Dear ACFRT Board of Trustees,

Please accept this letter as documentation of our final report for the "Novel multi-omic insight into evolution of antibiotic resistance in *Pseudomonas aeruginosa* (PA) in cystic fibrosis (CF) and relationship to clinical outcomes" for which we received an ACFRT Innovation Grant in 2018.

The aims for this project were to profile CF patient immune responses, profile *P. aeruginosa* virulence factors and the microbiome; and finally, to use whole genome sequencing (WGS) to study ABX resistance in CF patients infected with epidemic versus non-epidemic strains of P. aeruginosa and link this to disease outcomes.

Some of the data from this study were presented as an oral abstract at the 2019 Australasian CF Conference ("The evolution of antibiotic resistance in *Pseudomonas aeruginosa* in Cystic Fibrosis and its relationship to clinical outcomes"), which received a lot of interest and robust discussion. In addition, the novel and exciting results from this study will shortly be written up for publication in a relevant high impact factor journal.

Briefly, the major outcomes from the study are:

- ✓ Profiling *P. aeruginosa* virulence factors across multiple patients demonstrates that multiple virulence factors may be highly (or lowly) expressed by Pseudomonas in one exacerbation episode, but not in another, illustrating the opportunistic nature of the bacteria.
- ✓ Our microbiome analysis corroborates what has been previously shown, that the CF microbiome is significantly changed from healthy individuals, but importantly we show for the first time how the CF microbiome changes during different stages of disease i.e during acute exacerbation versus post exacerbation and treatment. These results will help advance understanding the immune responses of patients during these stages of disease.

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- ✓ Antibiotic testing of several strains of Pseudomonas over a 3-year period has revealed that treatment with a standard antibiotic such as Tobramycin may work for one exacerbation but not necessarily the next episode in the same patient, depending on which strain is colonising that patient at that specific time. This highlights how important it is to treat the specific bacterial strain, which is not currently done in clinical practice.
    - We also have data to show different antibiotics that are more effective at treating multiple Pseudomonas strains across several different CF patients.
- ✓ Whole genome sequencing (WGS) has revealed the majority of the Pseudo strains isolated from these CF patients were AUST-01 or AUST-03 strains, which are both epidemic strains. AUST-01 (highly prevalent and dominant genotype) and AUST-03 are both associated with more severe and frequent infections/exacerbations, are multi-drug resistant and associated with poorer clinical outcomes.

One of our next steps is to identify the different mutations across patient groups and extract mutations that are relevant to infectivity and antibiotic resistance. This marks an exciting step towards developing novel therapies to improve patient outcomes.

We truly thank the Australian Cystic Fibrosis Research Trust (ACFRT) again for the support of this research.

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