

The Institute of Cancer Research PHD STUDENTSHIP PROJECT PROPOSAL : MRC iCASE SCHEME	
PROJECT DETAILS	
Project Title:	Elucidating resistance to AKT inhibition in metastatic breast cancer
SUPERVISORY TEAM	
Primary Supervisor(s):	Prof Nicholas Turner
Associate Supervisor(s):	Dr Alex Pearson
Industry supervisor:	Dr Elza de Bruin
Backup Supervisor:	Prof Chris Lord
DIVISIONAL AFFILIATION	
Primary Division:	Breast Cancer Research
Primary Team:	Molecular Oncology
PROJECT PROPOSAL	
SHORT ABSTRACT	
<p>AKT inhibition has emerged as one of the most promising targeted therapies for breast cancer. In triple negative breast cancer with pathway mutations, AKT inhibition has substantial efficacy with paclitaxel. In ER positive breast cancer, capivasertib has substantial activity in <i>AKT1</i> mutant breast cancer and in combination with fulvestrant. Yet there is very limited understanding of clinically relevant resistance mechanisms. We will use breast cancer patient derived models, both organoid and xenograft, to study mechanisms of acquired resistance to AKT inhibitor resistance, combined with patients' material from AKT inhibitor clinical trials, to identify and validate mechanisms of resistance.</p>	
BACKGROUND TO THE PROJECT	
<p>Resistance of metastatic cancer to targeted therapies inevitably develops despite marked clinical responses often being observed in the first instance. As a result, identifying and characterising these mechanisms of resistance is critical to understanding how to prolong the clinical utility of specific targeted therapies. In the last two years AKT inhibition has emerged as one of the most promising clinical therapies for breast cancer. Two phase II trials with ipatasertib (Barry et al., 2018) and capivasertib (Turner et al., 2019) have generated evidence of substantial efficacy for AKT inhibition in combination with paclitaxel in patients with triple negative breast cancer (TNBC) with</p>	

pathway mutations (*PIK3CA*, *AKT1*, or *PTEN*). Pathway mutations occur in approximately 30% of metastatic TNBC, representing a substantial subset of the disease.

In ER positive breast cancer, the AKT inhibitor capivasertib has substantial efficacy in combination with fulvestrant (Jones et al., 2019), and in *AKT1* mutant breast cancer (Hyman et al., 2017). Mutations in *AKT1* have been identified in approximately 4% of metastatic breast cancers (MBC). In MBC patients with *PIK3CA* mutations, capivasertib demonstrated a pattern of response followed progression (Hrebien et al., 2019), suggesting resistance was predominantly clinically ‘acquired’ as opposed to intrinsic.

The mechanisms of resistance, and in particularly acquired resistance, are largely unknown to AKT inhibitors. AKT inhibition is known to upregulated ER signalling, which may be countered by ER downregulation with fulvestrant, and may lead to receptor tyrosine kinase upregulation. Whether these mechanisms are relevant in acquired resistance is unknown.

We have developed unique models of *AKT1* mutant breast cancer, using patient derived tumour samples from patients treated with capivasertib. We will use these as a model of AKT dependent breast cancer to study mechanism of resistance to capivasertib, through chronic exposure of MBC models to capivasertib. Through identifying mechanisms of acquired resistance we will then develop novel therapeutic strategies that will be validated in resistant models for their potential anti-tumour efficacy compared to existing standard of care treatment options. Successful strategies and drug combinations will be developed into future clinical trials.

PROJECT AIMS

1. To develop models of *AKT1* mutant metastatic breast cancer including models established from patient derived tumour material.
2. To elucidate the mechanisms of resistance to AKT inhibition in *AKT1* mutant metastatic breast cancer.
3. To identify potential drug combinations to overcome emergent resistance in these *AKT1* mutant models.
4. To validate putative AKT inhibitor resistance mechanisms in clinical trial samples

RESEARCH PROPOSAL

Develop *AKT1* mutant breast cancer models

We aim to establish at least 4 *AKT1* mutant patient derived models in which to study resistance mechanisms to capivasertib. Tumour material for these models will be obtained from patients identified through the ABCBio tissue collection study, in which patient’s tumour samples undergo sequencing using a targeted NGS panel. We have already established 1 *AKT1* (c.49G>A; p.E17K) PDX model from a patient with metastatic breast cancer

who was treated with capivasertib in the clinic. In addition we have developed patient derived organoid (PDO) cultures from this model. Two further *AKT1* mutant models are available in collaboration with Violette Serra (Val D'Hebron).

In addition to patient derived models, existing breast cancer cell lines will be engineered using CRISPR-Cas9 to generate isogenic cell lines with specific *AKT1* mutations (e.g. E17K, L52R, and Q79K). Breast cancer cell lines will be chosen based on their insensitivity to capivasertib, lack of pathway mutations, and for both ER positive and TNBC lines. Similarly to the patient derived models, paired isogenic cell lines will receive chronic exposure to establish drug resistance. Together, the PDO/PDX models and the isogenic cell line models will provide multiple options to explore mechanisms of resistance to AKT inhibitors.

Patient derived organoids and engineered cell lines will be exposed to continuous capivasertib to generate acquired resistance. In ER positive models resistance will also be generated with fulvestrant, and fulvestrant plus capivasertib, and in all models with paclitaxel and paclitaxel plus capivasertib, to study the clinically relevant combinations. Multiple independent clones will be derived, aiming for at least ten per model, to study heterogeneity in mechanisms of resistance.

The mechanisms of resistance to AKT inhibitors are unknown, as is the extent to which that may be influenced by stromal interactions. As well as using PDO models, we will treat PDX with the treatment combinations above in collaboration with AZ, to develop resistant PDX, allowing us to investigate multiple diverse mechanisms of resistance.

Elucidation of mechanisms of resistance to AKT inhibition

To elucidate the mechanisms of resistance to AKT inhibition, and combinations, we will explore both hypothesis driven and hypothesis free approaches.

For hypothesis free approaches we will profile resistant models with exome sequencing and RNAseq, paired with baseline, to identify acquired resistant mutations and phenotypes. In parallel, we will screen resistant PDOs with kinome siRNA modifier screens to identify kinases that mediate resistance, and use CRISPRn screening to identify guide RNA that mediate resistance to AKT inhibition in sensitive PDOs. We also anticipate using CRISPRa screens to investigate gain-of-function effects. Through these approaches we will functionally confirm resistance mechanisms, overlaying the data from the functional screens with exome and RNA sequencing. Using PDOs in these strategies is technically challenging and we will employ the isogenic breast cancer cell line models as backups.

Further, in collaboration with AZ we will perform combinatorial drug screens in resistant PDOs, to identify potential drug combinations to overcome resistance to AKT inhibition. We will use commercial drug libraries and compound libraries from Astra Zeneca for these screens.

In addition, we will investigate the following hypothesis driven approaches:

- Gatekeeper mutations in AKT1 through deep sequencing of *AKT1* mutant breast cancers to assess whether gatekeeper mutations influence the response to AKT1 inhibition. If potential gate-keeper mutations are found, we will introduce these mutations in PDOs by homology-directed CRISPR-Cas9 mutagenesis.
- Whether modulation of down-stream signalling modifies sensitivity to AKT inhibition. We will generate small libraries of CRISPRn, CRISPRi and CRISPRa optimised guide RNAs to either inactivate (CRISPRn, CRISPRi) or activate (CRISPRa) components of downstream AKT signalling pathways, and investigate for modification of AKT inhibitor sensitivity.
- Phospho-proteomic screening in response to AKT inhibition in *AKT1* mutant PDOs, to identify key pathway proteins that undergo differential protein phosphorylation in the acute response to AKT inhibition, and in derived resistant PDOs.
- ER signalling to assess whether fulvestrant insufficiently degrades ER to block AKTi induced ER upregulation.

Validate putative AKT inhibitor resistance mechanisms in clinical trials

We will validate the pre-clinical findings in available clinical trial material. Through our leadership of multiple clinical trials we have multiple sample sets available including:

- (1) ~30 patients with AKT1 mutant breast cancer treated in the plasmaMATCH clinical trial. Baseline biopsies, paired ctDNA samples sequentially from treatment and through every cycle to progression, and optional progression biopsies (samples at ICR);
- (2) Baseline and progression plasma samples for ctDNA analysis from the BEECH clinical trial of paclitaxel +/- capivasertib in ER positive breast cancer (samples at ICR);
- (3) Available NGS data (AZ600 gene panel) from baseline and progression plasma samples for ctDNA analysis from the PAKT clinical trial of paclitaxel +/- capivasertib in TNBC (in collaboration with Peter Schmid), analysis currently underway in AZ with the potential to extend to additional genes if required in the future.

We will use exome or targeted sequencing and RNAseq of tumour samples, or exome capture RNAseq for formalin fixed samples. For plasma DNA we will use molecular bar-code unique molecular identifier sequencing either with a targeted panel focused on pre-clinical resistance mechanisms or wider approach using the RMH200 cancer gene panel.

During the studentship, phase III studies with capivasertib are anticipated to read out (NCT03997123 in TNBC and in ER positive breast cancer), and there will be a unique opportunity to validate the findings of this studentship in clinical material.

Industry collaboration

Our laboratory is among the leading academic cancer research labs in breast cancer translational oncology with a proven track record in translation research and significant expertise in the study of resistance mechanisms, development of biomarkers, and bioinformatics analysis. We have extensive industry collaboration experience. AstraZeneca is developing capivasertib. The ICR was involved in the initial drug discovery of capivasertib (AZD5363)

This work will sit within a broader collaboration between our group and AZ on AKT inhibitor resistance in general, with other projects focused on resistance to AKT inhibitor in *PIK3CA* and *PTEN* mutant breast cancer. The study on *AKT1* mutant breast cancer, studies the cancer type with the 'hardest' addiction to AKT signalling, and it is anticipated the findings of this project will be beneficial to understanding AKT inhibitor resistance in general.

The collaboration will be integrated across all aspects of the work, in particular greatly facilitating the development of *in vivo* PDX models, and the potential for drug screens to be carried out in collaboration with AZ. Under a collaboration agreement we will initiate generation of acquired resistant models with *AKT1* mutant PDX at AZ, prior to the studentship, to allow the student to start work on analysing derived resistant samples early in the first year.

We plan a comprehensive set of studies to investigate the underlying mechanisms of resistance of *AKT1* mutant breast cancer to inhibitors of AKT. We will use patient derived models, employing cutting edge technology and techniques to test drug combinations

PLEASE INDICATE WHETHER THIS PROJECT ALIGNS WITH ANY OF THE MRC STRATEGIC SKILLS PRIORITY AREAS?

Quantitative skills Interdisciplinary skills Whole organism physiology

See the link below for further details: <https://mrc.ukri.org/documents/pdf/mrc-strategic-skill-priorities/>

LITERATURE REFERENCES

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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:
e.g. BSc or equivalent in specific subject
area(s)

BSc, First

Intended learning outcomes (including those
arising from the industry collaboration):

On completion of the studentship program the candidate will
have developed an deep understanding of:

1. Drug development
2. Translational Science

And an expertise in:

3. Development of patient derived models
4. High throughput genetic and drug screens
5. CRISPR-cas9 system
6. Next generation sequencing and analysis

Potential publications arising from project:

3 papers

At least 2 periods of 3months

ADVERTISING DETAILS

- Biological Sciences
 Physics or Engineering
 Chemistry
 Maths, Statistics or Epidemiology
 Computer Science
 Other (provide details)

Keywords:

1. Patient derived organoids/xenografts
2. Next generation sequencing

	3. Clonal evolution
	4. CRISPR
	5. Breast cancer
	6.