# Next-generation micro-bead signalling systems for T-cell generation and cancer treatment.

Supervisors: Professor Richard Boyd, Professor Graham Jenkin
Co-supervisors: Dr. Nicholas Boyd, Dr. Roland Shu

**Summary:**
*The ability to genetically enhance a T lymphocyte with a cancer tracking surface antibody that activates a killing cascade upon binding to the target cancer cell, has revolutionised immunotherapy. This project aims to overcome a major practical problem – generating sufficient supply of these genetically "supercharged T cells" from stem cells.*

**Aim:**
The ability to create an unlimited supply of CAR-T cells from iPSC unlocks access cancer immunotherapy to the masses. This requires an efficient iPSC to T-cell differentiation tissue culture system that is applicable to up-scale manufacture and clinical translation. The aim of this project is to provide a crucial element to this differentiation system by translating the highly coordinated set of signals provided by epithelial support cells within the thymus, into synthetic delivery system using microbeads and surface engineering.

**Background**:
Chimeric Antigen Receptor (CAR-) T cell immunotherapy is revolutionising cancer treatment, however autologous treatments will inevitably struggle to reach mass adoption given the cost (~US$400,000 per treatment), not to mention the time to manufacture of which patients often don't have and variability particularly associated with compromised immune systems following cancer assault and chemotherapy. Logically, a precisely defined, consistent, 'off-the-shelf' CAR-T product with broad histocompatibility is the future of this technology.

*In vitro* directed differentiation of T-cells from stem cells sources, such as induced pluripotent stem cells (iPSCs), provides a platform to generate a 'limitless' supply of CAR-T cells. Indeed, it has been shown that co-culture of iPSCs with genetically-engineered mouse support cells can be used to direct differentiation into hematopoietic lineage then into T-cells [1, 2]. However, the current manufacturing system is inherently inconsistent and not suitable for clinical use, given the support lines are of mouse origin. Human cell line analogues have been attempted with limited success, although 3D cultures have been shown to boost T-cell development [3]. Small molecule patterning has been shown to create HSCs from iPSCs, although conversion of these HSCs to T-cells remains challenging. Accordingly, we have been moving toward a molecularly defined, xeno-free, stroma-free, serum-free T cell differentiation culture system suitable for upscale manufacture.

**Proposal outline:**
The primary driving cue for T-cell development occurs through NOTCH signalling. Specifically, delta-like ligand-4 (DLL4) has shown to have the highest affinity to NOTCH-1 receptors presented by progenitor T-cells, and ultimately most potent differentiation stimuli. However there a number of co-stimulatory molecules that affect NOTCH signalling intensity, mechanical interaction, ligand clustering and cell motility, where presentation of DLL4 alone does not create T-cells. One recent publication in Nature Methods, demonstrated that adsorbing Fc-tagged DLL4 with Fc-VCAM-1 can drive differentiation of HSC to Progenitor T-cells [4]. This project will be centred around optimising how to best present DLL4 with other co-stimulatory molecules such as VCAM1 which are tethered to microbeads to drive optimal T-cell development when applied in conjunction with soluble cytokine combinations. Protein orientation is critical to ensure the notch ligand is presented in the correct

confirmation to developing T-cells. Protein engineering and biomaterial surface conjugation are thus required for project success.

NOTCH-signalling is required throughout multiple time-points during T-cell differentiation. Firstly, the NOTCH-signalling drives the induction of T-cells from hematopoietic stem cells and T-cell progenitors (which Cartherics is creating from iPSC). Beyond being able to make T-cell development possible, subtle notch signalling variations have been shown to effect the type of T-cell receptor (TCR) [5]. The bead design needs to be tuned to trigger and sustain develop of TCR $\alpha\beta$T-cells (which are most effective weapon we have in the immune system to attack foreign bodies), as opposed to TCR $\alpha\beta$T-cells. Lastly conversion of naïve cytotoxic T-cells to effector and central memory T-cells can be triggered via NOTCH-signalling [6]. Thus, the beads will also be used to control development of central memory and effector memory T-cells which can enhance CAR-T cell longevity and persistence, ultimately providing a more effective cancer treatment.

There are a number of components we anticipate may be required to enable high efficiency conversion of iPSC to T-cells. These include transcription factors, small molecules, and genetically-engineered mesenchymal stem cells, which will be compared to the bead approach. The beads need to be amenable to these culture inputs and ultimately provide complimentary differentiation stimuli. The killing potency of in vitro generated T-cells is the primary target endpoint of this project and a major hurdle the field currently faces, beyond clinical applicability and T-cell conversion efficiency from iPSC. The ability for these in vitro generated T-cells to kill host different adenocarcinomas, in vitro and in mice will be assessed, and crucial for applying this technology into human clinical trials.

**References**


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