Report for the Australian Head and Neck Cancer Society Research Foundation (ANZHNCS) Project Grant 2018/2019.

Investigator: Professor Robert Ramsay, Peter MacCallum Cancer Centre, VCCC Melbourne.

Title: Novel Immunological Targeting of the Rare Cancer Adenoid Cystic Carcinoma

Project Details

Summary

Adenoid Cystic Carcinoma (AdCC) is a rare malignancy arising in the salivary gland, breast and other organs and is characterised by the display of a slow, relentlessly progressive course. Approximately 1,200 Americans and around 85 Australians are diagnosed per year (Australian Institute for Health and Welfare). For a significant proportion of patients, the slow, relentless progression of this cancer leads to local relapse and the development of distant metastases. Most patients will succumb to the disease within 10-15 years. For patients with this slow recurring cancer, there are currently no treatments available and progression to fatal metastatic disease is an inevitable feature of a patient's clinical course. Because of its rarity and distinct clinical characteristics, AdCC remains an understudied malignancy and this has resulted in few evidence-based studies aimed at developing therapies to treat AdCC.

We have developed three aims to which results have been generated: -

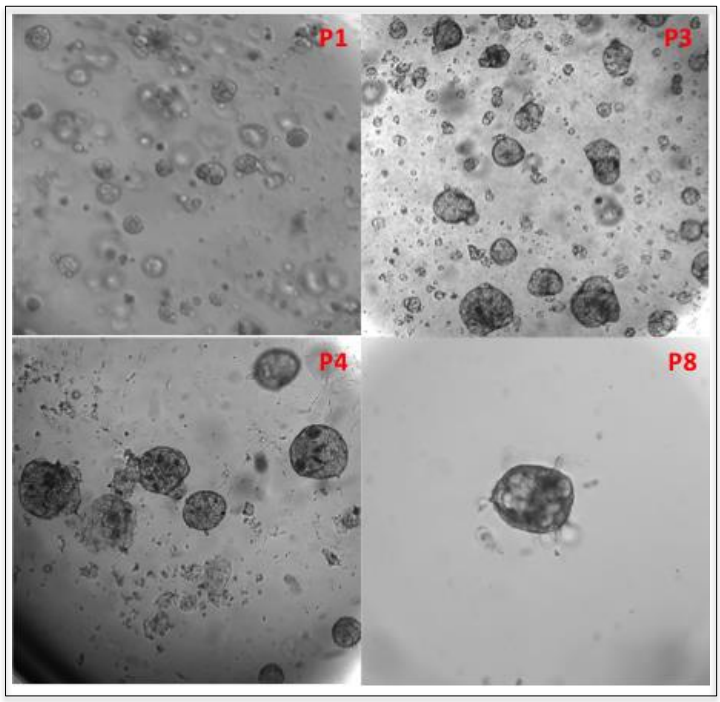
There is a substantive clinical need to offer novel targeted treatment options for patients with AdCC. This project has sought to bring additional insights into immunotherapy opportunities to treat AdCC. This project had three aims directed at defining the immune landscape of AdCC:

Aim 1. To establish 3D tumouroids from fresh AdCC biopsies or surgical resection specimens along with the expansion of tumour infiltrating lymphocytes (TILs).

Aim 2. To combine AdCC tumouroids and TILs to evaluate the functional cytotoxicity of the TILs against the matched tumour with and without immune checkpoint blockade antibodies.

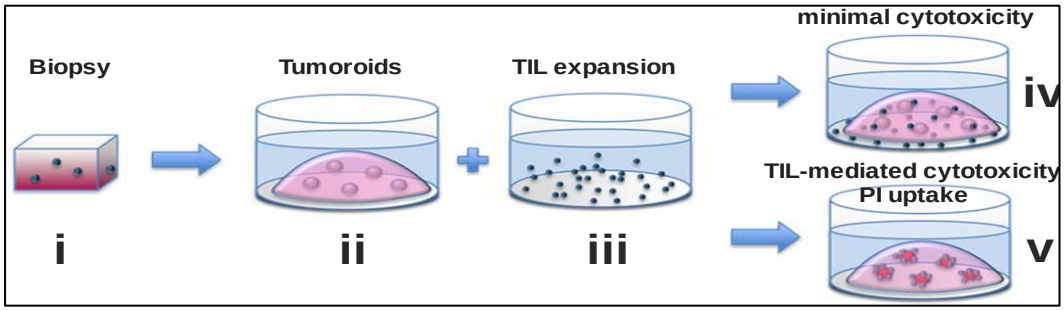
Aim 3. To define the topological landscape of immune cells within, at the AdCC tumour margin and in the stroma using multiplex immuno-fluorescence (OPAL).

Aim 1. *Success in growing AdCC tumouroids*



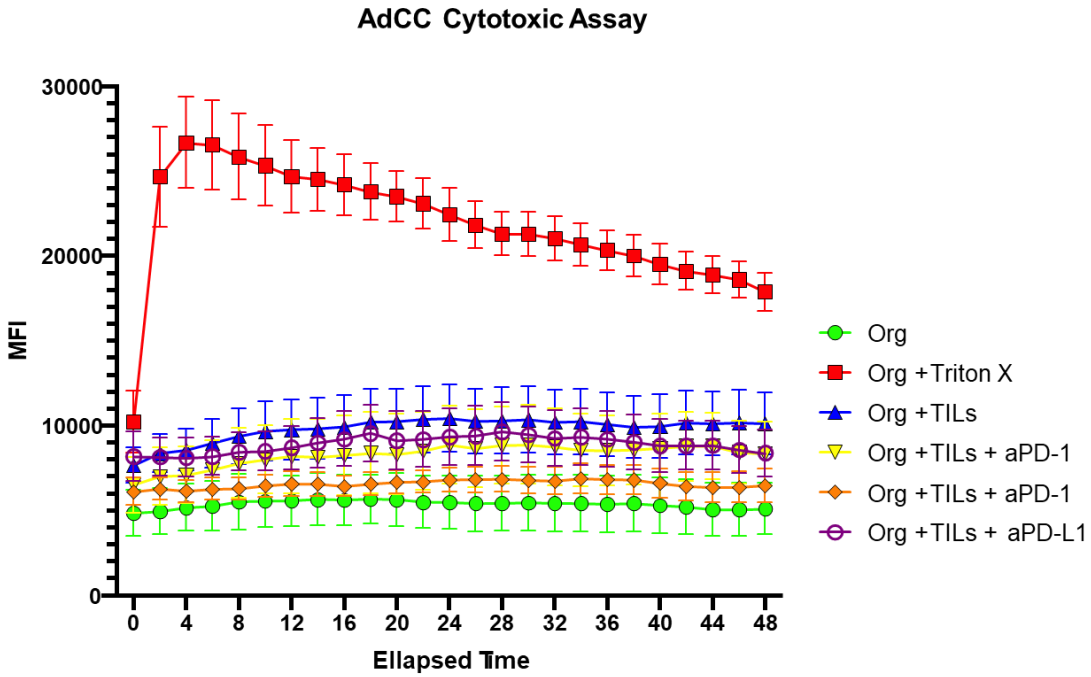
AdCC patient biopsy derived tumouroids successfully grown in Matrigel

Aim 2. *Testing of tumour-associated immune cells confirm they are dysfunctional*

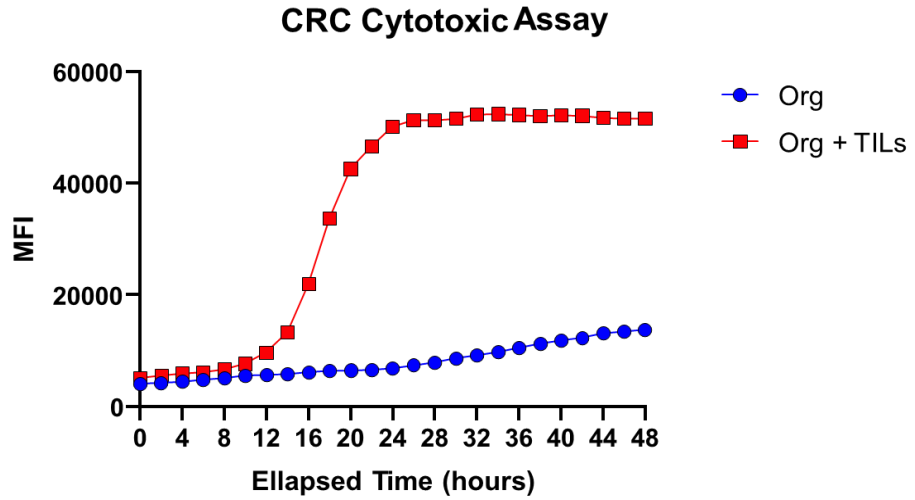


Cytotoxic assay format. Co-culture of AdCC patient biopsy derived tumouroids in Matrigel with patient derived with matched TILs.

A

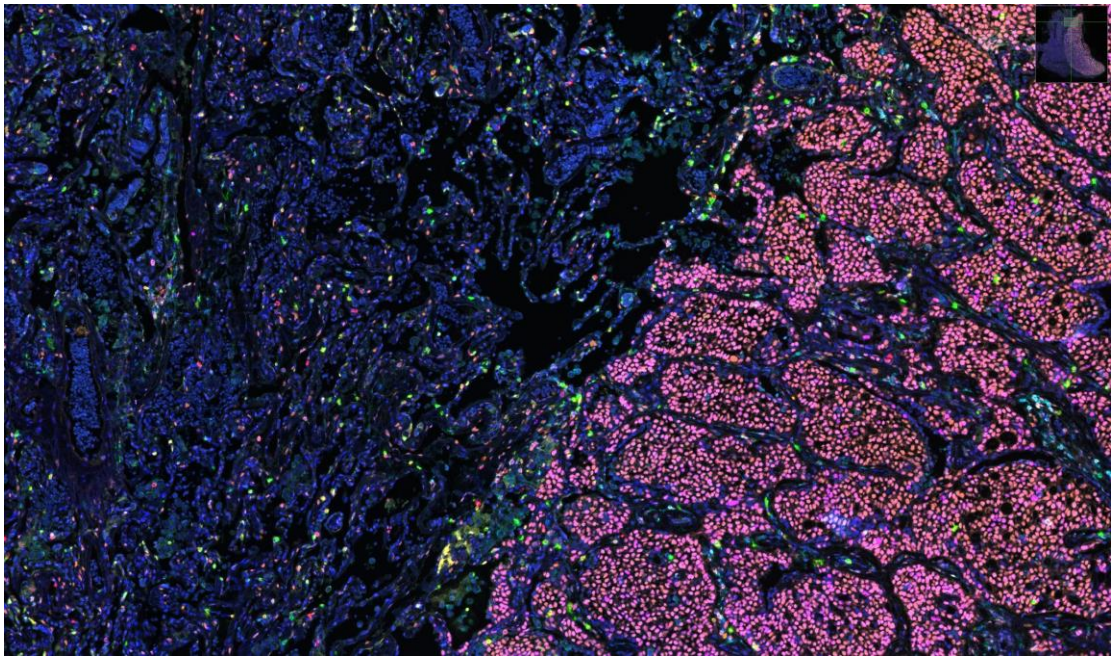


B



Cytotoxic assay. (A) Co-culture of AdCC patient biopsy derived tumouroids in matrigel with patient derived, matched TILs. Results from one of three AdCC patients is shown where no cytotoxicity was evident. (B) A similar assay using tumouroids from a colorectal cancer with matched TILs used to show positive killing. Mean fluorescent intensity (MFI) tracks the propidium iodide uptake. Inclusion of Triton X (red) was used as a positive control to measure MFI levels. Anti-PD1 checkpoint inhibitor antibodies were also included.

Aim 3. *Topological landscape of immune cells within a AdCC tumour demonstrates absence of TILs within the tumour.*



Surrounding tumour stroma **Tumour**

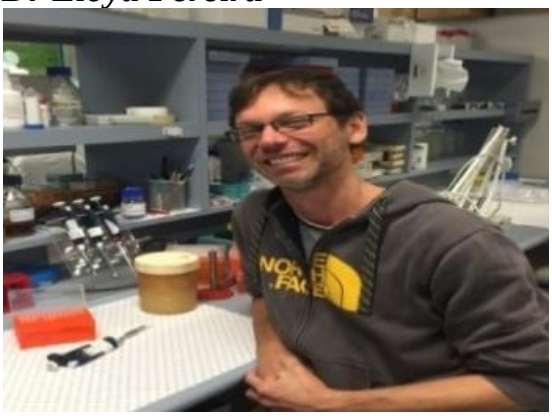
OPAL analysis of AdCC tumour. AdCC tumour (lung metastasis) and surrounding lung tissue (stroma) was analysed for markers of AdCC (-pink) and for the distribution and exclusion of immune cells (-green).

Summary and conclusions

We have successfully established an OPAL staining platform for AdCC tumours using a panel of AdCC tumour and immune cell markers. Our future goals will be to extend this approach to additional patient samples in order to develop a wider picture of the AdCC immune status. This process is currently underway using additional patient (x3 patients) biopsy material obtained in collaboration with A/Prof David Wiesenfeld). In addition, we will apply our OPAL panels to biopsy material obtained from our MYPHISMO clinical trial. This is a unique opportunity that will allow us to assess the abundance and function of AdCC TILs in response to immunotherapy.

Prof Rob Ramsay

Dr Lloyd Pereira



Dr Toan Pham