SMALL MOLECULE INHIBITORS OF AUTOTAXIN

- Autotaxin is overexpressed in a variety of human cancers
- Programme in Lead Optimisation with two potent, patented Autotaxin inhibitor series
- Assay screening cascade with validated biomarker assays in place
- Lead compounds have been validated in vivo in an orthotopic metastatic 4T1 breast cancer mouse model, with concomitant inhibition of metastasis and modulation of LPA
- Co-crystal structures of mouse ATX with inhibitors from both chemical series have been solved

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 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 (LPA₁-₆), 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 LPA₁-₃ in transgenic mouse models promotes breast tumour initiation, progression and metastasis (1). Through the generation of LPA, autotaxin is implicated in a number of tumours as a direct driver of tumour growth and in particular, tumour spread. ATX inhibition in mice results in a rapid decrease in plasma LPA levels (2).

AUTOTAXIN IN IMMUNO-ONCOLOGY

In addition, LPA is also known to exert a range of effects on both stromal and immune cells which implicate that its presence in the tumour microenvironment could contribute to immune suppression. LPA inhibits T-cell activation in vitro and in vivo (through LPA₁) and transfer of LPAR₁-deficient CD8⁺ T cells suppress melanoma progression in mice (3). ATX inhibitors may therefore function to boost tumour-infiltrating lymphocyte activity, while suppressing tumour cell motility. In addition LPA is chemorepulsive to the migration of both T and B cells in vitro, highlighting further the potential value of ATX inhibitors in cancer immunotherapy, particularly in combination with checkpoint therapies (4, 5). Cancer Research Technology (CRT) is currently investigating the effects of its ATX inhibitors in enhancing T cell activation/migration and tumour reactivity.

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

The Discovery Laboratories of CRT have 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 was carried out to optimise potency and pharmacokinetic (PK) properties of the two lead series. In vitro compound efficacy was determined by two biochemical assays; the FS3 assay and the Amplex Red assay. A number of the lead compounds show sub-10 nM potency against ATX and have drug-like physico-chemical properties, as summarised in Table 1. A crystallography platform using mouse ATX was established to guide the medicinal chemistry. Mouse ATX protein was prepared and x-ray crystal structures with lead compounds have been solved.
Two pharmacodynamic (PD) biomarker assays were validated and implemented in the Lead Optimisation screening cascade. The choline release assay is a high throughput assay and 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. PK studies were carried out and demonstrate that compounds from the lead series have good oral bioavailability and are well tolerated at therapeutic doses.

Lead compounds have been validated in vivo in an orthotopic metastatic 4T1 breast cancer mouse model, with concomitant inhibition of metastasis and modulation of LPA (Figure 2).

References:

ORIGINATING INSTITUTE
This programme is under development in collaboration with Prof Wouter Mooyaart and Dr Huib Ovaa from the Netherlands Cancer Institute, who provide invaluable expertise on the target and biological models. Prof Wouter Mooyaart is a world-leading expert in the field of ATX biology. Crystallographic input has been provided by Tassos Perrakis (6), also from the Netherlands Cancer Institute.

INTELLECTUAL PROPERTY
Lead series chemistry is protected under two patent applications filed on 4th February 2016 (PCT/ GB2016/050268, PCT/GB2016/050267).

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

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