PhD Project 6

Re-engineering the function of natural killer cell receptors via CRISPR/Cas9: a new approach for 'off-the-shelf' immunotherapy

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Cellular immunotherapy with chimeric antigen receptors (CARs) have provided unprecedented results in treatment of liquid cancers. However, the few FDA approved autologous based therapies have been priced around $400,000 per patient. Inherently these face major challenges to reach mass adoption. Furthermore, autologous CAR-T treatments can require ~2 months to manufacture (often time patients don't have) and produce variable (often insufficient) cell numbers as a result of poor immune systems hampered by chemotherapy. An on-demand, highly defined, universal product, which is compatible with multiple patients is required to unlock cellular immunotherapy therapy for the public. The answer lies in the utilisation of stem cells.

Cartherics is focused on developing a scalable, clinically applicable manufacture system to differentiate induced pluripotent stem cells (iPSC) to CAR+ cytotoxic cells: T-cells and Natural killer (NK) cells. NK cells offer unique advantages over T-cells in that they do not react against HLA-mismatch, therefore enabling NK-cells to be more compatible with a broad range of hosts effectively matched at the major HLA-C locus (which only has two variants). NK cells also have receptors which can identify and kill some cancer cells. However, they lack the precision of cancer specificity. Accordingly, NK cells have been recently transduced with cancer specific CAR's to complement their more general reactivity. However, the killing efficacy of CAR+ NK cells has been shown to be highly variable based on the CAR construct design with specific reference to which co-stimulatory domains are included. A further limitation of using NK-derived products is their reduced persistence in vivo, which therefore reduces the potential for long-term therapeutic effects and avoiding relapse. To enhance the potency and longevity of NK immune-therapeutics, this PhD will investigate a new alternative to inserting an entire synthetic CAR signalling system into the NK cells. Via CRISPR/Cas9 gene-editing, the terminal binding domain of NK surface receptors will be replaced with single chain variable fragments (scFV) that work as targets for cancer cells. Upon binding, all the natural activation and killing mechanisms related to that NK surface receptor will be engaged, giving the NK cell the potential to alleviate short-falls of CAR-triggered cytotoxicity and enhance the effect of tumour specific NK cell killing.

Aim 1
Use CRISPR-Cas9 editing of the NK92 cell line (which has been used clinically) to establish proof-of-concept knock-in (KI) of cancer specific scFv fragments into different NK membrane signalling receptors. The comparative impact on NK function and numerical expansion potential will be assessed and the strategy then applied to NK cells isolated from PBMCs.

Aim 2
Compare killing efficiency of NK cells with different KI scFv receptors (scFv-Rs) against CAR designs with co-stimulatory domains variations, on cancer cell lines *in vitro*.

Aim 3
Apply scFv-R gene-editing to iPSCs and assess their capacity to differentiate into an off-the-shelf scFv-R NK cell. Upon successful conversion to mature NK cells characterised via flow cytometry, the *in vitro* and *in vivo* activity of iPSC derived scFv-R-NKs compared with PBMC scFv-R NKs will be investigated.