The development of macrophages from iPSC (iMacs) for novel cancer immunotherapy

Background:

Immunotherapy has provided a potentially revolutionary approach to combatting cancer. Prototypical immunotherapies are antibodies aimed at blocking checkpoint receptors such as CTLA-4 and PD-1/PD-1L. By releasing the "immune hand brakes", the patient's endogenous immune system is given freedom to attack the cancer. This approach has been particularly successful for melanoma and lung cancer and is being tested in a multitude of other cancers, with variable success. While sufficient to warrant Nobel Prize recognition, the essential problem is that these checkpoint inhibitors rely on the quality and quantity of the patient's endogenous immune system. Chimeric Antigen Receptor (CAR) T cells have provided a much more sophisticated level of immunotherapy, conveying upon T cells a cancer "track and kill" capacity. CAR-T cells have been particularly successful in the treatment of B cell-based haematological malignancies but to date have had poor impact clinically on solid tumours. Since the vast majority are autologous CAR-T cells, their efficacy is linked to, and limited by, the functionality of the patient's immune system. This is commonly severely compromised by prior assault by the cancer, even before diagnosis, treatment with chemotherapy and even natural ageing. To a large degree these deficiencies are being addressed by the development of allogeneic "off-the-shelf" CAR-T cells and very recently CAR-NK cells. While CAR technology has provided unheralded advances in cancer treatment, current approaches fail to engage the advantages of the patient's "polyvalent" immune system.

Despite these advances, logic dictates that a comprehensive assault on cancer should mimic the mechanisms developed through evolution to eradicate infection: effectively engage both the innate and adaptive immune systems. Currently approaches centre on T cells and, more recently, NK cells. However, the first line of immune defence actually centres around the more functionally "primitive" but ultimately critical, macrophage lineage cells. While their basic capacity is to remove dead, dying and infected cells, it is now abundantly clear that cells from the myeloid lineage have the important function of promoting or retarding immune responses through cytokine networks; in addition, dendritic cells trigger adaptive T cell immune responses by presenting antigenic peptides in the context of MHC Class I and II molecules. Clearly the ability to enhance T cells, NK cells and macrophages with specific cancer targeting would be the ideal weaponry to attack cancer. Macrophages therefore represent a major, underutilised, pillar in cancer immunotherapy. If they can be adequately harnessed, the same principles could be applied to treating severe infections.
Furthermore, macrophages themselves are heterogenous with two main functionally distinct subsets: M1 (pro-inflammatory) and M2 (anti-inflammatory). M1 are clearly the most important for attacking cancer and are the focus of this project. Indeed monocyte-derived chemoattractant 1 (MCP-1) has been shown to play a role in cytotoxic T cell development and function (1).

However, one unifying property of all these cells (T cells, NK cells, macrophages) is the need to produce them in sufficient number and functional capacity. In particular macrophages have a very short half-life but can be produced effectively and efficiently from haemopoietic stem (HSC) cells. Very recently two groups have addressed this problem by using iPSC technology (2,3). iPSCs have effectively limitless capacity for self-renewal. If they can be successfully differentiated into macrophages this could overcome the major shortfall of their life span. Present studies still indicate a relatively short life in vivo (NSG mice): expansion for 3 days, persistence 20-30 days which was still sufficient allow to short term, limited reduction in a solid ovarian cancer model (14 days). More specifically they need to be M1 macrophages. This project takes its lead from Cartherics platform which uses iPSC derived from homozygous haplotype donors, to minimise MHC mismatch and immune rejection.

Aims:

1. To develop the technology for inducing macrophages from iPSC (iMacs).
2. To preferentially induce M1 macrophages by varying the differentiating conditions to block the M2 pathway or promote the M1 pathway (e.g. by addition of pro-inflammatory cytokines).
3. To characterise these cells phenotypically and functionally (e.g. pro- versus anti–inflammatory cytokines; CD surface expression).
4. To endow these cells with a cancer specific CAR such as a TAG-72 CAR.
5. To develop stable TAG-72 CAR-expressing iPSC clones.
6. To genetically engineer the IPSC to enhance iMac function and longevity.
7. To show that engineered iMac/CAR-iMac/ gene KO iMac possess essential properties of PBMC-derived macrophages, such as homing capacity and cytotoxity activity (phagocytosis). iPSC-derived macrophages capacity to eliminate human ovarian cancer will be assessed both in vitro and in vivo. An in vivo bioluminescence imaging method will be used to evaluate the capacity of iMacs to kill human ovarian cancer in NSG mice.
8. To explore the additive benefits of iMacs followed by, or coupled with, iNK or iT cells for cancer killing capacity in NSG mice.

Supervision:

Main supervisor: Professor Alan Trounson
Co-Supervisor: Dr Frederico Calhabeu
Associate Supervisor: Professor Richard Boyd

Sample of References:

1. The Role for Monocyte Chemoattractant Protein-1 in the Generation and Function of Memory CD8+ T Cells. Tao Wang et al J Immunol (2008); 180:2886-2893.
2. Pluripotent stem cell-derived CAR-macrophage cells with antigen-dependent anti-cancer cell functions. Li Zhang et al. J Hematol Oncol (2020) 13:153.