Microenvironmental determinants of Aplastic Anaemia progression to MDS/AML

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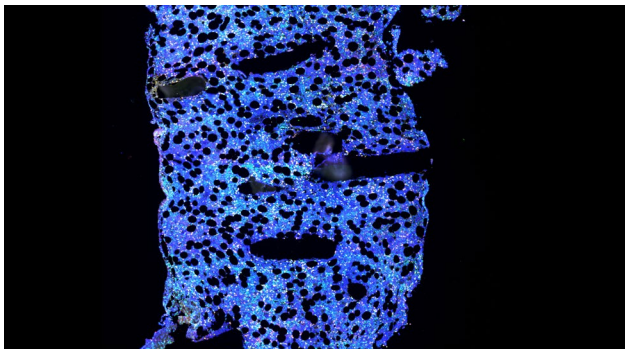
The Royal Melbourne Hospital

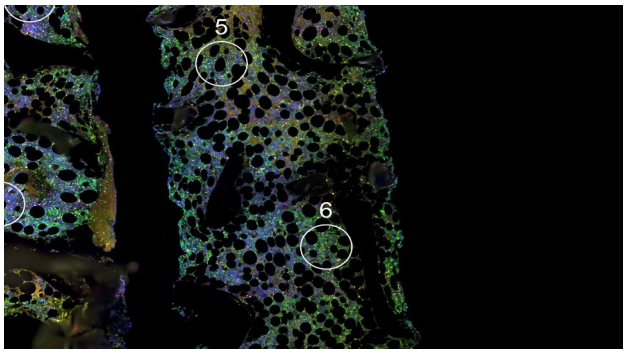
The Australian Cancer Research Foundation (ACRF) Translational Research Laboratory is a unique collaborative environment bringing together research scientists, higher degree students (both clinical and science based), nursing and clinical staff. Established in 2013 by Professor David Ritchie (Director) and Dr Rachel Koldej (Senior Scientist), the laboratory has the mission of undertaking projects utilising clinically derived samples from well-defined clinical cohorts of patients, applying high quality investigational assays and investigating the factors that determine clinical outcomes, treatment efficacy and exploring ways of improving the treatment of disease. Ultimately, the laboratory's goal is to improve the lives of people with blood conditions including Bone Marrow Failure Syndromes (BMFS).

One of the single biggest issues facing researchers in the field of BMFS is that the low incidence of these diseases means that patient samples for research are very difficult to obtain in large enough numbers for meaningful research discoveries to be made and translated into improved patient care.

One of the founding principles of the ACRF Translational Research Laboratory is to unlock the potential of existing sample sets for correlative translational research. The laboratory identified that while fresh samples from patients with BMFS are very rare and therefore slow to collect, there is often stored specimens collected over many years as part of routine care. At the ACRF laboratory, enormous effort has been made to identify these stored specimens as a means of fast tracking research that might otherwise take many years to complete. This approach was applied to Dr Lynette Chee's project into the use of circulating microRNA as a determinant of outcome in Aplastic Anaemia. The approach was further extended by accessing a repository of bone marrow samples (trephines) collected from patients as a part of routine clinical care to diagnose and monitor their disease. These trephine samples collectively represent over 30 years of patients with full



Bone Marrow Trephine tissue stained with immunofluorescent markers to allow selection of regions of interest for analysis using digital spatial profiling



Bone Marrow Trephine tissue stained with immunofluorescent markers to allow selection of regions of interest (circled) for analysis using digital spatial profiling

clinical outcome data and are an untapped resource for BMF research. However, the way that they had been processed has previously limited the ways in which they could be analysed.

In a world first study Dr Koldej demonstrated that a new technique, Digital Spatial Profiling (DSP), could be used to simultaneously examine the expression of multiple proteins in BM trephine samples (Koldej and Ritchie, Immuno-oncology Technology, 2020). This exciting discovery has allowed the incorporation of DSP into a number of studies to analyse the immune microenvironment in trephine samples including:

- Dr Koldej's project Microenvironmental determinants of Aplastic Anaemia progression to MDS/AML,
- Dr Ashvind Prabahran's International Travelling Fellowship project Novel immunological assessment of Aplastic Anaemia and post bone marrow transplant Graft Dysfunction for the purposes of targeted therapeutic intervention, and
- The Royal Melbourne Hospital/Fight Cancer Foundation Fellowship awarded to Dr Koldej for her project High multiplex analysis of the immune microenvironment in BM trephine samples using DSP.

Dr Rachel Koldej, ACRF

The central hypothesis to these studies is that acquired BMF and graft dysfunction post bone marrow transplantation, whereby there is evidence of donor engraftment but persistent cytopenias (low blood cell counts), occur via similar mechanisms and treatments that are applicable to one condition could also be used to treat the other. To this end, DSP is also being incorporated as an analysis technique into the prospective RESELECT clinical trial to treat both Aplastic Anaemia and Graft Dysfunction with combinations of drugs designed to target the dysfunctional immune microenvironment in these conditions.

As this project utilises archival BM trephines, which exist in pathology departments in their thousands, there are large numbers of patient samples that could be accessed to perform validation and translation studies in the future without requiring new patient samples to be collected, which would take a significant amount of time. In this way, the use of DSP will significantly increase the speed at which new treatments and monitoring methods are developed for BMFS and translated to the clinic.

Ultimately, this project will lead to a greater understanding of the contribution of the immune microenvironment to the biology and clinical course of BMF disorders. It will identify new therapeutic targets and new improved methods of disease monitoring that complement other techniques currently under development and those already used in the clinic.