CANCER RESEARCH UK | CONVERGENCE SCIENCE CENTRE

ICR The Institute of Cancer Research | Imperial College London

PhD Project Proposal

Funder details	
Studentship funded by:	CRUK
Project details	
Project title:	Super-resolution microscopy to investigate epigenetic evolution in ovarian high grade serous carcinoma.
Supervisory team	
Supervisor 1:	Professor Iain McNeish (Imperial – Surgery and Cancer)
Supervisor 2:	Professor Paul French (Imperial – Physics)
Supervisor:	Professor Jessica Downes (ICR)
Departmental affiliation	
Primary Departments:	Imperial: Surgery and Cancer; Physics. ICR – Cancer Biology
Primary Team:	ICR – Epigenetics and Cancer Stability
Locations:	Hammersmith, South Kensington, Sutton

Project background

High grade serous carcinoma (HGSC) is the commonest type of ovarian cancer (OC) and causes 80% OC mortality. HGSC has near-universal *TP53* mutation (>97%) and extensive genomic instability[1]. HGSC is treated with platinum-based chemotherapy, with initial response rates of c.80%[2]. However, up to 75% women relapse and ultimately acquire fatal platinum resistance, and true cure rates remain <30%. However, there are no consistent or recurrent changes in SNV, somatic copy number alterations or copy number signatures between diagnosis and relapse in HGSC[3]. These data indicate that acquired resistance in HGSC cannot be explained by purely genomic mechanisms.

Numerous pieces of evidence suggest that platinum resistance is associated with accumulated epigenetic change[4], the causal link between the two remains unclear. Emerging evidence suggests that platinum treatment induces a degree of plasticity, or even the emergence of stem-like states, that allow emergence of multiple and diverse transcriptional changes all conferring fitness advantages in a changing environment.

Single molecule localisation microscopy (SMLM) methods allow imaging below the diffraction-limited resolution (200nm) in both fixed and live cells[5]. In particular, (direct)stochastic optical reconstruction microscopy [(d)STORM] can directly visualise sub-cellular organisation at a resolution of ~20-30 nm in both single cells and tissue samples. The Photonics Group (Imperial Department of Physics) has developed a robust, low-cost method called "*easySTORM*"[6], which utilises high power multimode laser diode sources to provide large (>120 μm) fields of view. Moreover, to support higher throughput SMLM

assays, the group has developed a novel, multi-component pair-correlation function-fitting algorithm that can analyse SMLM data from 100 nuclei in 40 minutes per nucleus. Using *easySTORM*, preliminary data from the host labs indicate global changes in chromatin architecture between paired sensitive and resistant HGSC cell lines. Moreover, platinum resistance induces a chromatin structure similar to that seen in iPSC cells.

This PhD will combine super-resolution microscopy and biochemical assays to map the epigenetic landscape of platinum-resistant HGSC. Use of super resolution imaging systems in this project is motivated by their ability to detect epigenetic characteristics at single-cell level and provide combined structural and genomic insight into drug resistance.

Project aims

The student will investigate changes in chromatin structure, addressing three key questions:

1. Are the changes in chromatin organisation that arise during progression of HGSC global or localised to specific genomic regions?
2. Do changes evolve across the whole population or are they present in a subset of cells at diagnosis that are evolutionarily selected?
3. Are changes in architecture similar to those seen in pluripotent stem cells?

Research proposal

Aim 1

Previous STORM data were acquired using established cell lines. Using *easySTORM*, the student will image global chromatin structure (Histone H3) in primary HGSC cells growing in 3D following treatment with platinum chemotherapy. Cultures from four chemotherapy naïve HGSC patients will be treated with three cycles of carboplatin (50 µM for 6h followed by 7 days of recovery to model *in vivo* pharmacokinetics) or vehicle prior to imaging. Cells will be treated without trypsinisation to minimise additional stress or selection bias. DNA will be labelled with EdU and visualised with Alexa-688 azide, whilst chromatin will be stained with appropriate primary and Alexa-647 conjugated secondary antibodies[7]. Imaging will be performed in Photonics following fixation and sectioning. Structural changes in chromatin will be quantified using previously devised cluster analysis methods, and assays will be repeated using both active (H3K9ac and H3K27ac) and repressive (H3K9me3 and H3K27me3) chromatin marks.

To address the global–vs–local change, the student will use FISH in conjunction with STORM to image specific genomic regions. Preliminary data from the McNeish lab has identified upregulation of genes associated with Epithelial-Mesenchymal Transition (EMT) following platinum exposure in primary and established HGSC cells, (e.g. *CLDN18, WNT11*), with matched alterations in chromatin accessibility on ATAC-sequencing. An association between chemoresistance and the acquisition of EMT in ovarian cancer cells that attain a stem cell-like phenotype has been demonstrated[8]. In addition, platinum was able to induce stress responses via the MAPK/PI3K/Ras/TNF signalling pathway that may converge on the AP-1 family of transcription factors. The student will also use STORM to assess specific histone (H3K9ac, H3K27ac, H3K9me3, H3K27me3) modifications in treated and untreated cells. In parallel, the student will extract DNA and RNA for ChIP-seq to assess key differentially expressed genes between sensitive and resistant cells as well as other genomic loci whose association with specific histone modifications alters following platinum exposure.

Aim 2

Preliminary data from cells growing in 2D suggest maximum changes in gene expression (RNAseq) and chromatin structure (ATAC-seq) are observed following three cycles of carboplatin treatment. However, the kinetics of this change, and also whether it is recapitulated in physiologically relevant 3D spheroid structures is unclear. Furthermore, it remains unclear whether there is clonal (or subclonal selection) or whether platinum induces population-wide changes.

To address this, we will use DNA barcoding. Single cells from primary cultures will be labelled using technology that allows scRNAseq and barcode reading to be performed on the same cells. Barcoded cells will be grown as spheroids and treated with carboplatin as per aim 1, allowing assessment of population distribution and whether treatment selects pre-existing resistant clones or if epigenetic changes are induced by therapy and subsequently inherited in daughter cells. In parallel, cells will undergo serial imaging following each cycle of platinum to assess if any chromatin changes occur, if these changes are consistent after each cycle and if there is an initial stress response that occurs before any definite changes.

The Photonics Group has recently implemented STORM of both FFPE and frozen sections. Therefore, to assess whether changes seen in primary 3D cultures are recapitulated *in vivo*, the student will also interrogate matched samples from women at primary and secondary (first relapse) surgery.

Aim 3

Preliminary data indicated that global chromatin architecture in platinum-resistant IVR-01 cells was similar to that in iPSC cells, suggesting that platinum may induce a stem-like state. The final aim of this studentship will be to explore this in primary HGSC cells, and to map specific histone modifications associated with plutipotency, including an increase in H3K4me3, H3K36me3 and global histone acetylation.

Literature references

1 TCGA. Integrated genomic analyses of ovarian carcinoma. *Nature* **474**, 609-615 (2011).

2 Morgan, R. D. *et al.* Objective responses to first-line neoadjuvant carboplatin-paclitaxel regimens for ovarian, fallopian tube, or primary peritoneal carcinoma (ICON8): post-hoc exploratory analysis of a randomised, phase 3 trial. *Lancet Oncol* **22**, 277-288 (2021). https://doi.org:10.1016/s1470-2045(20)30591-x

3 Smith, P. *et al.* The copy number and mutational landscape of recurrent ovarian high-grade serous carcinoma. *Nat Commun* **14**, 4387 (2023). https://doi.org:10.1038/s41467-023-39867-7

4 Fang, F. *et al.* Genomic and Epigenomic Signatures in Ovarian Cancer Associated with Resensitization to Platinum Drugs. *Cancer Res* **78**, 631-644 (2018). https://doi.org:10.1158/0008-5472.Can-17-1492

5 Xu, J. *et al.* Super-resolution imaging reveals the evolution of higher-order chromatin folding in early carcinogenesis. *Nature Communications* **11**, 1899 (2020). https://doi.org:10.1038/s41467-020-15718-7

6 Kwakwa, K. *et al.* easySTORM: a robust, lower-cost approach to localisation and TIRF microscopy. *J Biophotonics* **9**, 948-957 (2016). https://doi.org:10.1002/jbio.201500324

7 Ma, H., Xu, J., Jin, J., Huang, Y. & Liu, Y. A Simple Marker-Assisted 3D Nanometer Drift Correction Method for Superresolution Microscopy. *Biophys J* **112**, 2196-2208 (2017). https://doi.org:10.1016/j.bpj.2017.04.025

8 Leung, D. *et al.* Platinum-resistance in epithelial ovarian cancer: an interplay of epithelial-mesenchymal transition interlinked with reprogrammed metabolism. *Journal of translational medicine* **20**, 556 (2022). https://doi.org:10.1186/s12967-022-03776-y

Candidate profile

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1).

Pre-requisite qualifications of applicants:	1st or 2:1 in either a biological sciences or physics/engineering (essential) MSc/MRes in either cancer biology or photonics (desirable)
Intended learning outcomes:	To develop understanding of biology of ovarian cancer, epigenetics, and chemotherapy-induced changes in epigenome and gene expression, including methods for analysing gene expression and chromatin structure. To develop understanding of super-resolution microscopy and methods for analysing complex imaging datasets To embrace the culture of convergence science, using engineering, physical and data sciences to address critical questions in cancer biology and cancer medicine.

Advertising details

Project suitable for a student with a background in:	☒ Biological Sciences ☒ Physics or Engineering ☐ Chemistry ☐ Maths, Statistics or Epidemiology ☐ Computer Science