CRUK Cambridge Centre MRes rotation project

Rotation Project Title	**Why are some neuroblastoma tumours resistant to differentiation?**
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Second supervisor if applicable	N/A
Programme	Neurological and Brain Cancers
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Laboratory Location	Hutchison/MRC Research Centre Stem Cell Institute

Project Outline	**Aims and objectives** The paediatric tumour neuroblastoma is the deadliest solid paediatric tumour, most often due to relapse and resistance to treatment. We have uncovered a new pathway of developmental regulation of the proliferation/differentiation axis in thus tumour, driven by post-translational regulation of a key transcriptional regulator of this process, Ascl1, which can be exploited as a new pathway for treatment for this devastating disease. However, we have yet to determine whether reactivation of differentiation as a therapeutic approach works for all neuroblastoma subtypes; in fact, we have evidence that some tumours may be resistant to differentiation. An understanding of the mechanism of resistance will give further insight into the underlying biology driving this devastating disease.
Experimental plan	You will test up to 10 neuroblastoma lines spanning the different subtypes for susceptibility to differentiation driven by lentiviral-mediated transduction of two different hyperactive forms of Ascl1, assaying cell proliferation and differentiation by semi-automated microscopy, morphological monitoring and immunocytochemistry. You will compare Ascl1 binding to key cell cycle exit and differentiation transcriptional targets in these lines by chromatin immunoprecipitation and compare target gene activation by qPCR. Based on solid preliminary data, we expect to find that some lines differentiate under the influence of activated Ascl1 while others are resistant. We will investigate addition of epigenetic modifying compounds to resistant cells to determine whether we can confer susceptibility to resistant lines by changing their chromatin landscape. Time permitting, you will compare the transcriptional response of neuroblastoma cells to that of hyperactive Ascl1 with glioblastoma cells that we have already shown to be resistant to Ascl1-mediated differentiation.
Main Techniques	• tissue culture • lentiviral-mediated gene transduction • Immunocytochemistry • chromatin immunoprecipitation • quantitative PCR • you will as well receive a good grounding in our understanding of transcriptional regulation of cell proliferation and differentiation in normal development and how it is perturbed in cancer.

Key References	**Fahad Ali, Daniel Marcos, Luke Wylie, Igor Chernukhin, Eva Papachristou, Tatiana Papkovskaia, Liam Lee, Christopher Weekes, Clive D'Santos, Suzanne Turner, Jason Carroll, Anna Philpott** *A phosphorylation switch between proliferation and differentiation in neuroblastoma. (2017). (*Manuscript submitted and available on request.) **L.A. Wylie, L.J.A. Hardwick, T. D. Papkovskaia, C. J. Thiele and A. Philpott.** *Ascl1 phospho-status regulates neuronal differentiation in a Xenopus developmental model of neuroblastoma. Dis Model Mech. 8:429-41. (2015)* **F.R. Ali, K. Cheng, P. Kirwan, S. Metcalfe, F.J. Livesey, R.A. Barker and A. Philpott.** *The phosphorylation status of Ascl1 is a key determinant of neuronal differentiation and maturation in vivo and in vitro.(2014)* ***Development****. 141, 2216-24.*