Developing a Clinically Translatable iPSC-derived Immunotherapy

Background:

The use of Chimeric Antigen Receptor (CAR) immunotherapies has shown impressive potential in treating liquid tumours, exemplified by complete remission of multiple patients in several recent clinical trials (1, 2), however success with solid tumours has been far more problematic. Despite the promise that CAR-T cells represent, there are still major problems with the practicalities of their manufacture, given that the vast majority of trials involve the use of autologous cells which suffer from poor quality and number of T cells and by the high manufacture cost-per-patient. Coupled with lengthy development times for a patient-specific therapy and potential for failed manufacture runs, large-scale implementation of autologous immunotherapies is practically and financially challenging. One way to address these issues is through the manufacture of an allogeneic immunotherapy, in which induced pluripotent stem cells (iPSCs) are derived from a widely immune-compatible donor, then differentiated at clinical scale into cytotoxic cells (Natural Killer (NK) and T cells) capable of targeting a variety of cancer antigens. Such an allogeneic immunotherapy will combine the potent anti-tumour activity of current autologous products with the clinically scalable manufacture potential of iPSCs, to provide a universal, highly defined, 'off-the-shelf' therapy to the public.

While CAR-T cells have been the forerunner for autologous immunotherapies, the complex signalling required for their derivation from iPSCs has hampered their transition into an allogeneic therapy. While not impossible, current methods of deriving T cells from iPSCs rely heavily on the use of feeder cells, many of which are not approved for use in a clinical setting (3, 4). In contrast, NK cells show far greater ease of derivation from iPSCs and, as such, are rapidly gaining momentum as the preferred candidate for allogeneic immunotherapies. However, attaining an expansion rate feasible for clinical scale is still dependent on the use of feeder cells (5). To circumvent the reliance on feeder cells in both T and NK cell derivation from iPSCs, the development of a feeder-free system would streamline the transition of proposed allogeneic therapies from the laboratory into the clinic. This PhD will assist in the development of a cost-effective feeder-free method for the scale-up of gene-edited iPSCs and their differentiation into effector T and NK cells, at a number suitable for use as a clinical immunotherapy.

Allogeneic approaches offer the unique ability to perform gene edits at an iPSC stage, such as the insertion of CAR constructs, which provide anti-tumour specificity and cytotoxicity, and knock out of genes leading to enhanced killer cell function and persistence *in vivo*. In theory, this provides an unlimited resource of gene-edited progenitor cells which can be readily differentiated into cytotoxic lymphocytes. However, iPSCs are notoriously difficult to gene edit, with the specificity and nature of CRISPR/Cas9 insertions dictating transgene expression and persistence (6-8). In order for a potential immunotherapy to be adopted for clinical use, absolute confidence of the fidelity of gene editing is required, to ensure on-target editing and retention of transgene expression in the proposed immunotherapy. Thus, this PhD will also investigate how aberrant transgene insertion can impact gene edit stability in iPSC passaging and differentiation, and what is required to ensure complete retention of the transgene in the final cell product.

Aim 1:

Use CRISPR/Cas9 to perform gene knockout and CAR knock-in of iPSCs, assess off-target and aberrant knockout/insertion frequency through PCR and sequencing, and determine how gene insertion status can influence transgene stability throughout iPSC passaging and differentiation.

Aim 2:

Investigate heterogeneity of iPSC cultures with respect to clonal variation and the impact this plays on differentiation propensity towards NK and T cells, and how this potential can be defined at an iPSC stage to better predict successful clones for continued investigation as immunotherapy candidates.

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