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 #1: 07-31-2024 – 09-01-2025

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:
There has been an unavoidable delay in the progress of this project due to several factors, outlined below.

Establishment of the *Ctns* Colony at UCC
In March 2024, I relocated to Ireland to establish my laboratory at University College Cork (UCC). Prior to my departure from New Zealand, embryos were successfully extracted from a cystinotic female rat. These embryos were shipped to Charles River Laboratories (CRL) in France in December 2023 for rederivation.

In June 2024, a litter of cystinotic rats was born at CRL, and in July 2024 the live animals were transferred to UCC, Cork. This litter comprised four males and seven females, all heterozygous for the *Ctns* mutation.

Following approval of our animal ethics authorization at UCC in August 2024, we commenced breeding to establish a *Ctns* colony in Cork. This colony is now well established, and we have had several rounds of successful breeding.

Laboratory Setup at UCC
The transfer of this grant from the University of Auckland to UCC was finalized in September 2024, enabling me to advertise for a postdoctoral fellow to advance the project aims. In November 2024, a postdoctural fellow joined the laboratory and initiated the procurement of the necessary reagents and equipment to begin the preclinical drug study. I provided training in essential animal techniques, including handling, blood and urine collection, tail vein injections, and drug administration.

In January 2025, I commenced maternity leave with the postdoctural fellow well-prepared to continue the study in my absence. However, in February 2025 she resigned from the position after securing a permanent role in another department. This unexpected development left the laboratory without staff to continue the project, necessitating a pause in the study during my leave.

I resumed work in September 2025 and am grateful to the CRF for granting a no-cost extension to allow the completion of this study. Recruitment for a new postdoctoral researcher is currently underway, and I anticipate appointing a candidate in the coming weeks.

Once this appointment is finalized, we will proceed with project aims 2 and 3.