**Theme:** Diagnosis and therapy

**Targeting glycolysis as an anti-cancer strategy: design and characterisation of Inhibitors of PFKFB3**

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**Background**

Many tumours have a demand for glucose as evidenced by FDG-PET imaging and therefore are predicted to have a high glycolytic flux. Therefore targeting the glycolysis pathway has emerged as an attractive therapeutic strategy in targeting tumour metabolism. Within glycolysis, Phosphofructokinase 1 catalyses the rate limiting conversion of F-6p and ATP to f1,6P. The enzymatic reaction of PFK1 can be allosterically regulated by a number of metabolites including f,2,6bp, the product of the PFK2 family of enzymes. PFKFB3 is a member of the PFK2 family and is reported to be overexpressed in a variety of cancers including breast, prostate and colon and in hypoxic studies and is considered an attractive target for blocking glycolysis in highly glycolytic tumour types. As part of the CRT/AstraZeneca metabolism alliance, a project was initiated to develop potent inhibitors of PFKFB3.

**Method**

A suitable HTS assay and robust crystallography platform were developed enabling a full HTS campaign against PFKFB3. In order to assess the cellular activity of the developed inhibitors a number of bespoke assays were developed to assess key metabolic intermediates from the glycolytic pathway.

**Results**

A full HTS campaign yielded 5 potential chemical series of interest. Crystallography revealed key interactions between compounds and PFKFB3 and enabled a structure based design approach to yield potent inhibitors of PFKFB3. Biochemical and cell-based assays confirmed the developed PFKFB3 inhibitors had a potent cellular activity against a range of defined pathway biomarkers.

**Conclusion**

In summary we have therefore developed potent cell active inhibitors of PFKFB3 for use in understanding the role of glycolytic pathways in cancer metabolism.

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