

Development of an *in vivo* gene repair kit: towards a cure for cystinosis

Lay Summary (Dec 2010)

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This project aims to develop a method to repair the defect in the *ctns* gene in a human cell model of cystinosis. The gene repair system requires the design of a pair of proteins (called ZFNs) to transiently break the chromosome containing the damaged gene, which then allows a so-called donor DNA molecule containing the correct gene sequence, to recombine with the two free ends and repair the gene defect. If the gene defect is corrected in a cell, that cell should no longer be cystinotic.

During the first 24 months of the project, two pairs of ZFNs have been successfully designed that specifically cleave the *ctns* gene in a test tube reaction. However, exhaustive testing has subsequently shown that these particular ZFN pairs do not efficiently function in cells. Since the start of the project, it has been indecently reported that typically only one pair of ZFNs in every three will lead to successful gene repair in cells. To this end, we are currently evaluating seven *new* ZFN pairs.

If one (or more) of the ZFN pairs can mediate *ctns* gene repair, the next stage of our proposal is to look at strategies to test this in an animal model. Recent studies suggest that a so-called "gene-modified autologous stem cell" transplantation method may be the most suitable approach. This involves removal of an animal's own bone marrow cells, genetically modifying them, and returning them to same animal. This is of potential benefit as the animal ultimately receives its own cells which significantly reduces the incidence of rejection. We have presented data of our gene repair strategy at two international cystinosis meetings in the last 12 months, and discussed how our method of gene repair offers potential advantages to the genetic modification step of this approach. We have already identified researchers in this field at these meetings, and are actively looking at ways to adapt our gene repair strategy to this approach. Whilst a long way from human trials, it should be noted that a similar approach has had some limited clinical success in the treatment of some blood disorders.

Closer to home, we have developed strong links with Professor Philip Newsholme's lab in UCD who has already offered provided initial training to Katrin Kaschig in assays to determine how quickly the gene correction reverses the cystinotic status of the cell. It should be noted that as well as correcting the *ctns* gene, this technology also allows us to generate cystinotic cell lines that will be of direct use to Prof Newsholme's study of the cause of diabetes seen in some older cystinosis patients.