Title: Evaluation of a novel drug combination treatment of CF10 and Everolimus for nephropathic cystinosis in a new cystinotic rat model
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Grant period: 07-05-2023 – 31-03-2027 (extension due to Dr Hollywood relocation to Ireland and maternity leave)
Progress report #3: 09-01-2025 – 03-31-2026

Background:
The lives of cystinosis patients could be improved by developing 1) a better variant of cysteamine that is more tolerable with less side-effects and 2) alternative therapies that target other pathways affected in cystinosis, such as autophagy, that are likely to play a role in the kidney failure but are not corrected by cysteamine alone. Towards these goals, we have used rodent models of cystinosis to develop a pro-drug version of cysteamine (CF10) that can be delivered in high doses with few side-effects, and found that the mTOR inhibitor Everolimus can ameliorate aspects of the cystinotic Fanconi syndrome. This work made use of a new rat model of cystinosis (Hollywood et al., 2022) that we have developed that has a phenotype that closely resembles the human disease. In this project we will **test the hypothesis that CF10/ low dose Everolimus combination therapy provides a more effective treatment for cystinotic rats than cysteamine/low dose Everolimus combination**. Specifically, we will determine if this new drug treatment has the potential to minimise the unpleasant side-effects seen with cysteamine, reduce the frequency and level of dosing, and determine if it is more effective at slowing, and potentially stopping the decline in kidney function. This preclinical study will provide the justification, and inform the appropriate dosing regime, for future human clinical trials.

The overall goal of this project is to conduct preclinical therapeutic drug intervention studies in *Cystinosin (Ctns)* knock-out (KO) rats to determine whether a combination treatment of CF10 and Everolimus is more efficacious at ameliorating the cystinosis phenotype than Cysteamine and Everolimus. To achieve this, we propose the following Aims:

Aim 1: Assess the long-term effectiveness of low dose Everolimus on renal function in *Ctns*$^{-/-}$ rats

Aim 2: Assess the effects of a Cysteamine and low dose Everolimus combination treatment on the renal defects in Ctns-/- rats.

Aim 3a: Evaluate CF10 and Everolimus drug-drug interactions in cystinotic rats.

Aim 3b: Assess the effects of a CF10 and Everolimus combination treatment on the renal defects in *Ctns*$^{-/-}$ rats.

Progress to date:

Completed
Aim 1: Assess the long-term effectiveness of low dose Everolimus on renal function in *Ctns*$^{-/-}$ rats-

In Progress
Aim 2: Assess the effects of a Cysteamine and low dose Everolimus combination treatment on the renal defects in Ctns-/- rats.

Aim 3a: Evaluate CF10 and Everolimus drug-drug interactions in cystinotic rats.

Aim 3b: Assess the effects of a CF10 and Everolimus combination treatment on the renal defects in *Ctns*$^{-/-}$ rats.

Project Progress Update:

Staff recruitment
Following successful application and interview Dr Lucia Nicosa was hired as a postdoctoral researcher on January 1st, 2026, to aid in completion of this project. Lucia is an exceptional researcher with a very strong background in molecular biology techniques and animal handling. Lucia has been trained in necessary animal techniques, and we are ready to begin our final aims of this project.

Project aims underway:
To allow better comparison of data we will perform aims 2 and 3 at the same time.

Aim 2, 3: Assess the effects of Cysteamine/low dose Everolimus and CF10/low dose Everolimus combination treatments on the renal defects in *Ctns*-/- rats.

Experimental animals were born on 03/04/2026 and acclimatization is due to begin on 03/30/2026. The animals will be handled daily to allow them to become comfortable with the researchers and they will be fed small jelly pills to acclimatize them to the drug delivery route we will perform.
Baseline urine and blood collections will be carried out at 5 weeks of age with drug delivery beginning at 6 weeks (04/13/2026) see table below for dosing schedule.

***Ctns* KO rat**	**Vehicle**	**Cysteamine (30mg/kg/twice daily) and Everolimus (2mg/kg/daily)**	**CF10 (82.5mg/kg/twice daily) and Everolimus (2mg/kg/daily)**
Males	6	6	6
Females	6	6	6

The study will run for 6 months, and we expect to have all treatments completed by October 2026. Once completed organs will be harvested for downstream analysis, such as cystine measurements, blood and urine composition, histology and immunohistochemistry.