PhD Project Proposal

Funder details

Studentship funded by:	MRC iCASE (AstraZeneca)

Project details

Project title:	Comprehensive multi-omic analyses of "islands of resistance" tumour cells to identify aromatase inhibitor resistant subclones in oestrogen receptor positive breast cancer

Supervisory team

Primary Supervisor:	Maggie Cheang
Associate Supervisor(s):	Christopher Morrow (AstraZeneca) Gene Schuster
Secondary Supervisor:	Nicholas Turner

Divisional affiliation

Primary Division:	Clinical Studies
Primary Team:	Integrative Genomics Analysis in Clinical Trials
Site:	Sutton

Project background

Over 80% of breast cancer (BC) patients in the developed western world have oestrogen receptor (ER)–positive disease; their treatment normally includes surgery and adjuvant endocrine therapy, and sometimes chemotherapy which greatly improves survival outcome (1). In postmenopausal women, the most effective endocrine therapy agents are aromatase inhibitors (AIs). Many patients recur because of de novo or acquired resistance to AI.

Currently, abemaciclib is approved for high-risk, node positive hormone receptor (HR)+/HER2- early BC in US and UK, supported based on the results from the phase 3 MonarchE randomised controlled trial (2). HR+/HER2+ BCs are molecularly heterogeneous with 30% of them are HER2-Enriched (HER2-E). We showed that HR+HER2-E is characterised by an immune-activated stroma with elevated tumour infiltrating lymphocytes, lower expression of luminal-related genes than the HR+/Luminal subtypes and benefit greatly from anti-HER2 therapies but poorly from endocrine therapy (ET) (3).

Resistance to endocrine therapies has been mainly studied in HR+/HER2- BC, but those mechanisms might differ between HER2+ and HER2- tumours, in part due to the differential distribution of intrinsic subtypes within each BC subgroup. Cells within a single tumour can be genetically diverse and respond differently to treatment. Even in tumours with good response to treatment, there may be a few resistant cells that remain growing and might be responsible for recurrences if not removed or killed by other methods.

The PeriOperative Endocrine-Therapy for Individualised Care (POETIC) trial (4) provides a framework to study endocrine resistance mechanisms in a large set of ER+ HER2+ and HER2- BC patients. The objective of this PhD project is work on method(s) to isolate these resistant tumour cells and discover genomic changes that are specific

to these resistant cells. This will allow us to understand why these cells do not respond to treatment and may allow additional personalised treatment targeting these resistant cells.

Project aims

- To determine whether the Ki67 (proliferating) persistent cells are different at a genomic (either genetic or epigenetic) from the quiescent cells isolated after peri-operative AI
- To determine whether the Ki67 rich "clones" drive growth in primary disease in neoadjuvant treatment?
- To determine whether those genomic markers in the Ki67 persistent cells after peri-operative AI at 2 weeks are found in distant recurrent disease?
- To identify new drug candidates by comparative analyses with the genomic profiles from target ER+ cell lines treated with investigational drugs in AstraZeneca

Research proposal

Mechanisms of resistance to aromatase inhibitors (AIs) vary between patients; a rational application of the emerging large number of targeted agents against resistance mechanisms that are present in individual tumours requires novel clinical approaches. We propose to analyse data from the POETIC (Peri-Operative Endocrine Therapy – Individualising Care) study (4). POETIC was an open-label, multicentre, parallel-group, randomised, phase 3 trial (done in 130 UK hospitals) in which 4480 postmenopausal women aged at least 50 years with WHO performance status 0–1 and hormone receptor-positive, operable breast cancer were randomly assigned (2:1) to POAI (letrozole 2·5 mg per day orally or anastrozole 1 mg per day orally) for 14 days before and following surgery or no POAI (control). Adjuvant treatment was given as per UK standard local practice. In this cohort, we found that KI67 levels and changes in the levels after perioperative AI treatment was associated with time to recurrence (TTR) and overall survival (OS) (4). The Ki67 value was measured using the standardised visual score method and they were as follows: all stained and unstained invasive tumour nuclei were counted in at least 5 high-power fields (i.e., the most representing field); the Ki67 staining index was calculated as the total number of stained nuclei counted/total number of all invasive nuclei counted.

Intratumor heterogeneity of Ki67 expression after neoadjuvant endocrine therapy is suggested to be associated with poor outcomes and resistance to treatment but datasets and techniques to identify genomic differences between the resistant and sensitive subclones has been limited. The key overall question is whether those persistent Ki67 cell populations identify clonal populations that will grow out under continued treatment pressure and drive the growth of micro-metastases to reveal themselves as recurrent disease. This question will be broken down into three sub questions.

Are the Ki67 persistent cells different at a genomic (either genetic or epigenetic) from the quiescent cells at 2 weeks?

Based on our pilot project, using multi-parameter fluorescence-activated cell (FACS) sorting of 26 AI treated FFPE POETIC samples (at 2weeks) allowed the separation of de novo AI-resistant from sensitive cell populations and whole genome sequencing revealed higher mutational burden in the AI resistant populations at the time of disease presentation (5). This suggests that interrogation of the mutational differences between the proliferative and non-proliferative cells may allow identification of putative drivers of resistance in individual tumours. This FACS method is an important bridge between methods that are low cost but give a limited view of tumour heterogeneity (needle microdissection) or are high cost and effort but full view of heterogeneity (single cell sequencing).

Further work is needed looking at various measures of genomic differences in many more samples.

In this PhD project, we would review the electronic record of the Ki67 score from each of the 5 fields to identify list of samples showing intra-tumour heterogeneity of Ki67 expression. We would apply case (recurrent) -control (no recurrent) study design within this list of samples (expected number of samples = 200).

We would apply either our established FACS method or DEPArray to cell sort the populations; however downstream analysis would be limited to WES.

We would also explore whether image analysis could be used to identify patients who had "pools" of resistant cells with techniques such as multiplex immunofluorescence or GeoMx Digital spatial profiling for proteomics and transcriptomic analysis.

As single cell omics techniques on FFPE tissues are rapidly evolving, we would explore emerging techniques such as Cyclic Immunofluorescence, 10X/Visium or Nanostring Digital Spatial Profiling and/or CosMx SMI. If this proof-of-concept study is successful, we will test this workflow further on other trials such as POETIC-A (recruiting).

Finally, we would integrate these data with bulk multi-omics data that are generated. We will aim to compare if these clones stratify by the HER2 status as well as intrinsic subtyping (PAM50 assay).

Do the Ki67 rich "clones" drive growth in primary disease in neoadjuvant treatment?

We would apply the same molecular characterisation technologies on samples collected from our various collections of neoadjuvant endocrine treatment studies in which 2 week and 12 or later week samples are available. This will be tested by assessing the genomic characteristics of that 12 or later week samples and whether it has been enriched for the genomic markers in the 2-week Ki67 persistent cells.

Are those genomic markers in the Ki67 persistent cells at 2 weeks found in distant recurrent disease? and whether different in locoregional disease?

POETIC is the ideal and unique study to study this question. Within the protocol, we have included an optional collection of recurrence samples when patient recur. Currently we have identified 15 samples with documented first recurrence being distant recurrence already in the lab based on 5-year follow-up time. We would also be retrieving loco-recurrence and positive lymph nodes samples, funded by another project, so we can also compare the genomics markers between primary and locoregional as opposed to the primary and distant recurrence comparison.

Are there better treatment approaches for these aromatase inhibitor resistant clones and cases?

To answer this question, we would collaborate with Translational Medicine and Bioscience colleagues at AstraZeneca, to compare the genomic characteristics of these AI resistant clones to the genomic profiles from cell lines and/or patient derived cancer models and compare the activity of AIs with next generation endocrine therapies or other emerging therapeutics/combinations to identify alternative therapeutic approaches. This would help to inform better trial design by identifying better biomarkers to stratify patients (predictive marker) and/or biological endpoints for Bayesian clinical trial (e.g., drug imposing changes to these characteristics).

By working with AZ, this PhD project would benefit significantly enhancing the clinical impact through looking for candidate drugs and therapeutic approaches for the ER+ AI resistant tumours. The student will gain experience and appreciate how forward and reverse translation can be made possible in next generation modern cancer trials.

Literature references

[1] Early Breast Cancer Trialists' Collaborative G.: Aromatase inhibitors versus tamoxifen in premenopausal women with oestrogen receptor-positive early-stage breast cancer treated with ovarian suppression: a patient-level meta-analysis of 7030 women from four randomised trials. Lancet Oncol. 2022;23(3):382-92

[2] Martin M, Hegg R, Kim SB, et al.: Treatment with adjuvant abemaciclib plus endocrine therapy in patients with high-risk early breast cancer who received neoadjuvant chemotherapy: a prespecified analysis of the monarchE randomized clinical trial. JAMA Oncol. 2022 Aug 1;8(8):1190-1194.

[3] Bergamino MA, López-Knowles E, Morani G, et al.: HER2-enriched subtype and novel molecular subgroups drive aromatase inhibitor resistance and an increased risk of relapse in early ER+/HER2+ breast cancer. eBioMedicine,2022.

[4] I Smith, J Robertson, L Kilburn, et al.: Long-term outcome and prognostic value of Ki67 after perioperative endocrine therapy in postmenopausal women with hormone-sensitive early breast cancer (POETIC): an open-label, multicentre, parallel-group, randomised, phase 3 trial Lancet Oncol, 21 (2020), pp. 1443-1454

[5] Schuster ER, Zabalgo L, Perou CP, et al.: Multi-parameter FACS sorting identifies higher mutational burden in aromatase inhibitor resistant subclones in estrogen positive breast cancer at diagnosis San Antonio Breast Cancer Symposium; 2019 Dec 10-14; San Antonio, TX. Philadelphia (PA)2019.

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:

- Candidates must have a First or 2:1 Honours degree or a master's in computer science, mathematics, engineering, statistics, or a related discipline.
- Academic knowledge in cancer biology, cell biology, or analytical chemistry
- Previous laboratory experience
- Good presentation and communication

Intended learning outcomes:	- Knowledge in next-generation sequencing, molecular pathology, molecular diagnostics. - Knowledge in experimental skills in molecular techniques including spatial omics and single-cell sequencing. - Advanced medical statistical skills. - Ability to apply machine learning and artificial intelligence to biomedical data - Ability to design, manage and progress a defined scientific project. - Scientific writing, presenting and communication skills. - Ability to read and process relevant literature.

Advertising details

Project suitable for a student with a background in:

- ☒ Biological Sciences
- ☐ Physics or Engineering
- ☐ Chemistry
- ☒ Maths, Statistics or Epidemiology
- ☒ Computer Science