Clinical PhD Project 2024

Investigating mechanisms of drug resistance in paediatric cancers

Principal supervisor's name: Prof **Suzanne Turner**
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Principal supervisor's CRUK CC theme: Paediatric Cancer Programme
Department for student registration: Department of Pathology

Department or institute where research will take place: Department of Pathology, Addenbrooke's campus

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

PhD project outline:

Resistance to targeted therapeutic agents is a universal issue in cancer whereby we are creating a chronic disease that is kept at bay by cycling through different generations of drugs. This is particularly the case for anaplastic lymphoma kinase (ALK) tyrosine kinase inhibitors (TKI) whereby the sub-clonal heterogeneity of tumours provides a backdrop in which clones of tumour cells possessing inherent and acquired resistance to ALK TKI ping pong through treatment regimens to emerge as the dominant clone. How this dynamic plays out in real terms and therefore how it can be overcome to eliminate all clones of a tumour remains to be investigated. In this project, the student will employ primary patient-derived tumours to model this process whereby each cell within the tumour has a unique DNA barcode by which it can be identified. In this manner, following the selective pressure of varying ALK TKI exposures, surviving sub-clones and their transcriptional programmes can be identified to hypothesise drug resistance mechanisms at the sub-clonal level. These data will be cross-compared to previously conducted CRISPR/dCas9 screens which were employed to identify potential bypass signalling tracks to ALK inhibitors. In this manner, it will be possible to derive the identity of therapy persister cells that lead to relapse in the patient setting. With this information in hand, the student will set out to investigate the relevance of these resistance mechanisms and to design therapeutic strategies to eliminate all cells within a tumour.

PhD experimental plan:

Year 1: Transduction of primary cells with a DNA barcode library, RNAseq of cell population: will require optimisation of transduction protocol for primary T cell cancers
Year 2: Exposure of barcoded cells to drugs including ALK kinase inhibitors: will require optimisation for in vitro culture of PDX cells, drug doses and in vivo assessment for the identification of therapy persister cells. Bioinformatics analysis of DNA sequencing of surviving barcoded cells, relate back to the transcriptional profiles of the barcoded cells. Identification of potential drug resistance mechanisms via cross-comparison of data to prior CRISPR bypass track screen results.
Year 3: Validation of potential drug resistance mechanisms - depending on the mechanisms identified this may involve biochemistry, apoptosis, cell cycle and proliferation assays of cell cultures as well as drug screens to identify targets for eradication of persister cells. This may be followed up with in vivo studies exposing PDX to drug regimens with assessment of tumour burden using imaging techniques.

Main techniques:

Primary cell culture
Patient derived xenograft propagation, characterisation and analysis
Proliferation/apoptosis/cell cycle assays
Drug screens
RNA sequencing and bioinformatic analysis

Key references:

Nina Prokoph†, Jamie D. Matthews†, Ricky M. Trigg, Leila Jahangiri, Ivonne A. Montes-Mojarro, G.A. Amos Burke, Falko Fen, Olaf Merkel, Lukas Kenner, Birgit Geoerger, Robert Johnston, Matthew J. Murray, Charlotte Riguad, Laurence Brugières, and Suzanne D. Turner. (2023) Patient-derived xenograft models of ALK+ ALCL reveal preclinical promise for therapy with Brigatinib. British Journal of Haematology, doi: 10.1111/bjh.18953

Perla Pucci, Liam Lee, Miaojun Han et al. Targeting NRAS via miR-1304-5p or farnesyltransferase inhibition confers collateral sensitivity to ALK inhibitors in high-risk neuroblastoma, 07 November 2022, PREPRINT (Version 1) available at Research Square [https://doi.org/10.21203/rs.3.rs-2167328/v1]

Prokoph, N., Probst, N.A., Lee, L.C., Monahan, J.M., Malik, V., Liang, H-C., Sharma, G.G., Montes-Mojarro, I., Mota, I., Larose, H., Forde, S.D., Imamoglu, R., Matthews, J.D., Trigg, R., Ceccon, M., Ducray, S.P., Lobello, C., Janikova, A., Gambacorti-Passerini, C., Pospisilova, S., Kenner, L., Klapper, W., Jauch, R., Woessmann, W., Chiarle, R., Mologni, L., Merkel, O., Brugières, L., Geoerger, B., Barbieri, I., and Turner, S.D. (2020) Inflammatory networks drive resistance to drugs in ALCL, ALK+; the IL10R is up-regulated in response to consistent exposure to ALK inhibition. Blood, 136(14):1657-1669. doi: 10.1182/blood.2019003793