Final Report to Cystic Fibrosis Australia

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Project Name: Development of new mucus-thinning drugs for CF patients

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Background:

The overproduction of a stickier-than-usual pulmonary mucus is a common symptom of cystic fibrosis (CF). This mucus is difficult to clear and reduces a patient's lung function through the formation of airway obstructions by increasing the risk of lung infections. Mucolytic drugs, which reduce mucus viscoelasticity, are used to address this issue. However, the leading mucolytic drug Pulmozyme® is a relatively old drug that only works in 70% of CF patients and provides only modest improvements in lung function. Better mucolytic therapies are needed for CF patients.

Research Progress:

Our laboratory has been studying the molecular interactions that define mucus viscoelasticity. This includes the trefoil factor (TFF) peptides, which cross-link mucin polymer chains to enhance mucus viscoelasticity. In COPD patients, the trefoil factor 3 (TFF3) peptide correlates with high mucus viscoelasticity and negatively correlates with lung function (as determined FEV_1). Using CF BALF samples obtained in collaboration with Dr Sarath Ranganathan (Murdoch Children's Research Institute, Melbourne) and the AREST-CF program, we set out to quantify TFF levels in CF patient pulmonary mucus. We found that TFF3 was consistently elevated in the BALF of most CF patients, typically in the range of 10-10,000 fold that of normal TFF3 levels. This suggested that antagonising TFF3 activity may represent a new strategy for reducing pulmonary mucus viscoelasticity in CF patients. To this end, we sought to develop antibodies as TFF3 antagonists.

Through a collaboration with CSL, we had identified 15 fully-human monoclonal antibodies that were capable of disrupting TFF3 function. By rigorously cataloguing the stability, solubility, predicted immunogenicity, affinity and specificity of these antibodies, we were able to narrow the field to five antibodies with characteristics that could make them amenable to further development as biologic drugs. We applied structural biology techniques to these antibodies and ultimately solved the structure of three antibodies in complex with human TFF3, thereby establishing functional epitopes capable of antagonising TFF3 function. Using a cone-and-plate rheometer, we next explored the potential of some of these antibodies to decrease the viscoelasticity of different mucus types.

Using a model mucus comprised of reconstituted mucin and recombinant TFF3, we were able to generate highly viscoelastic materials that could be effectively 'thinned' in a dose-dependent manner using our optimal TFF3-antagonising antibodies. However, available rheometers were not sensitive enough to detect differences in viscosity between healthy and CF BALF, presumably because of their highly diluted state. As such, a head-to-head comparison of TFF3 antagonists with Pulmozyme® was not possible with this experimental approach. Work is on-going to revisit this problem using induced sputum samples.

Outlook:

Since this project was initiated, the Trikafta® combination therapy has become available in the major markets around the world, including Australia. Anecdotally, Trikafta® significantly reduces the need for mucolytic therapy in those CF patients who are eligible for this drug: the SIMPLIFY trial in the U.S.A. will soon provide better guidance on this issue. Regardless, there are still many CF patients bearing nonsense mutations in CFTR who cannot benefit from this drug and who will continue to need improved mucolytic therapies. As such, we will continue to explore the role of TFF3 in CF and the possible application of TFF antagonists as novel mucolytics.

Thank you to Cystic Fibrosis Australia for this scholarship.

Signed: Shiyi Xi **Date:** 24/05/2022

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