CRUK Cambridge Centre MRes Rotation Project

Rotation Project Title	Targeting oestrogen receptors in myeloproliferative neoplasms
Head of Laboratory (PI) Name	Simon Mendez-Ferrer
Second Supervisor, if applicable	Jason Carroll
Programme	Haematological malignancies
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Laboratory Location	CSCI

Project Outline	**Aims and Objectives** The main interest of the Mendez-Ferrer lab is to understand the extrinsic regulation of normal and (pre)leukaemic haematopoietic stem cells (HSCs) (1). The group is studying the role of the bone marrow microenvironment in the development and treatment of myeloid malignancies (2) and has shown that the HSC niche can be a complementary therapeutic target in myeloproliferative neoplasms (3). The group found an expected role for oestrogen receptor (ER) in the regulation of HSC/progenitor survival and proliferation, and possible implications for the treatment of myeloproliferative neoplasms (4) currently under investigation in a multicentre clinical study. The Carroll lab is investigating how oestrogen receptor (ER) regulates gene transcription, and breast cancer progression and resistance to therapy (5). This project will merge the expertise of both laboratories to investigate the effects of selective oestrogen receptor modulators (SERMs) on normal and mutated haematopoietic progenitors driving myeloproliferative neoplasms.
Experimental Plan	The student will initially use cell lines to investigate the differential effects of SERMs on normal and mutated cells. Effects on cell survival, proliferation will be analysed by flow cytometry, the changes in gene expression will be studied by RNAseq and the metabolic effects will be analysed using Seahorse. CRISPR/CAS9 deletion of relevant receptors or coreceptors will be combined with the use of selective agonists and antagonists. CHIP-seq on ER-alpha (6) and, if needed, quantitative mass spectrometry (7) will be used to investigate the association of ER-alpha complexes to chromatin. The main results will be validated with primary human samples and will be contrasted with the effects of the SERM tamoxifen on mutated vs. non-mutated haematopoietic progenitors from human breast cancer and myeloproliferative neoplasms, using cutting-edge single cell genotyping and transcriptomics analysis.
Main Techniques	-CRISPR/CAS9 deletion of nuclear receptors.

	-Flow cytometry. -Seahorse. -Bulk RNA-seq. -CHIP-seq. -Mass spectrometry. -Combined single cell transcriptomics and genotyping for driver mutations.
Key References	(1) Sánchez-Aguilera A, **Méndez-Ferrer S**. The hematopoietic stem-cell niche in health and leukemia. **Cell Mol Life Sci**. 2017 Feb;74(4):579-590. doi: 10.1007/s00018-016-2306-y. (2) Korn C, **Méndez-Ferrer S**. Myeloid malignancies and the microenvironment. **Blood**. 2017 Feb 16;129(7):811-822. doi: 10.1182/blood-2016-09-670224. (3) Arranz L, Sánchez-Aguilera A, Martín-Pérez D, Isern J, Langa X, Tzankov A, Lundberg P, Muntión S, Tzeng YS, Lai DM, Schwaller J, Skoda RC, **Méndez-Ferrer S**. Neuropathy of haematopoietic stem cell niche is essential for myeloproliferative neoplasms. **Nature**. 2014 Aug 7;512(7512):78-81. doi: 10.1038/nature13383. (4) Sánchez-Aguilera A, Arranz L, Martín-Pérez D, García-García A, Stavropoulou V, Kubovcakova L, Isern J, Martín-Salamanca S, Langa X, Skoda RC, Schwaller J, **Méndez-Ferrer S**. Estrogen signaling selectively induces apoptosis of hematopoietic progenitors and myeloid neoplasms without harming steady-state hematopoiesis. **Cell Stem Cell**. 2014 Dec 4;15(6):791-804. doi: 10.1016/j.stem.2014.11.002. (5) **Carroll JS**, Hickey TE, Tarulli GA, Williams M, Tilley WD. Deciphering the divergent roles of progestogens in breast cancer. **Nat Rev Cancer**. 2017 Jan;17(1):54-64. doi: 10.1038/nrc.2016.116. (6) Serandour AA, Mohammed H, Miremadi A, Mulder KW, **Carroll JS**. TRPS1 regulates oestrogen receptor binding and histone acetylation at enhancers. **Oncogene**. 2018 Jun 12. doi: 10.1038/s41388-018-0312-2. (7) Papachristou EK, Kishore K, Holding AN, Harvey K, Roumeliotis TI, Chilamakuri CSR, Omarjee S, Chia KM, Swarbrick A, Lim E, Markowetz F, Eldridge M, Siersbaek R, D'Santos CS, **Carroll JS**. A quantitative mass spectrometry-based approach to monitor the dynamics of endogenous chromatin-associated protein complexes. **Nat Commun**. 2018 Jun 13;9(1):2311. doi: 10.1038/s41467-018-04619-5.