Pump-Priming Grant

Report

Matthew Brook

Title

Single cell analysis of the immune infiltrate in kidney transplant biopsy samples from patients receiving regulatory T cell therapy.

Objective

1) To generate a reliable protocol for isolation of single cell suspensions from kidney transplant biopsy samples.
2) Characterise the immune infiltrate and transcript in protocol kidney transplant biopsies from patients treated with regulatory T cell infusions as part of The TWO Study

Method

A literature search was performed to identify published methods of kidney transplant biopsy processing to single cell suspension for sequencing. Two methods were selected for further evaluation in our hands.

Following optimisation of processing to single cell suspension, we subsequently performed analysis of four biopsy samples taken from patients participating in The TWO Study both before and after regulatory T cell infusion. Single cell suspensions were generated and $CD45^+$ leucocytes isolated using FACS sorting. $CD45^+$ single cell suspensions were provided to collaborators at the Weatherall Institute for Molecular Medicine who generated single cell transcripts using 10X Genomics technology.

Results

Using kidney biopsy specimens from porcine kidneys we were able to compare methods for single cell isolation and yields of $CD45^+$ leukocytes to optimise a protocol we felt was suitable for clinical use.

We have processed four biopsy samples from patients taking part in the TWO Study. These samples were cores permitted by protocol in addition to a core that underwent routine histopathological analysis. In contrast to patients in the ONE Study, biopsies from the four patients analysed did not show significant dense focal infiltrates within the transplanted kidney. This is likely due to a different approach to immunosuppression and timing of regulatory T cell administration used in the TWO Study. In two samples we were unable to generate sufficient numbers of live cells to generate transcripts which we felt was due to excess DNase within the isolation protocol. However, we were able to adjust the isolation protocol and isolate 3451 and 2512 live $CD45^+$ leukocytes from two samples respectively that underwent sequencing. Sequencing transcripts are currently being analysed and we aim to have the final results of this by the end of 2021.

Outputs (publications/presentations)

Publications and presentations will depend on the final results obtained from completion of the complex analysis process. It is likely that these will feed into ongoing work on immune infiltrates following regulatory T cell therapy and form part of a publication on this basis.

Next Steps (what is it leading to)

The key outcome from this work has been the generation of a protocol for isolating single cell infiltrates from kidney transplant biopsy samples, sorting for $CD45^+$ cells of interest and successful transcript sequencing of such cells. We believe we now have a reliable protocol for this work that will allow us to develop an interest in single cell sequencing of transplant and native kidney biopsy samples in a variety of clinical settings. Furthermore, when the results of this novel analysis are finalised, we expect them to further contribute to our understanding of the impact of regulatory T cell therapy in kidney transplantation and inform ongoing comprehensive immune monitoring strategies.