Molecular barcodes for high-dimensional functionalization of cancer heterogeneity

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Project outline

Next-generation sequencing and immunotherapy have revolutionized cancer research. It is now widely accepted that cancer is a constantly evolving ecosystem composed of communities of genetically and transcriptionally different tumour cells that interact with the tumour microenvironment (TME) and adopt a multitude of strategies to deceit the immune system and develop resistance to therapies (1,2). With an unprecedented capacity to characterize the genetic of patients' tumours, and in light of an expanding array of available compounds and ready to be tested, there is a growing need for rapid, reliable and predictive model systems that can be interrogated through the lens of evolution. Prostate cancer (PCa) and its current clinical management perfectly exemplify this urgency. At disease initiation, PCa relies on the androgen receptor signalling pathway for growth. Hence, the mainstay of therapy is represented by androgen deprivation and, at relapse, the resulting castration resistant PCa is often driven by the restoration of AR signalling (3). The clinical introduction of next generation AR pathway inhibitors (ARPI) such as Abiraterone and Enzalutamide has increased the survival of patients, but at the same time, it has fuelled the occurrence of aggressive, metastatic, clones that rely on AR-indifferent compensatory mechanisms and display lineage plasticity and phenotypic switch towards alternative subtypes (4,5). At present, there is no established genetic driven therapy for the majority of these patients and, unfortunately, also the ongoing evaluation of immune checkpoint inhibitors shows low benefit.

Our knowledge of heterogeneity and evolution of solid tumours is largely based on phylogenetic reconstruction of clonal relationships, queried at a single endpoint in time. This retrospective analysis is performed by sequencing human patient samples and does not provide information on phenotypic and functional aspects of clones with specific genotypes. Prospective clonal tracing requires the introduction of a DNA barcode that uniquely and permanently label single cells. Its use is restricted to preclinical models and allows for tracing over time. The major limitation of current approaches is that the analysis is performed on bulk cells, meaning that single cell data cannot be collected. Clonal tracing approaches have been recently coupled with single-cell RNA sequencing (sRNA-seq) and enable high-content and unbiased clonal analysis. However, its throughput is limited by cost, protocol complexity and sequencing depth. Importantly, scRNA-seq does not allow simultaneous detection of protein-level phenotypic information, such as alteration in post-translational modifications (PTMs) for the direct detection of targetable signalling pathways.

Here we propose a project for the development of a novel barcoding strategy that has the potential to overcome many limitations of current technologies. Our system will be based on the combinatorial arrangements of unique molecular identifiers to enable simultaneous

cancer clonal tracing, multi-dimensional phenotyping, and functional screenings through mass cytometry analysis. The molecular barcodes will be used in combination with prostate cancer organoids for the characterization of clonal dynamics, phenotypes, and for the identification novel targets in PCa resistant to ARPI.

Mass cytometry and imaging mass cytometry are novel, rapidly expanding methodologies that couple high speed, single-cell throughput common to conventional flow cytometry and imaging analysis, with the detection capability of atomic mass spectrometry. More than one hundred metals can be resolved with minimal signal overlap, providing the researchers with an unparalleled ability to phenotypically and functionally profile cells (6). While they are largely used in clinical and preclinical settings as a tool for high-dimensional immune phenotyping, mass cytometry and imaging mass cytometry can also be used to facilitate discovery research into fundamental principles of cancer biology. The Brown's lab has recently shown a novel application of mass cytometry in cancer cell population tracing and phenotyping using a protein barcoding approach (Pro-Code) (7).

One of the major goals of this project is the generation and optimization of isotope-coupled oligo probes for the detection of the molecular barcode by mass cytometry and imaging mass cytometry. The development and optimization of the proposed technology require a convergence approach. The successful PhD candidate will benefit from the expertise and resources of the Tumour Functional Heterogeneity group (Bezzi Lab), part of the Centre for Evolution and Cancer at the ICR, and from the world-renowned team of Professor Takats (Imperial College of London), pioneer in analytical chemistry and mass spectrometry.

The PhD student will apply a broad range of cutting-edge techniques covering molecular biology, cellular biology, 3D culture, mouse modelling, proteomics, and biochemical methods. These include:

- Lentiviral vector design, cloning, library preparation, transfection
- CRISPR/Cas9-mediated gene editing
- Organoid cultures
- Generation and longitudinal characterization of syngeneic orthotopic models of prostate cancer

The above-mentioned experiments will be carried out at the ICR, where the candidate will receive extensive training in cancer biology, preclinical modelling and investigation of solid tumours. Crucial training in chemistry and mass cytometry will be given by the team of Prof. Takats at the ICL, where the student will perform:

- Isotope panel design for mass cytometry and imaging mass cytometry
- Oligo-isotope coupling and antibody-isotope coupling
- Mass cytometry and imaging mass cytometry data acquisition
- Mass cytometry and imaging mass cytometry data analysis

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Key Words
1. Tumour Heterogeneity
2. Mass Cytometry
3. Tumour Clonal Tracing
4. Imaging Mass Cytometry (IMC)
5. Organoids
6. Mouse Models

Person specification:

This project is suitable for a talented graduate or undergraduate student with life sciences or chemistry 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 Imperial College London with affiliate status at the Institute of Cancer Research. 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.