The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

FUNDER DETAILS	
Studentship funded by:	Medical Research Council industrial Collaborative Awards in Science and Engineering (MRC iCASE)
Funder specific requirements:	All MRC iCASE students will attend taught courses one day a week for the first nine months of the PhD. This training will cover computational and thematic science training as well as core and transferable skills. Students will spend the remainder of the four years on their PhD project full time with monthly cohort activities. In addition, students must spend a cumulative period of no less than three months working in the facilities of the industrial collaborator. This 3 month period can be at any point during the studentship and may consist of a number of shorter visits if appropriate.
Estimated amount and distribution of time spent with industrial partner:	We predict 50/50 split in time between ICR and Artios
PROJECT DETAILS	
Project Title:	**Understanding Polθ and ATR inhibitor sensitivity in cancer**
SUPERVISORY TEAM	
Primary Supervisor:	Professor Christopher Lord
Associate Supervisor(s):	Dr Stephen Pettitt Dr Diana Zatreanu
Secondary Supervisor:	Professor Andrew Tutt
Industry Supervisor:	Dr Graeme Smith, Artios
Lead contact person for the project:	Professor Christopher Lord
DIVISIONAL AFFILIATION	
Primary Division:	Breast Cancer Research
Primary Team:	Gene Function/Target Validation and DNA damage response

ICR The Institute of Cancer Research

UKRI Medical Research Council

SHORT ABSTRACT

New drugs are starting to change the way cancer is treated. With our partner Artios, we have recently identified a new class of cancer drugs, known as Polθ inhibitors, which work by targeting the DNA repair processes in cancer cells. These drugs kill tumour cells with BRCA1 or BRCA2 mutations but also kill tumour cells that have developed resistance to a commonly-used targeted therapy, PARP inhibitors. In order to ensure that this promising approach to cancer treatment is used in the most appropriate patients, we need to better understand at the molecular level how Polθ inhibitors work and how tumour cells might re-wire or evolve in response to Polθ inhibitor treatment. This PhD is aimed at answering these questions, and will give the candidate high-level training in functional genomics, drug discovery, cancer biology, genetic manipulation, high-content microscopy, image and data analysis, DNA repair biology, cancer drug resistance and synthetic lethality, forming the basis for a later career as a cancer researcher.

BACKGROUND TO THE PROJECT

The repair of double stranded DNA breaks (DSB) can be broadly classified into three main pathways; Non-Homologous End Joining (NHEJ), which preferentially repairs unresected DSB ends [1-3] and two processes that require nucleolytic resection of 5′ terminal strands generating DSBs with a 3′ ssDNA overhang [4-6]. These latter processes are termed homologous recombination (HR), a conservative template-dependent DNA repair process requiring the BRCA1 and BRCA2 tumour suppressor proteins, and an error-prone process, Theta-Mediated End Joining (TMEJ, also known as alt-NHEJ or Microhomology-Mediated End Joining, MMEJ). HR is a largely error-free mechanism of DSB repair, which utilises stand invasion into an intact sister chromatid or homologous chromosome followed by templated DNA synthesis to repair the damage. In cells that lack HR, such as *BRCA*-gene deficient cancer cells, TMEJ serves as an essential backup pathway to repair resected DSBs [7]. TMEJ is initiated by 5′ to 3′ resection factors, involves the Poly-(ADP-Ribose) Polymerase PARP1, DNA ligase III and the eponymous 290 kDa Polymerase A family enzyme, DNA Polymerase Theta (Polθ, encoded by *POLQ*) [8]. Polθ possesses a N-terminal helicase-like domain and a C-terminal DNA polymerase domain separated by a non-structured central amino acid sequence [9,10] and is only found in multicellular organisms, where it is relatively well-conserved [11]. The polymerase domain of Polθ includes three insertion amino acid loops, not conserved among other A-family DNA polymerases [9]. It is this distinct structure that allows for the interaction, annealing, and extension of short single-stranded (ss)DNA primers [12,13]. Biochemical studies have shown that the helicase domain of Polθ acts to displace RPA bound to the single strand DNA overhang and facilitate annealing of short tracts of microhomology (>1-2 bp) that flank a DSB,

potentially using distant DNA sites as templates [4,12,14,15]. Polθ then employs its polymerase domain to initiate DNA synthesis to fill in the gaps, prior to ligation of the annealed DSB ends.

The interest in Polθ as a therapeutic target in cancer has been piqued by a number of observations including synthetic lethal interactions between loss of the *POLQ* gene and deficiencies in DNA repair-related tumour suppressor genes that control DSB repair/HR, including *BRCA1, BRCA2*, *ATM* and *FANCD2,* observations perhaps best explained by the role TMEJ plays as a backup pathway in the absence of HR [7,13,16-18]. As for the vast majority of cancer-related synthetic lethal effects identified by genetic perturbation, the potential to exploit *POLQ*/HR-gene synthetic lethal effects have not, until very recently, been realized by the discovery of small molecule inhibitors[7]. In part at least, this might be due to the perceived complexity in identifying potent and selective inhibitors of DNA polymerases or helicases, as opposed to other drug targets such as protein kinases. However, working with Artios Pharma, we have recently described novel, drug-like Polθ inhibitors that not only elicit the synthetic lethality with *BRCA*-genes previously predicted by genetic studies, but also confer synthetic lethality with defects in the 53BP1/Shieldin DNA repair complex that are a source of PARP inhibitor resistance[19], effects also seen by others who have identified different Polθ inhibitors[20]. This suggests that Polθ inhibitors not only have clinical potential in targeting *BRCA*-gene defective cancers but could also be used to target PARP inhibitor resistance. This PhD project is designed to build on this work to understand: (i) the precise mechanism by which Polθ inhibitors work; and (ii) how resistance to Polθ inhibitors might emerge.

In other work, we have recently shown that drug-like inhibitors of the replication fork stress-related kinase, ATR, also synthetic lethal target tumour cells with defined tumour suppressor defects[21]. Other than resistance caused by loss of CDC25 proteins, very little is understood about how resistance to ATR inhibitors (now in clinical assessment) might emerge. This PhD project is designed to also assess how resistance to ATR inhibitors might emerge.

References (*key papers to read)

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PROJECT AIMS

1. Assess the possibility that mutations in the drug target Polθ cause Polθ inhibitor resistance
2. Assess the possibility that mutations in the drug target ATR cause ATR inhibitor resistance
3. Assess whether trapping of DNA repair proteins such as Polθ or ATR contribute to tumour cell death
4. Assess how resistance to Polθ inhibitors emerges in BRCA1/2 mutant cancers

RESEARCH PROPOSAL

This project focusses on the genomic stability research theme of the MRC iCase programme

Aims 1 and 2. Assess the possibility that mutations in the drug target Polθ cause Polθ inhibitor resistance and that mutations in the drug target ATR cause ATR inhibitor resistance.

Resistance to multiple different cancer drugs is often caused by mutations in the drug target that render the target protein functional, even in the presence of drug-like inhibitors. This is especially true of kinases, where inhibitors mimic the natural co-factor, ATP, and where "gatekeeper" mutations cause drug resistance. To address whether this is the case for Polθ and ATR, the candidate will take the following approaches:

(i) Using Artios' proprietary Polθ inhibitors and understanding of the inhibitor/target three dimensional structure, the candidate will use site directed mutagenesis of *POLQ* cDNA to mutate specific Polθ residues to assess whether these cause drug resistance in BRCA1 or BRCA2 mutant tumour cell lines, PDO and PDX;

(ii) Using a similar approach for ATRi, the candidate will use classical site-directed mutagenesis of ATR cDNA expression constructs to mutate ATR catalytic domain residues to determine if these drive drug resistance, initially focussing on a p.I800M/F930V double mutant, which is predicted from PI3Kδ and PI3Kα homology modeling to be a likely candidate for influencing interactions with multiple ATR inhibitors;

(iii) Addressing both targets in an unbiased fashion, the candidate will also use close to saturation mutagenesis of *ATR* or *POLQ* genes to identify residues in Polθ or ATR that cause resistance in BRCA1 or BRCA2 mutant tumours. Here the candidate will use catalytically-dead Cas9 fused to deaminase enzymes (and other similar approaches) to mutate endogenous *ATR* or *POLQ* genes, prior to selecting resistant clones in the presence of inhibitor, both in vitro and in vivo. Resistance-causing *ATR* or *POLQ* mutations in cells that survive drug exposure will be identified by Proton sequencing of each gene.

Where appropriate, the candidate will relate the site of drug resistance-causing mutations identified by these approaches to the known structure of existing ATR or Polθ inhibitors, with the intention of predicting the structure of novel inhibitors that could target drug resistant tumour cells.

Aim 3. Assess whether trapping of DNA repair proteins such as Polθ or ATR contribute to tumour cell death.

Our initial observations suggest that exposure of cells to Polθ inhibitor increase the amount of time Polθ is resident on damaged DNA. Our preliminary data from a genome-wide CRISPR screen for determinants of Polθ inhibitor resistance also indicate that loss of Polθ might also cause Polθ inhibitor resistance. Together, these observations raise the hypothesis that Polθ inhibitors impair the fitness of tumour cells by "trapping" Polθ on damaged DNA, providing a steric blockage for the effective repair of damaged DNA and/or the normal progression of replication forks. A similar

scenario is also the case for certain PARP inhibitors. The candidate will address whether Polθ (or indeed ATR) inhibitors mediate their therapeutic effects via trapping by:

(i) Assessing the validity of our initial observations by assessing whether loss of Polθ causes Polθ inhibitor resistance

(ii) Using CRISPaint to label endogenous *POLQ* and *ATR* genes with 5' GFP tags. Using the resultant cell lines, we will use laser-stripe and chromatin precipitation assays to assess whether inhibitors increase the residence time of Polθ or ATR on damaged DNA and/or whether the kinetics of recruitment and/or displacement from DNA are altered in the presence of inhibitor;

(iii) If "trapping" is observed, to then use this information to assess which other proteins associate with trapped Polθ or ATR as a means to understand how these inhibitors drive tumour cell death.

Aim 4. Assess how resistance to Polθ inhibitors emerges in BRCA1/2 mutant cancers. In parallel with the above aims, the candidate will also take more unbiased approaches to identifying mechanisms of Polθ inhibitor resistance in BRCA1 or BRCA2 mutant cancers. Already, we understand that reversion mutations in BRCA2 cause Polθ inhibitor resistance, suggesting that restoration of homologous recombination serves as one route to resistance. Using recent genome-wide CRISPR screens we have identified other, novel, candidate mechanisms of resistance. The candidate will therefore learn how to interpret this data, validate novel resistance-causing mechanisms and then seek to understand how these operate at the mechanistic level.

Approaches to be used: the candidate will learn the following approaches (learnt at Artios or ICR): functional genomics (ICR), drug discovery (Artios), cancer biology (ICR, Artios), genetic manipulation (ICR), high-content microscopy (Artios), image and data analysis (ICR, Artios), DNA repair biology (ICR, Artios), cancer drug resistance and synthetic lethality (ICR, Artios).

LITERATURE REFERENCES

Williamson CT, Miller R, Pemberton HN, Jones SE, Campbell J, Konde A, Badham N, Rafiq R, Brough R, Gulati A, Ryan CJ, Francis J, Vermulen PB, Reynolds AR, Reaper PM, Pollard JR, Ashworth A, Lord CJ. ATR inhibitors as a synthetic lethal therapy for tumours deficient in ARID1A. Nat Commun. 13;7:13837. (2016)

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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:	BSc or equivalent in natural sciences, biology, cell biology, genetics, biochemistry, medicine
Intended learning outcomes:	The candidate will learn the following approaches (learnt at Artios or ICR): • functional genomics (ICR) • drug discovery (Artios) • cancer biology (ICR, Artios) • genetic manipulation (ICR) • high-content microscopy (Artios) • image and data analysis (ICR, Artios) • DNA repair biology (ICR, Artios) • cancer drug resistance and synthetic lethality (ICR, Artios). • experimental design
ADVERTISING DETAILS	
Project suitable for a student with a background in:	☒ Biological Sciences ☐ Physics or Engineering ☐ Chemistry ☐ Maths, Statistics or Epidemiology ☐ Computer Science ☐ Other (provide details)
Keywords:	1. cancer
	2.genomics
	3.drug sensitivity and resistance
	4.synthetic lethality
	5.POLQ
	6.ATR