CRUK Cambridge Centre MRes rotation project

Rotation Project Title	**Investigating transcriptional dysregulation during the early stages of leukaemogenesis at single cell resolution**
Head of Laboratory (PI) Name	Bertie Gottgens
Second supervisor if applicable	Larissa Carnevalli
Programme	Haematological Cancers
Supervisor's Email	bg200@cam.ac.uk
Laboratory Location	Stem Cell Institute

Project Outline	**Aims and objectives** Initiating mutations in acute myeloid leukaemia commonly affect epigenetic and transcriptional regulators. The Gottgens group has a long track record in studying transcriptional networks in normal haematopoietic stem and progenitor cells (HSPCs), and how their dysregulation contributes to leukaemia development. The group pioneered the use single cell expression profiling to study both normal and malignant HSPCs [1-3], which for the first time allows us to study the process of malignant transformation at single cell resolution. The specific aims of this rotation project proposal is to analyse single cell RNA-Seq data from pre-leukaemic mouse models to identify consistent molecular changes shared across a range of oncogenes, and then validate the resulting candidate genes/pathways experimentally. Within the timeframe of a rotation project, the experimental work will focus on cell culture assays, giving priority to potentially druggable genes/pathways.
Experimental plan	1) Mine single cell RNA-Seq data to identify genes that are consistently up/downregulated in blood stem/progenitor cells expressing four different initiating leukaemogenic mutations 2) Overexpress/knock-out genes discovered in (1) in cell line models of acute myeloid leukaemia 3) Perform cell biological analysis of cell lines generated in (2), including proliferation, differentiation and cell death assays
Main Techniques	• single cell data analysis • cell culture including transfection and CrispR/Cas genome engineering • flow cytometry (FACS) to assess proliferation, differentiation and cell death
Key References	**Moignard V., Macaulay I.C., Swiers G., Buettner F., Schütte J., Calero-Nieto F.J., Kinston S., Joshi A., Hannah R., Theis F.J., Jacobsen S.E., de Bruijn M.F.T, Göttgens B.** (*2013) "Characterisation of transcriptional networks in blood stem and progenitor cells using high-throughput single cell gene expression analysis" Nature Cell Biology 15: 363-372* **Wilson N.K., Kent D.K., Buettner F., Shehata M., Macaulay I.C., Calero-Nieto F.J., Sánchez Castillo M., Oedekoven C.A., Diamanti E., Schulte R., Ponting C.P., Voet T., Caldas C., Stingl J., Green A.R., Theis F.J., Göttgens B.** (*2015). "Combined single cell functional and gene expression analysis resolves heterogeneity within stem cell populations" Cell Stem Cell 16(6): 712-724*

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