CRUK Cambridge Centre MRes rotation project

Rotation Project Title	**The use of Crispr scaring and scRNAseq to study cellular dynamics changes and cell fate decisions in TNBC**
Head of Laboratory (PI) Name	Dr. Walid T. Khaled – (Pharmacology)
Second supervisor if applicable	Dr. John Marioni – (CRUK, CI/EBI)
Programme	Breast/Quantitative
Supervisor's Email	wtk22@cam.ac.uk
Laboratory Location	Department of Pharmacology

Project Outline	**Aims and objectives** Understanding the molecular and cellular mechanisms of how epithelia maintain a homeostatic state throughout the lifespan of an animal is a major challenge for developmental and stem cell biology. From a developmental perspective, the epithelium of the mammary gland is unique as it undergoes most of its development during adulthood. Despite recent efforts of characterising the tissue homeostasis at a cellular level (1), (2) little is known about how this is affected by cancer predisposing mutations. In this study we wish to further our understanding of the differentiation dynamics of the mammary gland during tumour development by using single-cell RNA-sequencing and CRISPR-scaring.
Experimental plan	In this project we will combine the Crispr/Cas9 technology with scRNAseq to determine the early cellular dynamics and cell fate changes in a model of triple negative breast cancer (TNBC). In order to achieve this objective, we will have to adapt a genetic lineage tracing system that has been developed and deployed in the zebrafish, known as scGESTALT (3) to the murine model. The principle relies on the ability of the CAS9/sgRNA complex to recognise specific target DNA sequences and introduce a break. This break can be repaired randomly leading to DNA deletions of varying length. In the GESTALT system a tandem of 8-10 sgRNA target sequences are introduced in the cells – this is called the barcode (3). One of these target sequences is a perfect match to the sgRNA while the rest are predicted off-target sequences. If Cas9 and a single sgRNA (with perfect match to one of the tandem sequences in the barcode) are transiently expressed in cells carrying the barcode, random length of DNA deletions will be generated and retained in the genome of any progeny cells. Therefore, each cell will be genetically marked with a unique barcode in vivo allowing for cellular lineage maps to be charted in an unbiased fashion. For the rotation project we will perform a proof-of-principle study using the constructs for the gRNA and the array of target sequences developed by Raj B. et al. and introduce them together with Doxycycline (DOX)-inducible Cas9 and sgRNA into TNBC cell lines. We will then use these cell lines to optimise the GESTALT system in a mammalian system. With this we can test the efficiency of the genetic scaring as well as estimate a ratio of overlaps of the randomly generated barcodes between unrelated cells. The proof-of-principle studies will be essential before proceeding to murine based experiments.
Main Techniques	• Bioinformatics • Single cell RNA sequencing analysis • Tissue culture • Molecular biology

Key References	1. K. Bach *et al.*;**8;**2128;*Nature Communications* (2017) 2. B. Pal *et al.*;**8;**1627;*Nature Communications* (2017) 3. B. Raj *et al.*;*Nature Biotechnology* (2018)