Project title:

Studying the role of colorectal cancer associated fibroblasts (CAFs) on resistance to KRAS G12D inhibitors using multi-scale CRISPR screening co-culture systems and 3D vascularized microfluidic organoid models.

Project Summary:

Background

Colorectal cancer (CRC) is the second most common cancer worldwide. Approximately 40% of colorectal cancers have *KRAS* mutations, the most common being a KRAS G12D mutation. Multiple KRAS G12D or pan-KRAS inhibitors have been developed and are in clinical trials. However, treatment is not curative and only about half of the patients with *KRAS* G12D mutations respond to treatment. The tumour stroma contains multiple cells such as cancer associated fibroblasts (CAFs) which may secreted proteins can cause resistance to KRAS inhibitors due to paracrine signaling. The Banerji and Au groups have engineered a high-throughput recirculatory perfusion bioreactor system for CRISPR-screening that precisely controls the fluid exchange between cancer associated fibroblasts and cancer cells to can be used to determine genes associated with resistance or sensitivity of KRAS G12D.

Hypothesis

Cancer associated fibroblasts secrete proteins that cause resistance to KRAS G12D and pan-KRAS inhibitors in *KRAS* G12D mutated colorectal cancer cell lines.

Aim 1

Identify CAF genes associated with resistance, Using the recirculatory perfusion co-culture system, the candidate will run parallel CRISPR screens to study resistance caused by two KAS G12D inhibitors in a KRAS G12D mutated cell lines in medium contemporaneously exposed to cancer associated fibroblasts or cancer cells to delineate the contribution CAFs secretome causing resistance in KRAS mutated CRC.

Aim 2

To validate up to 10 genes identified by Aim 1 as a mechanism of resistance by findings in Aim 1 by conducting CRISPR knock out/knock in or pharmacological inhibition of genes in colorectal cancer cells. These *KRAS* G12D mutated cancer cells will be grown in co-culture with colorectal cancer CAFs or other colorectal cancer cell lines in the presence of KRAS G12D inhibitors.

Aim 3

To evaluate CAF induced change in vascularization *KRAS* G12D mutated colorectal cancer microfluidic organoid systems. We will build upon 3D vascularized microfluidic devices developed in the Au group to contain tri-cultures of CAFs, endothelial cells and CRC (cells/organoids) to study the effects of colorectal cancer CAFS on angiogenesis causing resistance to KRAS G12D inhibitors.

This exciting PhD project leverages recently engineered laboratory tools to study a common cancer and new classes of drugs i.e. KRAS inhibitors that are likely to change standard of care. Using new technology, the candidate will be at the forefront of studying mechanisms of resistance that include

cancer associated fibroblasts that can yield unique and new insights into drug resistance that can be translated into the clinic. The PhD project will be run in two labs who have worked together before; Prof Banerji (ICR/RM) and Dr Au (Imperial) which brings together medical/clinical oncology, molecular pharmacology and engineering under the auspices of the convergence science centre. The laboratory skills and critical thinking developed in this project are transferable and a very good basis for clinician scientists in development.

Supervisory Team:

Professor Udai Banerji ICR/RMH
Dr Sam Au Imperial College

Clinical Specialities:

Medical Oncology

Clinical Oncology

Clinical Pharmacology

Gasteroenterology