CRUK Cambridge Centre MRes rotation project

Rotation Project Title	**Investigating an infectious aetiology for paediatric lymphoma**
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Programme	Paediatric Cancers
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Laboratory Location	Department of Pathology

Project Outline	**Aims and objectives** Anaplastic Large Cell Lymphoma (ALCL) is a form of T cell lymphoma largely diagnosed in children and young adults and is driven by a translocation breakpoint product, Nucleophosmin-Anaplastic Lymphoma Kinase (NPM-ALK). The unusual immunophenotype of the tumour cells is largely accountable to NPM-ALK activity but is also shaped by the microenvironment which we propose constitutes an infectious agent. In this manner, antigenic stimulation of incipient tumour cells provides an evolutionary drive whereby the T cell receptor (TCR) must be down-regulated for cell survival. Hence, we hypothesise that the TCR is a tumour suppressor, that must be silenced following an infectious stimulus permitting tumour development. To address this hypothesis, we will determine the effects of co-expression of a TCR and NPM-ALK on T cell survival, the subsequent effects on proximal and distal TCR-induced cell signalling and potential therapeutic opportunities this poses for the treatment of ALCL.
Experimental plan	1. Primary T cells isolated from human buffy coats will be transduced to express NPM-ALK and the effects on expression of the TCR and signalling through it on stimulation monitored. 2. The Jurkat T cell line and variants of this that lack proximal TCR signalling proteins but expressing NPM-ALK will be assessed for the activation status of distal TCR-induced signalling. 3. T cells isolated from TCR transgenic mice (OTI) expressing NPM-ALK will be analysed for downstream TCR-induced signalling on ligation of the TCR by peptides of varying avidity.
Main Techniques	The student will develop skills in • tissue culture of primary human, mouse and established cell lines • isolation of T cells using magnetic bead-based technologies • Western blot • cell growth assays (e.g. MTT) • cell survival assays (e.g. Anexin V flow cytometry) and qRT-PCR.

Key References	1. **Malcolm TI, Villarese P, Fairbairn CJ, Lamant L, Trinquand A, Hook CE, Burke GA, Brugières L, Hughes K, Payet D, Merkel O, Schiefer AI, Ashankyty I, Mian S, Wasik M, Turner M, Kenner L, Asnafi V, Macintyre E, Turner SD** *Anaplastic large cell lymphoma arises in thymocytes and requires transient TCR expression for thymic egress.* *Nat Commun. 2016 Jan 12;7:10087. doi: 10.1038/ncomms10087.* 2. **Moti N, Malcolm T, Hamoudi R, Mian S, Garland G, Hook CE, Burke GA, Wasik MA, Merkel O, Kenner L, Laurenti E, Dick JE, Turner SD.** *Anaplastic large cell lymphoma-propagating cells are detectable by side population analysis and possess an expression profile reflective of a primitive origin.* *Oncogene. 2015 Apr 2;34(14):1843-52. doi: 10.1038/onc.2014.112. Epub 2014 May 12.* 3. **Turner SD, Yeung D, Hadfield K, Cook SJ, Alexander DR.** *The NPM-ALK tyrosine kinase mimics TCR signalling pathways, inducing NFAT and AP-1 by RAS-dependent mechanisms.* *Cell Signal. 2007 Apr;19(4):740-7. Epub 2006 Oct 4.*