ICR The Institute of Cancer Research

PhD Project Proposal

Funder details

Studentship funded by:	Christine O'Connell of *One More City*

Project details

Project title:	Evaluation of sub-clonal alterations mediating response and resistance to CDK7 inhibition in therapy resistant triple negative breast cancer

Supervisory team

Primary Supervisor:	Rachael Natrajan
Associate Supervisor(s):	Simak Ali
Secondary Supervisor:	Andrew Tutt

Divisional affiliation

Primary Division:	Breast Cancer Research
Primary Team:	Functional Genomics
Site:	Chelsea

Project background

Triple negative breast cancers (TNBC) with high levels of viable residual cancer cells post neoadjuvant chemotherapy (NAC) are highly aggressive with an average survival of less than 50% and a median progression free survival of 1.7 months for women who have therapy resistant metastatic disease1. Although immunotherapy is showing promising results in early TNBC, many patients do not respond, and chemotherapy remains the main option. High rates of recurrence in TNBC are in part due to its inherent molecular heterogeneity and sub-clonal diversity, in which cells present in minority sub-populations escape the pressures of therapy. Preliminary studies suggest these pre-existing sub-clones harbor distinct transcriptional signatures rather than genetic alterations that result in regulated changes in gene expression and can foster sub-clonal diversity, tumour evolution, and ultimately therapy failure and breast cancer metastasis2. Data from our lab highlight that subsets of distinct metastasis associated genes are seen as sub-clonal populations in primary untreated TNBC. These sub-clonal populations and are selected for upon chemotherapy resistance, leading to transcriptional re-wiring and upregulation of epithelial to mesenchymal transitional states (EMT).

Recent studies have highlighted that aggressive TNBC show "transcriptional addiction" and are uniquely sensitive to inhibition of the cyclin-dependent kinase CDK7 in vitro and in vivo. This effect is mediated through specific up-regulation of an "Achilles heel" set of genes which are regulated by super-enhancers, including regulation of genes that control EMT and ultimately cell fate3,4. The CDK7 specific inhibitor samuraciclib5 has demonstrated durable responses in metastatic TNBC patients in clinical trials and identifying patients a priori that may derive benefit is paramount. We hypothesise that specific sub-populations of cells that are selected for upon chemotherapy exposure are epigenetically defined, show "transcriptional addiction" and are the sub-populations of cells that are sensitive to CDK7 inhibition. Based on this, this project will address the following questions:

Project aims

- How does CDK7 inhibition alter the sub-clonal heterogeneity of TNBC?
- What are the sub-clonal populations responsible for resistance to CDK7i?
- Can CDK7i be combined with immunotherapeutic approaches in TNBC?

Research proposal

Our laboratory has developed several reagents for this project. These include i) molecularly characterised TNBC models derived from patients resistant to NAC and from distant metastatic sites, ii) implementation of molecular barcoding technologies and iii) experience in single cell sequencing from primary breast cancers and PDX models and complex bioinformatics for data analysis and interpretation.

Aim 1: How does CDK7 inhibition alter the sub-clonal heterogeneity of TNBC?

The project will use a unique panel of TNBC patient derived xenograft (PDX) models that have been derived post NAC and from metastatic disease that have been established as organoid cultures (PDxO) characterized in the lab. Firstly, the project will assess the sensitivity of this panel of PDxO models to the CDK7i samuraciclib, which has demonstrated promising activity in clinical trials in TNBC. Multi-ome single cell RNA-sequencing (scRNA-seq) and chromatin profiling (scATAC/H3K27ac) will be performed on these models before and after samuraciclib exposure to identify if sub-clonal populations of cells mediate sensitivity to CDK7i and if these genes are regulated by super-enhancers. Single cell profiles from sensitive versus resistant models will also be compared. Transcriptional alterations identified will be validated using both our in-house generated and published scRNA-seq data sets from TNBC patients to generate a potential biomarker of response.

Aim 2: Are these sub-clonal populations responsible for resistance to CDK7i?

As with many targeted agents, resistance can emerge over time. Identifying mechanisms of resistance is important to i) understand the biology that mediates sensitivity and ii) identify potential combination therapy approaches that may halt the emergence of resistance. To identify resistance mechanisms, samuraciclib sensitive PDxO models will be infected with complex expressed barcode libraries to molecularly tag cells with unique barcodes and exposed to samuraciclib over time to generate resistance. This approach has the advantage of tracking minority populations of cells without a priori knowledge of their genetic or transcriptomic make-up to evaluate the sub-clonal evolution of resistance at the single cell level. As each cell has a unique barcode integrated into the DNA, we can ascertain whether resistance is stochastic or conserved (i.e. enrichment of particular barcodes in replicate experiments will indicate pre-existing heritable sub-clones that have the capability to drive resistance). Random barcode enrichment will indicate adaptive evolution to metastatic disease. As the barcodes are expressed, we can additionally ascribe which barcoded cell has a particular chromatin and transcriptional landscape using sc-sequencing. Sub-clonal alterations enriched upon samuraciclib resistance will be validated through CRISPR/Cas9 editing of sub-populations of genes enriched in resistant cells to test if these re-sensitise cells to samuraciclib.

Aim 3: Can CDK7i be combined with immunotherapeutic approaches in TNBC?

Recent evidence suggests that CDK7i can potentiate a robust immune response via induction of IFN-γ signaling, which is further improved by anti-PD-1 and chemotherapy6. Addition of samuraciclib to chemotherapy and anti-PDL-1 may thus improve TNBC patient response to combinations of anti-PD-1 and chemotherapy and widen the number of TNBC patients that will respond to these agents. To test this, immune competent TNBC murine models will be treated with combinations of samuraciclib, anti-PDL-1 and taxane chemotherapies. Using single cell sequencing approaches, the effect on tumour sub-clones that are responsible for response/resistance to samuraciclib identified from aims 1 and 2 will be investigated. Additionally, the effects on the immune milleu will be investigated with the hypothesis that both the complement of immune cells and epithelial cells will govern response to the combination. This will be further validated using tissue slices of primary TNBC patient tumours grown in vitro as short-term cultures.

Literature references

[1] Symmans, W. F. et al. Long-Term Prognostic Risk After Neoadjuvant Chemotherapy Associated With Residual Cancer Burden and Breast Cancer Subtype. J Clin Oncol 35, 1049-1060, doi:10.1200/JCO.2015.63.1010 (2017).

[2] Kim, C. et al. Chemoresistance Evolution in Triple-Negative Breast Cancer Delineated by Single-Cell Sequencing. Cell 173, 879-893 e813, doi:10.1016/j.cell.2018.03.041 (2018).

[3] Wang, Y. et al. CDK7-dependent transcriptional addiction in triple-negative breast cancer. Cell 163, 174-186, doi:10.1016/j.cell.2015.08.063 (2015).

[4] Li, B. et al. Therapeutic Rationale to Target Highly Expressed CDK7 Conferring Poor Outcomes in Triple-Negative Breast Cancer. Cancer Res 77, 3834-3845, doi:10.1158/0008-5472.CAN-16-2546 (2017).
[5] Patel, H. et al. ICEC0942, an Orally Bioavailable Selective Inhibitor of CDK7 for Cancer Treatment. Mol Cancer Ther 17, 1156-1166, doi:10.1158/1535-7163.MCT-16-0847 (2018).
[6] Zhang, H. et al. CDK7 Inhibition Potentiates Genome Instability Triggering Anti-tumor Immunity in Small Cell Lung Cancer. Cancer Cell 37, 37-54 e39, doi:10.1016/j.ccell.2019.11.003 (2020).

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:

BSc. (1st or 2:1) or equivalent in biological sciences or computer science.

Intended learning outcomes:

- Knowledge of the molecular basis of cancer
- Advanced skills in a wide range of molecular, cellular and biochemical assays
- Experience in bioinformatics
- Project management skills
- Wide appreciation of cancer research
- Acquisition of skill-set relevant to future as postdoctoral research fellow
- Ability to productively liaise with internal and external collaborators

Advertising details

Project suitable for a student with a background in:

- [x] Biological Sciences
- [ ] Physics or Engineering
- [ ] Chemistry
- [ ] Maths, Statistics or Epidemiology
- [x] Computer Science