Cystinosis Gene Repair

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Overview of study progress against key milestones (Oct 2010 – Mar 2011)

Specific Goals

1) Development of 13 ZFN pairs/donor sequences to repair mutations/deletions

To date five pairs of ZFNs have been designed and tested *ex vivo*. Two pairs of ZFNs designed against exon 4 using the modular assembly (Liu et al. 2002) and rational design (Sera and Uranga) approaches and a pair of ZFNs target to exon 9, exon 11, and exon 12 using context dependent assembly (Sander et al. 2011). None of these ZFNs pairs displayed any measurable level of activity *ex vivo*.

An alternative method to ZFN design is Oligomerised Pool Engineering (OPEN). This method is based on a bacteria 2 hybrid screen of ZFN libraries (Maeder et al. 2008). This method has been shown to isolate ZFNs with high efficiency and specificity and the original study describing the method double the number of ZFN targeted genes in the literature. An OPEN screen is currently underway using a pool of 8 x 10^5 potential ZFNs to isolate ZFNs targeted to 8 regions of *CTNS* (intron 4, intron 8, exon 9, two sites in intron 10, exon 11, and two sites in exon 12). The goal is to have the majority of these ZFNs selected and tested in a β-galactosidase based assay within 12 months of the project start date allowing adequate time to carry out cellular based assays and to assess delivery of the ZFNs using AAV2.

Primers have been designed to amplify donor sequences from WT *CTNS* and cloning of donor PCR products into pAAV-GFP in underway.

2) Optimise delivery of ZFNs and donor sequence

pAAV-GFP will be used as an expression vector for ZFNs and for donor plasmids as it allows both the ZFNs and donor sequences to be packaged into rAAV for viral delivery. Cloning of donor sequences into pAAV-GFP is under way. Cloning of ZFNs into pAAV-GFP will commence once ZFN selection is successful.

3) Evaluate off target events

Off-target analysis will be carried out as described in Petek *et al.* 2010. The p2A-TOA vector required has been requested from the authors.

4) Exploit ZFNs through active collaborations

The PI and research fellow have met with other researchers in the field at the 6th International cystinsosis conference in Italy (2010) and has informally discussed possible collaborative projects with Dr. Paul Goodyer and Dr. Francesco Emma. The lab has also received cell lines from Prof. Elena Levtchenko. This network will allow for quick communication and availability of active ZFNs to the wider cystinosis research community. An update of the research will be presented at the UK cystinosis conference in September 2011 where further opportunities for collaboration will be explored.