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Metro South Health

A pre-clinical, personalised model for bladder cancer immunotherapy

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Summary

Bladder cancer (BC) affects approximately 3,000 Australians annually and has an average five-year survival rate of 54%. Despite being the first malignancy to have an immunotherapeutic standard-of-care (BCG), the potential of immune checkpoints and the inhibition of such is yet to be fully understood. With some success seen in lung and colorectal cancers, trials are emerging for these treatments in urological malignancies. Patient-derived organoids and explants present as potentially suitable platforms to model. The introduction of autologous components such as serum and PBMCs will increase the personalisation of the model and may reflect patient response at the cancer-immune axis more accurately.

Immune checkpoint binding is a means of controlling effector cell function. The basic theory is that checkpoint ligands are upregulated in various malignancies and inhibition of these interactions can restore a degree of anti-cancer activity in effectors such as B and T cells.

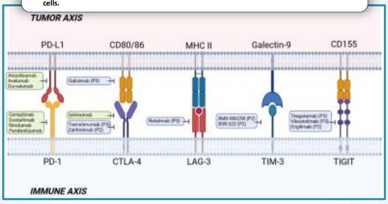
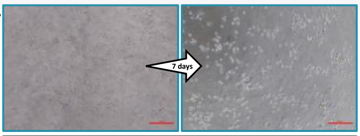


Figure 1. Clinically relevant immune checkpoints in BC with principal inhibitors. (P#) indicates clinical trial phase if applicable *Mackenzie et al., in preparation*

A readily deployable, robust 3D co-culturing platform for predicting response to immunotherapy in BC will inform personalised treatment selection and this potentially improve the clinical outcomes for people with BC.

Comparison of traditional vs novel culture media

A.



B.

PBMC Concentration	Control (RPMI)		3X		2X		1X		0.5X		0.25X	
2x10^5	1393	6227	3292	3026	15848	15191	19796	19403	20869	18674	13971	888
	31373	43028	2258	2666	17757	15543	21968	22383	23767	23378	29163	20354
1x10^5	4123	6576	1268	761	7210	6990	8716	10657	12413	12628	15328	9261
	14196	20942	1008	575	7574	6264	3008	7428	12024	12003	14015	9525
5x10^4	1639	2101	360	359	2522	2754	3190	4731	5690	5935	7245	2635
	5925	9484	1015	232	2392	2507	4367	4705	5748	5703	6740	2328
2.5x10^4	389	570	174	180	765	253	1753	2092	2596	2608	3172	685
	137	619	156	129	534	431	909	1300	1579	1026	523	198

(Column header group: Happy Cell Concentration)

Figure 2. RPMI combined with a suspension medium (i.e. Matrigel®) is commonly used to create a 3D culture environment. Happy Cell ASM is a novel and promising synthetic suspension medium with poorly understood effects on PBMCs **(A)** PBMCs in Happy Cell-ASM immediately after seeding and after 7 days. **(B)** Heat map showing ATP presence in relative luminance at t=7 following CellTiterGlo® assay. **Preliminary results show 1x and 0.5x retain a similar viability to RPMI. Several biological repeats are planned for statistical robustness.** Scale, 100 µm

Immunotherapeutic co-culture with primary tumour slices/organoids and autologous Peripheral blood mononuclear cells (PBMCs)

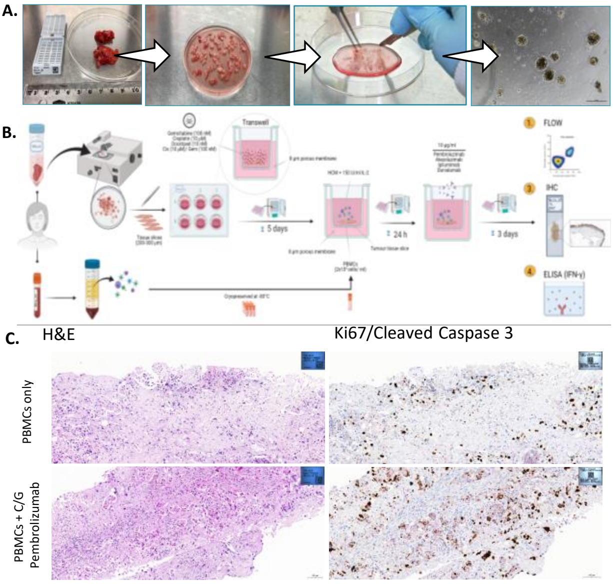


Figure 3. (A) Procedure for handling tumour tissue for organoid establishment. Freshly excised tumour samples are manually dissected into small fragments to assist with enzymatic digestion of tissue. This tumour digest is then filtered and cultured in organoid media, forming spherical clusters. **(B)** Flow diagram depicting co-culture process with tissue slices (note that tissue slices can be substituted for organoids in the procedure). **(C)** ***Left*** H&E stains of tissue slices in co-culture with PBMCs or PBMCs + cisplatin/gemcitabine and pembrolizumab (PD-1 blocker) ***Right*** Ki67 (***brown***) stain indicates proliferative cells and cleaved caspase 3 (*purple*) indicates apoptotic cells. **IHC staining for CD45, CD3, CD4, CD8, PanCK, PD-1, PD-L1, CTLA4, TIM-3, LAG-3, TIGIT and VISTA is planned for the histological characterisation of patient samples alongside IFN-γ quantification and flow cytometry analysis**

Scale, 100 µm

Multiparametric (21-colour, 18-parameter) Flow Cytometry analysis of PBMCs

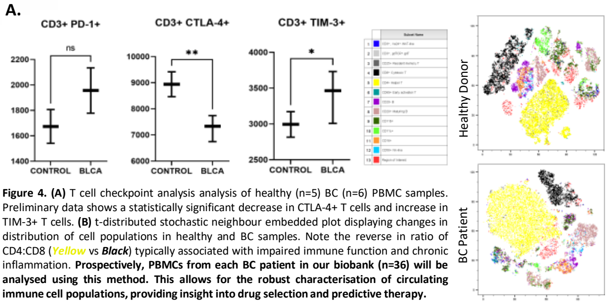


Figure 4. (A) T cell checkpoint analysis analysis of healthy (n=5) BC (n=6) PBMC samples. Preliminary data shows a statistically significant decrease in CTLA-4+ T cells and increase in TIM-3+ T cells. **(B)** t-distributed stochastic neighbour embedded plot displaying changes in distribution of cell populations in healthy and BC samples. Note the reverse in ratio of CD4:CD8 (*Yellow* vs ***Black***) typically associated with impaired immune function and chronic inflammation. **Prospectively, PBMCs from each BC patient in our biobank (n=36) will be analysed using this method. This allows for the robust characterisation of circulating immune cell populations, providing insight into drug selection and predictive therapy.**

Conclusions and challenges

Preliminary data shows that the platform remains viable for the length of treatment (t=10 days) and can be assessed for proliferation, immune cell infiltration and cell death when combined with checkpoint inhibitors and autologous components. Further optimisation and experimentation is required and planned with differing culture settings and drug combinations/concentrations.

- **Do tissue slices or organoids function as a more suitable platform for immunotherapy prediction?**
- **What is the potential for co-culture in rapidly comparing metastatic sites in patients for accurate, holistic treatments?**

References & Acknowledgements

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