Clinical PhD Project 2024

Generation and evaluation of non-activated chimeric antigen receptor (CAR) T cells for treatment of cancer

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Principal supervisor's CRUK CC theme: Cancer Immunology Programme

Department for student registration: Medicine
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Postgraduate scheme: **Clinical Research Training Fellow (3-year PhD)**

PhD project outline:

Adoptive cellular immunotherapy using T cells genetically modified to express a chimeric antigen receptor (CAR) has revolutionized the treatment of relapsed or refractory B-cell lymphomas, and has the potential to do the same for other haematological and solid malignancies. CAR T cells are typically manufactured ex vivo from primary human T cells by sequential activation of the endogenous T cell receptor, genetic modification using a lentiviral vector, then expansion in culture for several days. Nonetheless, this process of activation and expansion leads to progressive differentiation of the CAR T cells towards an effector phenotype, with an associated reduction in proliferative capacity and therapeutic potency. Avoiding this problem, the Matheson lab (https://www.citiid.cam.ac.uk/nick-matheson/) has developed a workflow for transduction of resting primary human T cells, without prior activation. In collaboration with the Chapman lab (https://crukcambridgecentre.org.uk/users/mac54), the aim of this project is therefore to generate and evaluate non-activated CAR T cells, with the potential to increase their efficacy for treatment of cancer. The student will: (1) optimize the workflow for generation of non-activated CAR T cells; (2) test the activity of these non-activated CAR T cells in vitro using cytotoxicity assays; (3) assess the potency of these non-activated CAR T cells in vivo using a standard mouse model; then (4) use this approach to generate and evaluate non-activated CAR T cells targeting a novel antigen for immunotherapy of myeloma. The project will therefore offer a broad training in molecular medicine, with the potential to develop new therapies benefitting patients in the clinic.

PhD experimental plan:

VSVg-pseudotyped lentiviral particles currently used for manufacture of CAR T cells are unable to transduce resting primary human T cells, without prior activation. This limitation may be overcome by using lentiviral particles pseudotyped with HIV Env (rather than VSVg), in combination with HIV Vpx-containing virus-like particles (Vpx-VLPs). Optimisation of the HIV Env protein has allowed us to develop a workflow which is now efficient enough to produce large numbers of genetically modified, non-activated T cells (Figure 1). At each stage in this project, non-activated CAR T cells generated using this workflow will be compared with equivalent CAR T cells generated by conventional means, including prior T cell activation.

(1) Generation of non-activated CAR T cells. To optimize the existing workflow for generation of non-activated CAR T cells, resting primary human T cells will be transduced with a well-described CAR targeting human CD19, for which an idiotype-specific antibody is commercially available.

(2) Characterization of non-activated CAR T cells in vitro. To assess their in vitro activity, non-activated CD19-specific CAR T cells from (1) will be co-cultured with CD19+ target cells, with quantification of cytotoxicty, markers of differentiation and exhaustion over sequential challenges.

(3) Evaluation of non-activated CAR T cells in vivo. To assess their in vivo anti-cancer activity, non-activated CD19-specific CAR T cells from (1) will be tested against CD19+ target cells using a standard mouse xenograft model, with quantification of persistence, proliferation and anti-cancer activity.

(4) Generation, characterization and evaluation of non-activated CAR T cells for immunotherapy of myeloma. Finally, building on proof-of-concept data from (1-3), a similar approach will be used to generate and evaluate non-activated CAR T cells targeting a novel antigen expressed by myeloma cells.

Main techniques:

Cell culture (including primary human T cells), molecular cloning, lentiviral transduction, flow cytometry, cytotoxicity assays and mouse xenograft models.

Key references:

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Geng X et al. Efficient delivery of lentiviral vectors into resting human CD4 T cells. Gene Ther. 2014 Apr;21(4):444-9. doi: 10.1038/gt.2014.5. PMID: 24572792

Melenhorst et al. Decade-long leukaemia remissions with persistence of CD4+ CAR T cells. Nature. 2022 Feb; 602(7897): 503–509. doi: 10.1038/s41586-021-04390-6. PMID: 35110735

Anderson GSF et al. Unbiased cell surface proteomics identifies SEMA4A as an effective immunotherapy target for myeloma. Blood. 2022 Apr 21;139(16):2471-2482. doi: 10.1182/blood.2021015161. PMID: 35134130