The development of eosinophils from iPSC (iEos) for novel cancer immunotherapy.

Background:

Immunotherapy is now recognized as the fifth major pillar of cancer treatment alongside chemotherapy, radiation, surgery and therapeutic small molecules. Despite successes in harnessing the adaptive immune system through specific monoclonal antibodies (mAbs) endowed with cancer killing payloads and enhanced cytotoxic T cells with checkpoint inhibitors (1), their value is limited by the function, and lack of breadth and cancer specificity of the patient's endogenous immune system, as well as the suppressive tumour microenvironment (TME). Autologous Chimeric Antigen Receptor (CAR) T Cells have provided a much more sophisticated level of immunotherapy, conveying upon T cells a cancer "tracking and kill" capacity (2,3). But they are expensive, time consuming to produce, have variable characteristics on manufacture and the quantity and quality of T cells that can be obtained from the patient are often severely compromised by prior chemotherapy. Furthermore, CAR-T cells have had a poor impact in clinical trials on solid tumours. Very recently, NK cells have emerged as an important alternative to CAR-T cells because they naturally possess multiple cancer recognising receptors, don't exhibit graft versus host disease and nor do they require activation (4). NK cells can also be transduced to become CAR-NK cells. Notwithstanding these collective successes, the ability to attack solid cancers remains a critical unmet clinical need.

The obvious question arises: why not combine the adaptive immune response with potentially potent contributions from the innate immune system (5)? One key innate cell type which has broad functions, including anti-cancer activity, is eosinophils (6). Eosinophils are commonly found within tumours and they form part of the TME of many human solid and haematological cancers (7,8). They produce a plethora of soluble mediators and, if their powerhouse of immune modulating factors can be appropriately harnessed to limit negative side effects, they could transform current immunotherapies, particularly against solid tumours.

The key feature of eosinophils infiltrating tumours early in the disease is based on their expression of multiple chemokine and alarmin receptors that enable them to directly lyse tumour cells. They also play a role in regulating the TME, providing anti-tumourigenic factors, in addition to recruiting T cells, dendritic cells and macrophages (9,10).

Thus eosinophils have obvious potential for playing an important role as anti-cancer therapeutics, notwithstanding the need to ablate or modulate their pro-tumour growth effects.The reason that eosinophils have, however, failed to gain widespread acceptance as potential targets for immunotherapy is primarily because they are traditionally linked to hypersensitivity and because of their low cell numbers and lack of accessibility (6). They also have a relatively short half-life (days) (9).

Proposal:

Deriving "off-the-shelf" cancer fighting immune effector cells from induced pluripotential stem cells (iPSC) offers a remarkable opportunity for overcoming these limitations (10). Under appropriate conditions, iPSC can be propagated indefinitely in vitro and hence they serve as a continual supply of cells to be strategically differentiated. Very recently, Lai et al (11) showed they could indeed generate eosinophils from human embryonic stem cells (hESC) and iPSC. The iEos were able to kill multiple cancer cells in vitro but had no effect on normal human mesenchymal stem cells, fibroblasts, or human umbilical vascular and endothelial cells, demonstrating safety.

Project:

This present project is a logical extension to these studies. We aim to fuse the respective abilities of the innate and adaptive immune systems to attack cancer by inducing cancer targeting iPSC derived iEos which will be further enhanced functionally by insertion of a cancer specific CAR and deletion of immune suppressive gene(s). They will be interrogated for their synergistic function with our current CAR- iNK and CAR-T cells.

The project takes its lead from the Cartherics platform which uses iPSC derived from homozygous HLA haplotype donors, to minimise MHC mismatch and bi-directional immune rejection.

Aims:

1. Develop clinic-ready technology for inducing eosinophils from human iPSC (iEos).
2. Characterise iEos cells phenotypically and functionally with a panel of reagents to validate their eosinophil authenticity.
3. Initially examine production of tumour inhibiting versus tumour promoting soluble factors by iEos.
4. Genetically edit the iPSC to endow these iEos cells with a cancer specific CAR.
5. Genetically engineer the IPSC to enhance iEos function by deleting key immune inhibitory genes
6. Examine the functional capacity of the genetically enhanced iEOS to kill human ovarian cancer cells in vitro or when transplanted into NSG mice.
7. Explore the additive benefits of iEos together with iNK or T cells for their cancer cell killing capacity in NSG mice. All of these effector cells would carry the same CAR to promote tumour targeting.

Supervision:

Main supervisor: Professor Alan Trounson
Co-Supervisor: Dr. Frederico Calhabeu
Associate Supervisor: Dr. Richard Boyd
Cartherics Education Program Director: Professor Graham Jenkin

Selected Publications

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11. Lai,W., Xie,H., Liu, Y., Zheng,F., Zhang,Y, LeI,Q., Lv, L., Dong, J., Song, J., Gao, X., Yin, M., Wang, C. and Deng, H. Human pluripotent stem cell-derived eosinophils reveal potent cytotoxicity against solid tumors. Stem Cell Reports, Stem Cell Reports (2021) 16: 1697–1704.