

20 April 2021

To
The Australian Cystic Fibrosis Research Trust

Interim progress report

Sponsor/Scheme: Cystic Fibrosis Australia/Conquer GI Innovation Grant 2020

Title: Towards understanding gut host-microbe interactions and personalised probiotic therapy in cystic fibrosis using organoid-derived 2d intestinal models

Briefly, we have been developing human 2D and 3D intestinal organoids from our patients with CF using primary human induced pluripotent stem cells. These patient-specific epithelial cell models are a close replica of the *in vivo* intestinal environment and provide a system for disease modelling. This grant will allow us to introduce specific bacterial species with known CF associations into the 2D epithelial culture systems to examine microbiota-induced changes in primary epithelial cells. It is our hope that results from the study will guide new and existing therapeutic treatments to better address GI symptoms and complications for patients with CF.

Aims

Our primary aim for the study is to quantitatively characterise host-microbe interactions in CF patient-specific intestinal organoids through the measurement of epithelial integrity, inflammatory markers, proteins and metabolites. We would like to determine if (i) the microenvironment of CF intestinal models drive immune and mucosal responses to microbiota significantly different to HC models and (ii) if *F. prausnitzii* and/or *A. muciniphila* are potential candidates for CF-specific probiotics.

Progress

Optimisation studies for cell models - complete

- Cell cultures have been used to establish optimal protocols for storage, dilution and sample preparation for biopsy swabs and patient stool samples.
- Results have confirmed significant differences between CF and HC cytokine responses to CF and HC stool sample preparations in epithelial cells.

Organoid culturing and cell differentiation optimisation - ongoing

- Intestinal organoids have successfully cultured
- Differentiation of gut organoids to create 2D models amenable to infection with microbial agent was tested on 3 occasions. Optimal seeding cell density was confirmed, monolayer formation was successful albeit without apical mucus development.

Bacteria culturing - ongoing

- Bacteria cultures *F. prausnitzii* and *A. muciniphila* have been sourced from Germany

- Optimum anaerobic media specific to the *F. prausnitzii* and *A. muciniphila* cultures has been sourced and prepared.
- Once successfully growing to desired density, cultures will be introduced into 2D organoid culture systems.

I would like to thank CF Australia, Conquer CF Committee and all the donors for their support and look forward to communicating the final findings of the study.

Yours sincerely,

A handwritten signature in black ink, appearing to be 'K. Ooi', written inside a simple, hand-drawn outline of a head and neck profile.

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