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TITLE: Developing Novel Strategies to Uncouple Immunotherapy-Induced Autoimmunity from Antitumor Immunity

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CONTRACTING ORGANIZATION: University of Western Australia

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14. ABSTRACT

The project aims to study underlying mechanisms of cancer immunotherapy driven adverse events. These adverse events affect tissue and organs similar to autoimmune disease, and is T cell driven. As human organ biopsies are challenging to collect, we propose studying organ infiltrating T cells in animals treated with immunotherapy. By comparing organ and tumour infiltrating T cells, we aim to identify druggable targets that can be targeted to manage these adverse events without affecting the anti-tumour immune response. We report that gene expression of T cells from affected organs and tumours are very different, suggesting that there are targets that we could potentially focus on. We successfully performed flow cytometry and bulk RNA sequencing on T cells, and are in the process of completing single cell sequencing and analysis.

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TABLE OF CONTENTS

1. INTRODUCTION:

The aim is to study underlying mechanisms of cancer immunotherapy driven adverse events. These adverse events affect tissue and organs similar to autoimmune disease, and is T cell driven. As organ biopsies are challenging for humans, we propose studying organ infiltrating T cells in animals treated with immunotherapy. By comparing organ and tumour infiltrating T cells, we hope to identify druggable targets that will prevent these adverse events without affecting the anti-tumour immune response.

2. KEYWORDS:

Immunotherapy, mesothelioma, immune related adverse events, autoimmunity

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1 – Single cell sequencing of tumor and organ infiltrating T cells

- Writing and obtaining animal ethics approval (1-2 months; complete)
- Setting up in vivo experiments to collect tumor, liver, pancreas and colon samples from two murine models. (1-2 months; Complete)
- Single cell sequencing of collected samples (3-6 months; 50% complete)
- Single cell data analysis - Cell annotation, alignment of transcriptome TCR sequences, differential transcriptome analysis between tumor and organ infiltrating cells. Analysis to determine targets for downstream validation. (7-12 months; 50% complete)
- Comparative analysis between different murine models of irAE. Selection of targets for downstream in vivo assessment (7-12 months; 25% complete)

What was accomplished under these goals?

1. Major activities and key outcomes
    - Successfully obtained ethics approval (AE236) from the Harry Perkins Institute of Medical Research to perform animal experiments.
    - Successfully imported INS-HA mice as a second model to interrogate immune checkpoint inhibitor mediated autoimmunity.
    - Successfully collected 156 samples from liver, pancreas, colon and tumors (effector sites) from a murine model (Foxp3.DTR). Mice had Tregs partially depleted and/or were administered with immune checkpoint inhibitors (anti-CTLA-4 + anti-PD-L1) at doses known to cause organ toxicity. This particular combination immunotherapy was used to model lowered peripheral tolerance in the context of irAE generation.

Finding 1 – Different T cell subsets increase at effector sites post immunotherapy. We compared the frequency and phenotype of organ infiltrating T cells by flow cytometry and found that immunotherapy increased $CD4^+$ T cell frequencies in pancreas, and $CD8^+$ T cell frequencies in tumours and liver (Figure 1). Increase in $CD8^+$ T cells expressing inhibitory receptors was only found in tumours, suggesting that T cell activation and differentiation in the presence of chronic antigen exposure is only present in the tumours. Different mechanisms drive T cell activation in other organs.

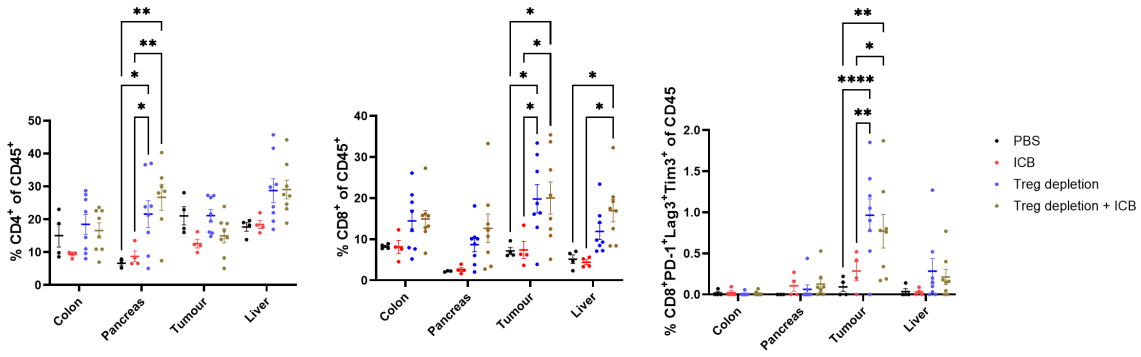

Figure 1. Proportions of $CD4^+$, $CD8^+$ and activated $CD8^+PD\text{-}1^+LAG\text{-}3^+TIM3^+$ T cells in pancreas, liver, colon and tumour samples after anti-CTLA-4 + anti-PD-L1 treatment (ICB) and transient Treg depletion (4 x 10ng/g DTX) in Foxp3.DTR mice inoculated with AB1-HA mesothelioma tumour cells.

Unmet goal – We sought to further understand the differences in immune cells from different effector sites through single cell analysis. We encountered issues with the 10x genomics platforms at our local sequencing facility (Institute of Immunology and Infectious Diseases, Murdoch University), as they had to be replaced and delivery was delayed. CI-Chee also had to take parental leave from October to December 2022, and was unable to expedite the process. We mitigated this risk by performing sequencing with a different facility (GenomicsWA), and this took an additional 3 months to setup. We are in the process of completing single cell sequencing of 24 samples. We attach evidence of preliminary data from single cell sequencing below (Figure 2).

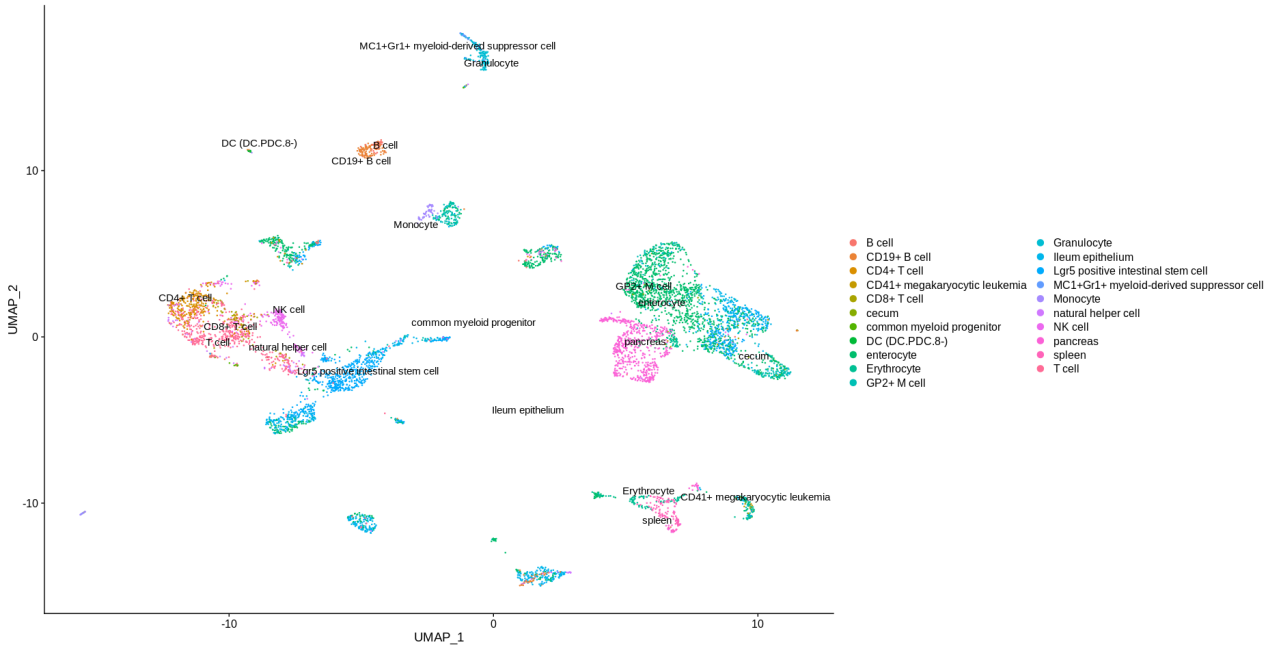

Figure 2. Preliminary data of single cell 5'VDJ and RNA sequencing of samples derived from Foxp3.DTR model. UMPA data representative of first run, which consists of 4 samples and 6800 cells.

Finding 2 – T cells from different effector sites post immunotherapy are transcriptionally different. As we encountered difficulties with the single cell analysis, we performed complementary bulk RNA sequencing of equal numbers of sorted $CD3^+$ T cells enriched from different effector sites. Preliminary analysis suggests that the gene expression profile of T cells from organs are different. We are in the process of performing pathway analysis on differentially expressed genes, Gene Set Enrichment Analysis and upstream regulator analysis to identify common pathways enriched in each site from different analyses methods (Figure 3).

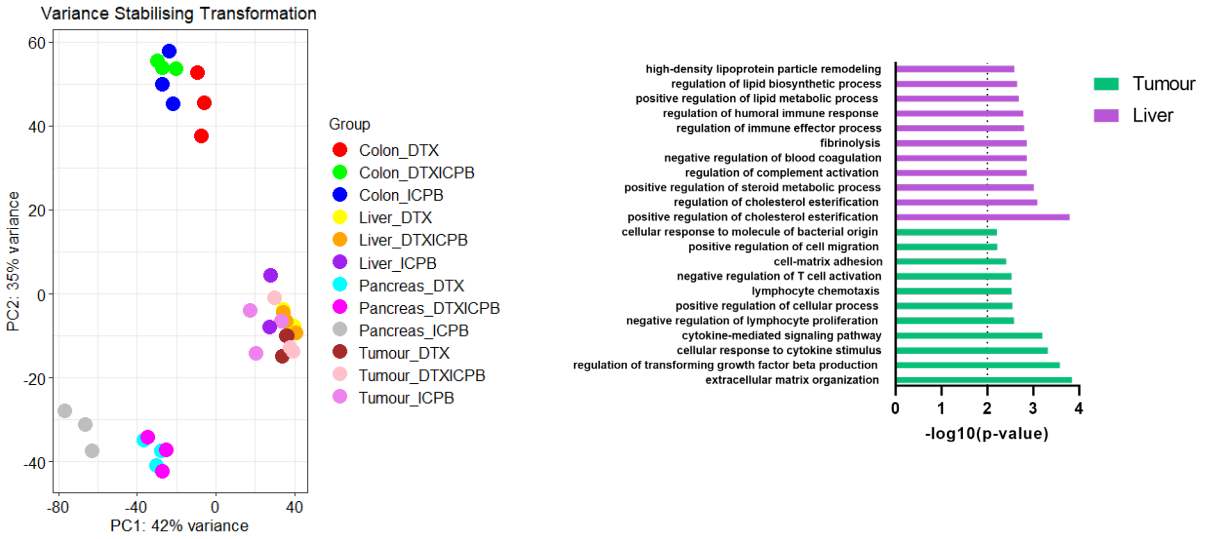

Figure 3. Bulk RNA sequencing of CD3 T cells enriched from organs and tumours reveal distinct profiles for each organ post immunotherapy. PCA of gene expression data of different samples (left), and example of pathway analysis comparing tumour versus liver infiltrating T cells (right).

Finding 3 – Tumor bearing INS-HA mice failed to develop autoimmunity after immune checkpoint inhibitor treatment. Our second model seeks to mimic shared antigen between target organ and tumor. We utilized a transgenic mouse model that expressed model antigen hemagglutinin (HA) under the insulin promotor (RIP), and a tumour cell line that expresses HA antigen on the surface. In a previous study of a similar model (RIP-OVA), seeding mice with a population of naïve OVA-specific T cells followed by immune checkpoint inhibitor treatment causes fulminant diabetes. We failed to recapitulate these results in the RIP-HA model (0/10 mice developed diabetes).

To check that INS-HA mice could develop autoimmune diabetes, we adoptively transferred activated HA-specific T cells as a positive control and mice developed autoimmune diabetes in the absence of a tumour (Figure 4). However, this was not the case when activated HA-specific T cells were administered to tumour-bearing animals. This suggests that a tumour could be systemically suppressing the autoimmune response. Although results are potentially interesting for a different field, the results from this model does not fit the overall goal of our project.

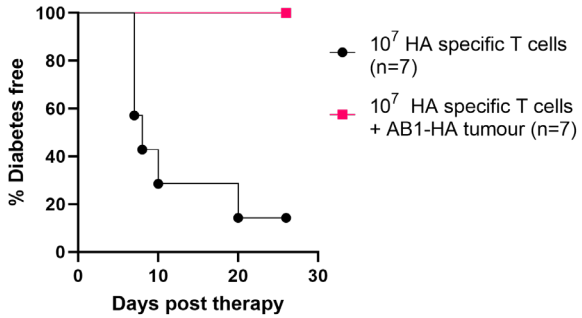

Figure 4. Diabetes incidence of INS-HA mice administered 10^7 activated HA-specific $CD8^+$ T cells, in the presence and absence of AB1-HA tumours

What opportunities for training and professional development has the project provided?

Ms Amber-Lee Phung attended RNAseq and coding (R) courses organized by the Australasian Society of Immunology in 2022.

Dr Kofi Stevens and Dr Jonathan Chee will be presenting and attending the International Mesothelioma Interest Group Meeting on 24th to 26th June 2023. Dr Chee is an invited speaker and Dr Stevens has received an interview request from media covering this conference.

How were the results disseminated to communities of interest?

This project and preliminary results were presented to the consumer advisory panel through regular bi-annual meetings. The panel members include family members and carers of patients with mesothelioma, as well as key organisations involved in patient advocacy and support who disseminate information on our research. This is an important forum to reach non-scientific audiences, enhance public understanding and increase interest in this important therapeutic area. The project has received consumer support from concerns about immunotherapy related side effects.

Dr Chee presented early findings at the 2022 National Centre for Asbestos Related Diseases annual scientific meeting. This brings together key researchers in the field from across Australia and collaborating international sites.

What do you plan to do during the next reporting period to accomplish the goals?

1. Complete single cell sequencing and analysis from the Foxp3.DTR model.
2. Identify key pathways that can be targeted from our sequencing data, and validate these pathways with in vivo experiments
3. Import a different model of shared antigenic target (RIP-OVA)

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Our results to date suggest that organ infiltrating T cells are different from tumor infiltrating T cells in mice affected by immunotherapy side effects. In-depth understanding of these differences is the next step to developing novel targets to prevent these side effects.

What was the impact on other disciplines?

One of our models failed to develop autoimmune diabetes in the presence of a tumor. Though unexpected, this is very interesting as the underlying mechanisms can be studied and exploited to treat autoimmune diseases. This will be considered in consultation with Prof Thomas and A/Prof Arabella Young (AIs on the grant), both experts in autoimmune diabetes (type 1 diabetes).

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report in changes to approach.

Actual or anticipated problems or delays and actions or plans to resolve them

Delays encountered

1. The fluidics platform for single cell library preparation was not available to our local sequencing facility from April 2022 – July 2022. We had to enlist the services of a different collaborator, and the work has continued since. The costs remain as the kits, reagents are still the same
2. I (CI-Chee) had to take parental leave from October 2022 – December 2022. This meant that I was unable to expedite certain processes, such as data analysis and finding a new collaborator for single cell sequencing. We anticipate that a no-cost extension will be required to complete all proposed analyses.

Problems encountered

1. One of our models did not develop autoimmunity with immune checkpoint inhibition, this meant that we were not able to study organ-infiltrating T cells in this model. To mitigate this, we are importing an analogous model (RIP-OVA) from our collaborators in Melbourne.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

We are in the process of applying for animal ethics approval to add RIP-OVA, OT-I mice to our ethics.

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

Publications, conference papers, and presentations
Journal publications.

Nothing to report

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Nothing to report

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other Products

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Dr Jonathan Chee
Project Role:	PI
Nearest person month worked:	9
Contribution to Project:	Dr Chee is the PI of this proposal and responsible for the overall direction of the Specific Aims and supervision of the analyses related to the proposal.
Funding support:	NA

Name:	Ms Amber Lee Phung
Project Role:	Research Assistant
Nearest person month worked:	4
Contribution to Project:	Ms. Phung has performed mouse experiments and sample collection
Funding support:	NA

Name:	Dr Kofi Stevens
Project Role:	Junior postdoc
Nearest person month worked:	3
Contribution to project:	Sample collection and in vivo experiments
Funding support:	NA

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Organization Name:	Genomics WA
Location of Organization:	Australia
Partner's contribution to the project:	Assistance with single cell library preparation and sequencing.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:
Nothing to report
QUAD CHARTS:
Nothing to report

9. APPENDICES:

None