened a library of diverse small molecule compounds for inhibitors of ATX. This screen revealed several distinct series of nanomolar inhibitors, two of which are currently being progressed.

An extensive medicinal chemistry effort is being carried out to optimise potency and PK properties of the two lead series. *In vitro* compound efficacy is being determined by two biochemical assays, the F53 assay and the Amplex Red assay. A number of the lead compounds show sub-10nM potency against ATX and have drug-like physicochemical properties, as summarised in Table 1. Crystallography studies have also been initiated to guide

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medicinal chemistry. Rat ATX protein has been prepared and will be used to obtain x-ray crystal structures of ATX with the lead compounds.

Table 1: Properties of ATX lead series inhibitors

	Series A	Series B
ATX IC50 Amplex Red Assay (nM)	<10	<10
PD Biomarker - choline release assay ( $\mu$ M)	<0.1	<1
Molecular weight (Da)	450-500	400-520
Polar surface area	70-100	70-100
cLogD	3-4	2-3
Orally bioavailable	Yes	Yes

Two PD biomarker assays have been validated and implemented in the Lead Optimisation screening cascade. The choline release assay is a high throughput assay and is a marker for ATX activity measuring choline released from LPC in human plasma. The LPA assay is a low throughput LC/MS/MS assay validated for the analysis of LPA in human plasma to confirm ATX inhibition and in mouse plasma to support *in vivo* PK/PD and efficacy studies. There is good correlation between the two assays, supporting the use of the high throughput choline release assay as the primary biomarker assay. Preliminary PK studies have been carried out and demonstrate that compounds from the lead series are well tolerated in mice, and some have good oral bioavailability.

Two *in vivo* mouse models, an orthotopic breast and an orthotopic ovarian cancer model, are currently being optimised to enable the determination of tumour growth inhibition and modulation of plasma and tumour levels of LPA.

## Originating Institute

This programme is under development in collaboration with Prof Wouter Moolenaar and Dr Huib Ovaa from the Netherlands Cancer Institute, who provide invaluable expertise on the target and biological models. Prof Wouter Moolenaar is a world-leading expert in the field of Autotaxin biology. Crystallographic input has been provided by Tassos Perrakis (8), also from the Netherlands Cancer Institute.

## Commercial Opportunity

CRT is seeking a commercial partner interested in pursuing a co-development or direct licensing arrangement.

## References

1. Liu S. *et al.* 2009. Expression of autotaxin and lysophosphatidic acid receptors increases mammary tumorigenesis, invasion, and metastases. *Cancer Cell* 15(6):539-550.
2. Li H. *et al.* (2009). Lysophosphatidic acid stimulates

cell migration, invasion, and colony formation as well as tumorigenesis/metastasis of mouse ovarian cancer in immunocompetent mice. *Mol. Cancer Ther.* 8(6):1692-1701.

3. Baker D.L. *et al.* 2006. Carba analogs of cyclic phosphatidic acid are selective inhibitors of autotaxin and cancer cell invasion and metastasis. *J. Biol. Chem.* 281(32):22786-22793.
4. Altman M.K. *et al.* (2010). Targeting melanoma growth and viability reveals dualistic functionality of the phosphonothionate analogue of carba cyclic phosphatidic acid. *Mol. Cancer* 9:140.
5. Xu X. and Prestwich G.D. 2010. Inhibition of tumor growth and angiogenesis by a lysophosphatidic acid antagonist in an engineered three-dimensional lung cancer xenograft model. *Cancer* 116(7):1739-1750.
6. Zhang *et al.* 2009. Dual activity lysophosphatidic acid receptor pan-antagonist/autotaxin inhibitor reduces breast cancer cell migration *in vitro* and causes tumor regression *in vivo*. *Cancer Res.* 69(13):5541-5549.
7. Albers H. *et al.* 2010. Boronic acid-based inhibitor of autotaxin reveals rapid turnover of LPA in the circulation. *PNAS* 107(16):7257-7262
8. Hausmann J. *et al.* (2011). Structural basis of substrate discrimination and integrin binding by autotaxin. *Nat. Struct. Mol. Biol.* 18(2):198-204.

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