MRes Rotation Project 2024

Innate immune control of kidney cancer

Principal supervisor's name: **Prof James Nathan**
Principal supervisor's email address: jan33@cam.ac.uk

Principal supervisor's CRUK CC theme: Urological Malignancies and Cell & Molecular Biology Programmes

Department for student registration: Department of Medicine

Department or institute where research will take place: Cambridge Institute of Therapeutic Immunology and Infectious Disease (CITIID)

Co-supervisor's name: Prof Grant Stewart
Co-supervisor's email address:

Postgraduate scheme: **MRes + PhD (1 + 3-year non-clinical applicants only)**

MRes project outline:

Approximately 13,000 new cases of kidney cancer are diagnosed each year in the UK, of which around 80% are clear cell renal cell carcinomas (ccRCCs). While surgery offers good outcomes for early-stage tumours, treatment options for metastatic ccRCC remain limited and largely ineffective. Genetically, over 90% of ccRCCs are typically initiated by biallelic loss of *VHL*, which encodes the E3 ubiquitin ligase von Hippel Lindau (pVHL), and inherited loss-of-heterozygosity of *VHL* also underlies the eponymous hereditary cancer syndrome. Targeting cancer cells harbouring *VHL* mutations could provide an effective therapeutic strategy to treat the cancer with minimal off-target effects. We have exploited this 'synthetic lethality' approach using our expertise in forward genetic screens and uncovered a novel interferon response for controlling *VHL* mutant ccRCCs. This project aims to characterise this innate immune control of ccRCCs, and explore whether it functions in the control of other solid organ tumours. The work is ideally suited to a rotation project as all the tools required for the analysis of this interferon response are established. However, it will also serve as the basis for further doctoral studies on innate immune surveillance of kidney cancer, and the candidate will benefit from both the fundamental biological (Prof Nathan) and clinical translational (Prof Stewart) expertise of the supervisors.

MRes experimental plan:

We have already developed tools and methods to activate this interferon response in ccRCCs, which involve CRISPRs/Cas9 depletion of the transcription factor controlling this pathway, and tools to manipulate the cGAS-STING arm of the interferon response.

Studies will involve activating cGAS-STING in *VHL* mutant and reconstituted *VHL* ccRCCs and measuring interferon signalling using flow cytometry, imaging, and biochemistry techniques.

The second part of the project will involve characterising this pathway in patient-derived samples. We already have tissue sections from *VHL* mutant ccRCCs and control tissues, and the

candidate will use immunohistochemistry to examine how interferon signalling occurs in metastatic and non-metastatic ccRCCs. This will be paired with existing RNA-seq data used to explore whether expression of these proteins correlates with disease progression.

It is envisaged that these initial aims will form the basis of a substantial PhD project, where work will be extended to characterise this immune response to tumours in other solid cancers, and whether it can be harnessed as a therapeutic strategy

Main techniques:

- Cutting edge CRISPR/Cas9 genome-wide screens and genetics
- High throughput sequencing
- large data analysis
- flow cytometry, molecular biology, and immunohistochemistry.
- Incucyte imaging

Key references:

1) The HIF complex recruits the histone methyltransferase SET1B to activate specific hypoxia-inducible genes. Ortmann BM, Burrows N, Lobb IT, Arnaiz E, Wit N, Bailey PSJ, Jordon LH, Lombardi O, Peñalver A, McCaffrey J, Seear R, Mole DR, Ratcliffe PJ, Maxwell PH, **Nathan JA**. *Nature Genetics* (2021). DOI: 10.21203/rs.3.rs-85295/v1. PMC7611696

2) Hypoxia Regulates Endogenous Double-Stranded RNA Production via Reduced Mitochondrial DNA Transcription. Arnaiz E, Miar A, Dias Junior AG, Prasad N, Schulze U, Waithe D, **Nathan JA**, Rehwinkel J, Harris AL. *Front Oncol.* (2021) Nov 24;11:779739. doi: 10.3389/fonc.2021.779739. PMID: 34900733

3) Metabolite-driven antitumor immunity. **Nathan JA.** *Science* (2022) Sep 30;377(6614):1488-1489. doi: 10.1126/science.ade3697. PMID: 36173838

4) **G.D. Stewart,** T. Klatte, L. Cosmai, A. Bex, B.W. Lamb, H. Moch, E. Sala, S. Siva, C. Porta, M. Gallieni. The multi-speciality approach to management of localised kidney cancer. *The Lancet*. 2022. 400(10351):523-534.

5) S. Ursprung, H. Mossop, F.A. Gallagher, E. Sala, R. Skells, J.A.N. Sipple, T.J. Mitchell, A. Chhabra, K. Fife, A. Matakidou, G. Young, A. Walker, M.G. Thomas, M. Crispin Ortuzar, M. Sullivan, A. Protheroe, G. Oades, B. Venugopal, A.Y .Warren, J. Stone, T. Eisen, J. Wason, S.J. Welsh, **G.D. Stewart**. The WIRE study a phase II, multi-arm, multi-centre, non-randomised window-of-opportunity clinical trial platform using a Bayesian adaptive design for proof-of-mechanism of novel treatment strategies in operable renal cell cancer – a study protocol. BMC Cancer. 2021. In press.