Clinical PhD Project 2024

## Gene Therapy of Human Glioblastoma Tumour-Associated Macrophages

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Principal supervisor's CRUK CC theme: Brain Cancer Virtual Institute

Department for student registration: Wellcome MRC Cambridge Stem Cell Institute
Department or institute where research will take place: Jeffrey Cheah Building

Postgraduate scheme: **Clinical Research Training Fellow (3-year PhD)**

### PhD project outline:

Glioblastoma, the most common primary malignant brain tumour, remains fatal with a 6.8% 5-year survival rate and is recognized by CRUK as a key tumour of unmet clinical need.
To date targeted therapy and immunotherapy approaches, including checkpoint inhibition, cancer vaccines and adoptive T cell therapy have failed to achieve consistent survival benefit. This reflects an 'immune-cold' glioblastoma microenvironment dominated by tumour-associated macrophages (TAMs), which adopt anti-inflammatory/immune-suppressive activation states specific to glioblastoma. This environment prevents adaptive immune recruitment and is associated with trophic/pro-proliferative effects on normal and cancer stem and progenitor cells.

The goal of this project is to demonstrate proof-of-principle for TAM-directed immunomodulatory gene therapy, to render the human GBM microenvironment permissive for a range of immunotherapy approaches. To do this we aim to harness endogenous pro-inflammatory mechanisms driven by cGAS-STING signalling, a potent driver of innate immune responses to pathogens and cancer. STING detects cyclic dinucleotides produced by pathogens themselves or by cGAS in response to cytoplasmic double strand DNA. The downstream cascade induces a pro-inflammatory state including cytokine and interferon secretion. By expressing a mutant constitutively-active STING R842M in patient TAMs, we aim to force adoption of pro-inflammatory/immune-permissive states, in transduced patient TAMs directly and in neighbouring TAMs and GSCs through paracrine cytokine signalling. Human TAM cell biology is not well modelled in rodents, so our work will focus on primary human patient-derived GBM explant and purified TAM cell fractions, models which have been extensively developed and refined in our lab over recent years.

### PhD experimental plan:

The project will begin with comprehensive profiling and comparison of cell states in primary patient GBM tissue and in sorted CD11b+ cell fractions, across a representative panel of patient GBMs, with mutation data available through existing collaborations. Based on our preliminary data we hypothesise that we can re-establish baseline tumour-permissive/supportive states following TAM isolation, by exposing our cultures to glioma stem cell (GSC) conditioned media or defined combinations of recombinant cytokines (IL-4, TNF-a). In this way the student will optimise modelling of patient tissue anti-inflammatory/ immunosuppressive TAM cell states. For control purposes the student will also generate induced microglia-like (iMGL) populations from pluripotent stem cells using standard protocols.

Secondly, we will generate viral vectors incorporating tetracycline-inducible STING R842M, tet-responsive element and fluorescent reporter based on existing constructs (Tutt lab;

addgene.org/190027/). We will codeliver lentivirus together with a Simian immunodeficiency virus non-structural protein VPX-Vpl, packaged in lentiviral-like particles. VPX-Vpl blocks SAMHD1 activity to dramatically improve lentivirus transduction efficiency in human induced microglia-like cells, which have traditionally been difficult to infect (doi:10.1101/2022.05.02.490100v1). In the event of technical difficulties additional strategies have been developed by existing collaborators for targeting TAMs, including novel transfection approaches and gene therapy adeno-associated viral vectors bearing custom capsid sequences.

Combining the tools and approaches refined above, the student will go on to profile the TAM cell state changes associated with STING gene therapy, and validate these in human organotypic slice cultures and demonstrate functional effects on cytotoxic effector activity using coculture of glioma stem cells, TAMs and human peripheral blood mononuclear cells.

**Main techniques:**

The project will entail a range of ex vivo tissue, primary cell and cell line culture techniques, including isolation, magnetic antibody cell sorting, FACS, organotypic slice culture, glioma cell/ TAM/ PBMC coculture and live cell imaging.
The student will apply a range of routine molecular biology assays, as well as RNA-smFISH, mass cytometry and single cell sequencing.
The student will become familiar with lentiviral cloning and transduction techniques.

**Key references:**

1. Hambardzumyan D, Gutmann DH, Kettenmann H. The role of microglia and macrophages in glioma maintenance and progression. Nature neuroscience. 2016 Jan;19(1):20-7.
2. Gangoso E, Southgate B, Bradley L, Rus S, Galvez-Cancino F, McGivern N, Güç E, Kapourani CA, Byron A, Ferguson KM, Alfazema N. Glioblastomas acquire myeloid-affiliated transcriptional programs via epigenetic immunoediting to elicit immune evasion. Cell. 2021 Apr 29;184(9):2454-70.
3. Bohlen, C.J., Bennett, F.C., Tucker, A.F., Collins, H.Y., Mulinyawe, S.B. and Barres, B.A. (2017) “Diverse Requirements for Microglial Survival, Specification, and Function Revealed by Defined-Medium Cultures, Neuron, 94(4), pp. 759-773.e8. Available at: https://doi.org/10.1016/j.neuron.2017.04.043.
4. Bulstrode H, Girdler GC, Gracia T, Aivazidis A, Moutsopoulos I, Young AM, Hancock J, He X, Ridley K, Xu Z, Stockley JH. Myeloid cell interferon secretion restricts Zika flavivirus infection of developing and malignant human neural progenitor cells. Neuron. 2022 Dec 7;110(23):3936-51.