Spatial and temporal high resolution quantitative live imaging techniques to determine mechanisms of chromosomal instability and drug resistance in breast cancer patient-derived organoids

Supervisors:

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Research Summary:

This project is based on the following:

1. Triple-negative breast cancer (TNBC) is a molecularly heterogenous disease, where few effective therapies exist. Although patients are commonly prescribed anti-mitotic chemotherapies, which prevent normal mitosis by either inhibiting microtubule assembly or disassembly, good predictive biomarkers for response to these drugs do not exist.
2. Microtubule-targeting chemotherapies induce mitotic arrest by causing mis- oriented microtubule attachments (which activate the error correction (EC) pathway) or unattached chromosomes (which active the spindle assembly checkpoint, SAC). In some contexts, both the EC and SAC can be circumvented, with cells prematurely exiting mitosis with mis-segregated chromosomes (mitotic slippage), a process that can lead to chromosomal instability and tumourigenesis. To date, these processes have been observed in 2D cell culture, yet without determining their spatial and temporal distributions and dynamics at the nanoscale. Moreover, they are poorly understood in more physiologically- and clinically- relevant settings.
3. We are interested in how well-established and newly-identified cancer driver genes in breast cancer (such as HORMAD1) alter the mitotic process and chromosomal segregation, with the intention of using this information to understand how to better treat people diagnosed with the disease.
4. Using live cell imaging methods, we will be able to (a) resolve chromosome organisation processes in space and time, (b) resolve chromosomes and protein structures at the nanoscale level, and (c) visualise and quantify the dynamics of protein:protein and chromosome:protein interactions at the micro and nanoscale level.

This proposal aims to understand how the SAC and EC controls chromosomal segregation and how this is impacted by breast cancer driver genes and drug exposure in model systems relevant to clinical context. In doing so, we aim to generate information that informs the identification of predictive, patient-stratification, biomarkers for microtubule-targeting chemotherapies and mitotic checkpoint kinase inhibitors (currently under investigation in Phase 2 clinical trials). To do this, we will utilise a convergence approach, combining the use of **Patient Derived Organoids** (PDO), a focus of the Convergence Centre**,** alongside drug **the use of novel live cell super-resolved spatiotemporal quantitative imaging methods** applied to EC, the SAC and chromosomal segregation in these patient derived models.

Specifically, we will apply these novel methods (single-molecule fluorescence spectroscopy, fluctuation correlations and image correlation spectroscopy in diffraction- limited and super-resolution mode) to address the following discrete questions:

- Is the relative activity of the SAC indicative of the extent of chromosomal segregation errors, mitotic cell death, cell fitness and drug sensitivity?
- Does the expression of breast cancer driver genes such correlate with the activity of the SAC and/or drug response?
- Where do ostensibly meiotic breast cancer driver proteins localise in mitotic cells?
- Which mitotic proteins interact with ostensibly meiotic breast cancer driver proteins?

The student will use a variety of microscopy approaches to measure the nanoscale location of breast cancer driver proteins and their interaction with mitotic proteins. breast cancer driver proteins will be monitored via the doxycycline-inducible fluorophore-tagged lentiviruses in PDOs combined with:

- super-resolution stimulated emission depletion (STED) nanoscopy
- live cell imaging quantitative single-molecule fluorescence spectroscopy
- *in silico* (deconvolution-based) high-resolution laser-free imaging used with image correlation variants (ICS), STED fluorescence correlation microscopy (STED FCS), STED raster imaging correlation spectroscopy (STED RICS) and STED fluorescence lifetime correlation spectroscopy (STED FLCS).

Literature references:

Watkins J, Weekes D, Shah V et al. Genomic Complexity Profiling Reveals That HORMAD1 Overexpression Contributes to Homologous Recombination Deficiency in Triple-Negative Breast Cancers. Cancer Discovery 2015; 5: 488-505

Person specification:

This project is suitable for a talented graduate or undergraduate student with life sciences, engineering or physics background. The standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1) and our full eligibility criteria can be found here: https://www.icr.ac.uk/studying-and-training/phds-for-science-graduates/entry-requirements

The studentship will be registered at the Institute of Cancer Research with affiliate status at Imperial College London. The student will have access to both institutions and benefit from the world class research infrastructure and expertise across the two institutions. The student will become a member of the CRUK Convergence Science Centre PhD cohort which is a unique group of students working across distinct disciplines to tackle the big problems in cancer. A unique convergence science training programme will provide the skills and language to navigate different disciplines.

Funding and Duration:

Studentships will be for four years commencing in October 2021. Applications for PhDs are invited from talented UK graduates or final year undergraduates. International

students are also invited to apply subject to outlining how they will meet the difference in tuition fees.

We look forward to receiving applications from all candidates and will select those who display the potential to become the world leading cancer researchers of the future based on their application and performance at interview. However, we are particularly to welcome UK applicants from Black and ethnic minority backgrounds, as they are underrepresented at PhD level within the ICR and Imperial.

Successful candidates will undertake a four-year research training programme under the guidance of a supervisory team of world-class researchers. Students will receive an annual stipend, currently £21,000 per annum, and project costs paid for the four-year duration. Convergence Science PhDs cover tuition fees for UK students only. Funding for overseas fees is not provided.