Optimising the function of anti-cancer killer T cells: the role of endogenous TCR in CAR-T function and overcoming exhaustion to supercharge CAR-T cells

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Summary:
Chimeric Antigen Receptor T cells are providing extraordinary results in the clinic, particularly for haematological malignancies. As exciting and tantalising as this immunotherapy revolution is, there are still major hurdles to be overcome in optimising their clinical utility. This project will apply the rules that govern normal endogenous T cell function to CAR-T cells, to help their functional impact across a range of cancers and to increase their longevity after transplantation.

Aim:
Recent studies have shown that T cell exhaustion significantly impacts the ability for chimeric antigen receptor (CAR-) T cells to remain potent killers. Overall, this project will aim to characterise how T cell receptor (TCR) mediated activation and ultimately modulation of T cell exhaustion will enhance CAR-T potency *in vitro* and *in vivo*.

Background:
CAR-T cells are designed to exploit the intrinsic cytotoxic function of T cells whilst manipulating specificity by expressing an antigen-specific receptor and cytoplasmic activation domain. Such cells have recently revealed remarkable clinical success with multiple studies reporting the ability to unburden $CD19^{+}$ malignancies (1). However, transitioning this technology to the treatment of solid tumours is a recognised hurdle in the field (2,3).

T cell activation is essential for the CAR-T production process however it is a double-edged sword. Prolonged activation, either through endogenous TCR or continued CAR/antigen interaction, gives rise to exhausted T cells ultimately diminishing their cytotoxic function. This phenomenon has been attributed to reduced persistence of CAR-T cells and poor tumour response ultimately resulting in disease relapse *in vivo*. It is therefore critical to understand the role of CAR-T cell activation and its association with exhaustion to improve CAR-T therapies in solid tumour indications.

A number of approaches are being explored to overcome activation induced exhaustion. One strategy generates TCR knockout (TCRko) CAR-T therefore limiting endogenous TCR activation whilst still allowing antigen specific activation and function. Whilst this may limit exhaustion, there are safety concerns. It has been documented that misregulated TCR bearing T cells can give rise to lymphoma disease *in vivo* (4). It also potentially eliminates a mechanism by which CAR-T cells can be supercharged to induce improved tumour response. An alternative, and perhaps safer option would be to ameliorate exhaustion through immune checkpoint inhibition. The use of small molecules and antibodies targeting either PD-1 or CTLA-4 for immune checkpoint regulation has been FDA approved in select cancer indications since 2011 (5). Determining whether these strategies could be used synergistically with CAR-T treatment to overcome reduced function due to exhaustion may be essential for the use of CAR-T in solid tumour indications.

Proposed project outline:

Pre-clinical studies conducted by Cartherics to date have demonstrated that T cell hyper-activation leads to the potent, indiscriminate elimination of target cells induced by both CAR-T and non-transduced T cells. Through real-time cell monitoring we have identified a collection of culture conditions which have the ability to augment CAR-T function *in vitro*. Importantly, manipulation of exogenous growth factors and cytokines significantly enhances target cell elimination to the detriment of target-antigen specificity. This project would use these findings as a springboard to further explore activation/exhaustion and how we could manipulate these elements to generate CAR-T cells that can reduce tumour burden AND persist indefinitely to ultimately improve the efficacy of CAR-T treatment. Embedded within this, there are three main questions to be addressed:

1. *What type of T cells make the best CAR-T cells?*

As traditional killer T cells, it is assumed that $CD8^+$ CAR-T cells would be the most potent killers. However, we have generated limited *in vitro* evidence to the contrary suggesting that both $CD4^+$ and $CD8^+$ CAR-T have the ability to eliminate target cells. Initial stages of this project would further investigate the impact of diverse CD4:CD8 ratios both *in vitro* and *in vivo.* Linked with this is the consideration of T cell subtype – central memory vs. effector memory vs. naïve – with a focus on highlighting which cells are the most potent killers.

2. *How does T cell activation impact the potency of CAR-T cells* in vitro *and* in vivo*?*

Endogenous TCRs provide killing faculties in addition to CAR/antigen mediated toxicity. It has been suggested that highly activated T cells may be required to rapidly de-bulk tumours in the first instance however their longevity is short lived. In contrast, rested T cells may be slower, short term killers which may persist and provide long term protection.

3. *How can we overcome activation-induced CAR-T cell exhaustion to ensure they remain potent killers?*

To address this question two approaches would be implemented: i) small molecule modulation and, ii) genetic modulation of immune checkpoint proteins with a particular focus on PD-1/PD-L1 interaction. It is hypothesised that these modulations can overcome T cell exhaustion allowing them to continue to function *in vitro* and *in vivo* even in the presence of continued antigen interrogation.

At the conclusion of this project, you would have identified the T cell subset that gives rise to the most potent, persistent CAR-T cells whose function can be supercharged by exploitation of endogenous TCR and modulation of immune checkpoint signals.

References:

1. CAR T cells — what have we learnt? Nat Rev Clin Oncol [Internet]. 2017;15(1):1–1. Available from: http://www.nature.com/doifinder/10.1038/nrclinonc.2017.196
2. Mirzaei HR, Rodriguez A, Shepphird J, Brown CE, Badie B. Chimeric Antigen Receptors T Cell Therapy in Solid Tumor: Challenges and Clinical Applications. Front Immunol [Internet]. 2017;8. Available from: http://journal.frontiersin.org/article/10.3389/fimmu.2017.01850/full
3. D'Aloia MM, Zizzari IG, Sacchetti B, Pierelli L, Alimandi M. CAR-T cells: The long and winding road to solid tumors review-article. Cell Death Dis. 2018;9(3).
4. Serwold T, Hochedlinger K, Swindle J, Hedgpeth J, Jaenisch R, Weissman IL. T-cell receptor-driven lymphomagenesis in mice derived from a reprogrammed T cell. Proc Natl Acad Sci [Internet]. 2010;107(44):18939–43. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.1013230107
5. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252–64.