CRUK Cambridge Centre MRes Rotation Project

Rotation Project Title	Identification of RNA binding protein required for Lymphoma cells survival and proliferation.
Head of Laboratory (PI) Name	Isaia Barbieri
Second Supervisor, if applicable	
Programme	Haematological malignancies
Supervisor's Email	Ib318@cam.ac.uk
Laboratory Location	*Addenbrooke's Hospital Lab Block Level 3*

Project Outline	**Aims and Objectives** The main interest of the Barbieri lab is to better understand the biology of blood cancers and to identify new potential therapeutic targets. Lymphoma is a heterogeneous family of diseases originating from Lymphocytes. The most common type of high-grade lymphoma (NHL) is diffuse large B-cell lymphoma (DLBCL), But there are a number of different types, including Hodgkin lymphoma, Burkitt lymphoma and peripheral T-cell lymphoma (PTCL). We are particularly interested in studying the role of RNA binders in these diseases. Coding and non-coding RNAs can be regulated through several mechanism including chemical modifications and protein binding. These mechanisms can affect RNA processing, translation and stability. RNA binding proteins (RBP) represent the major mediators of these regulatory processes. We recently identified RNA methyltransferases required for the growth of Acute myeloid leukaemia cells using a domain specific CRISPR-CAS9 screen. Using the same approach, we will perform CRISPR-CAS9 dropout screens in Lymphoma models, targeting specifically the RNA-binding domain of RBPs. Ultimately the project will identify a number of RNA-binding proteins involved in the proliferation of lymphoma cells and identify new molecular pathways necessary for the growth of lymphoma cells.
Experimental Plan	The starting point of the project is the screening of RBPs in lymphoma ex vivo models derived from transgenic mice. We will perform domain-specific CRISPR-CAS9 targeting of RNA binders. A selected number of dropouts will be identified and validated through individual CRISPR targeting. The dropout targets will be filtered for genes generally required for viability and a restricted group of lymphoma-specific dropouts will be identified. The next step of the project will involve the characterization of one or few of the identified RBPs. This will be achieved through the generation of loss of function models through CRISP/CAS9 or RNAi
Main Techniques	-CRISPR/CAS9 dropout screens -Generation of CRISPR/CAS9 and RNAi loss of function models. -Characterization of Loss of function models.
Key References	Pereira B, Billaud M, Almeida R. RNA-Binding Proteins in Cancer: Old Players and New Actors. Trends Cancer. 2017 Jul;3(7):506-528. doi: 10.1016/j.trecan.2017.05.003. Epub 2017 Jun 20.

	Shi J, Wang E, Milazzo JP, Wang Z, Kinney J, Vakoc CR. Discovery of cancer drug targets by CRISPR-Cas9 screening of protein domains. Nat Biotechnol. 2015 Jun;33(6):661-7. doi: 10.1038/nbt.3235. Epub 2015 May 11. Barbieri, I., Tzelepis, K., Pandolfini, L., Shi, J., Millán-Zambrano, G., Robson, S. C., … Kouzarides, T. (2017). Promoter-bound METTL3 maintains myeloid leukaemia by m6A-dependent translation control. Nature, 552(7683), 126–131. https://doi.org/10.1038/nature24678.