The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS	
Project Title:	Understanding therapeutic responses in *BRCA* mutant and BRCAness triple negative breast cancer
Short Project Title:	**BRCAness in TNBC**

SUPERVISORY TEAM	
Primary Supervisor(s):	Prof. Christopher Lord and Prof. Andrew Tutt
Other supervisory team members:	Dr. Stephen Pettitt Prof. Nicholas Turner

DIVISIONAL AFFILIATION	
Primary Division:	Breast Cancer Research
Primary Team:	Gene Function

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Our experimental work originally identified the profound sensitivity of *BRCA1* or *BRCA2* mutant tumour cells to drugs that inhibit the DNA repair enzyme PARP1. This work eventually led to the development of a completely novel approach to treating cancer that exploits a "synthetic lethal" vulnerability in tumour cells based upon their ability to effectively repair specific types of DNA damage. To date, three different PARP1 inhibitors are now used in the treatment of ovarian cancer and PARP inhibitors are also showing promise in the treatment of *BRCA1/2* mutant triple negative breast cancer (TNBC). Our clinical work has also demonstrated that a subset of *BRCA1/2* TNBCs also show a favourable response to the commonly used chemotherapy, cisplatin. These clinical responses to cisplatin in TNBC are likely caused by the same DNA repair defect that causes PARP inhibitor sensitivity.

Despite these advances, many patients, especially those with advanced forms of the disease, eventually develop resistance to PARP inhibitors and cisplatin. Although we have identified some causes of drug resistance, in most patients, the precise cause of this effect is unknown. Furthermore, how one might use additional drugs to delay the development of PARP inhibitor/cisplatin resistance in patients is not known, nor are effective treatments for patients who have developed this form of drug resistance.

This project is aimed at addressing these issues. The PhD candidate will use cutting edge technology, such as the use of CRISPR-Cas9 perturbation screens and patient derived organoid systems to identify novel causes of drug resistance as well as investigating how combining different drugs could be used to delay or avoid drug resistance. Ultimately, the intention is to use the information that the PhD candidate generates to understand how people with cancer respond to treatment and to use this information to design novel ways to treat the disease, assessing these in clinical trials to be carried out with our hospital partner the Royal Marsden Hospital.

PROJECT AIMS

- Use CRISPR-Cas9 mutagenesis genetic screens and a computational approach to map, on a genome-wide level, the multiple molecular mechanisms that cause PARPi resistance in tumour cells with either *BRCA1/2* or *BRCAness* gene defects.

- To dissect how these molecular mechanisms operate.

- Using an analysis of patient-derived material, to assess whether mechanisms of PARPi resistance identified in genetic screens also operate in the clinical disease

- Using *in vitro* and *in vivo* model systems, to identify drug combination strategies that either delay or prevent the emergence of PARPi resistance

- To use all of the above analyses to inform the design of novel, mechanism-based, clinical trials aimed at targeting PARPi resistance.

RESEARCH PROPOSAL

The PhD candidate will use genetic approaches to address the following research questions:

- Which undiscovered causes of PARPi sensitivity and resistance exist?
- Do mechanisms of PARPi resistance differ depending upon the initial mechanism of drug sensitivity (e.g. *BRCA1, BRCA2, ATM, CDK12*, *PALB2* or *RAD51C,D* mutation)?
- Do additional targets exist that could re-sensitise PARPi resistant cells to PARPi?
- What additional genes are synthetic lethal with *BRCA1* or *BRCA2* mutations?

To address these questions, the PhD candidate will initially carry out a series of genetic perturbation screens (**year one**) and use the information gained from these screens to validate and mechanistically dissect novel mechanisms of therapy resistance (**years two and three**).

1. CRISPR-Cas9 mutagenesis genetic screens to map mechanisms that cause PARPi resistance (year one).

Recent reports, and our own pilot data, indicate that CRISPR-Cas9 (CRISPR) genetic screening provides a very effective approach to identifying mechanisms of drug response. This suggests that a reappraisal of PARPi sensitivity/resistance using a CRISPR-based approach could be informative. In year one, the PhD candidate will work with a core team of three technical staff and one bioinformatician to carry out a series of *in vitro* PARPi chemosensitivity/resistance genome-wide CRISPR screens in variety of TNBC-relevant model systems, including:

(i) human tumour cells with naturally occurring *BRCA1* or *BRCA2* mutations;
(ii) already-generated daughter clones derived from these tumour cells that have PARPi resistant-causing mutations in either *BRCA1, BRCA2, 53BP1, REV7* or *PARP1* (generated by CRISPR mutagenesis), and;
(iii) novel *BRCA1, BRCA2, RAD51C, RAD51D, ATM, CDK12* and *PALB2* defective cell lines derived from p53 mutant non-tumour epithelial HMLE3 cells

Parallel screens in these models (20 in total) will therefore generate a map of what might drive PARPi responses in a series of clinically relevant contexts, identifying shared mechanisms of resistance as well

as those effects specific to particular genotypes. In addition, the comparison of essential genes in *BRCA* mutant compared to *BRCA* wild type cell line models, will identify novel *BRCA*-synthetic lethal genes.

During this year the learning objectives for the PhD candidate will include:

- Techniques - Classical and high-throughput tissue culture
- Techniques - Classical molecular biology techniques: DNA isolation, cloning and manipulation, PCR, Sanger sequencing, western blotting
- Techniques - Deep sequencing analysis
- Techniques - Analysis of high-throughput cell based screens
- Theory skills – BRCA and PARP biology
- Research skills - Laboratory notebook keeping, scientific writing and presentation
- Research skills - Critical reading skills
- Research skills – Research ethics

Subsequent to this activity and the analysis of the screens, the PhD candidate will select one/two observations identified in the screens to analyse in greater detail in years two and three. Here we will select events that are seen in multiple screens, minimising the false positive rate and requirement for extensive post-screen validation. In general, this subsequent work will focus on three areas:

- Mechanistic dissection of PARPi resistance-causing mechanisms or mechanisms of *BRCA*-synthetic lethality
- Integrating functional genomics data with clinical response data to assess mechanisms of drug resistance
- Identifying drug combination approaches for minimising the impact of PARP inhibitor resistance

It is intended that the PhD candidate will follow one or potentially two of these subsequent research streams, with the relative focus being defined by the precise “hit” genes selected.

2. Mechanistic dissection of PARPi resistance (year two/three).

Known mechanisms of PARPi resistance include restoration of DNA repair by homologous recombination, restoration of DNA resection, stabilisation of replication fork stability or pharmacological mechanisms, including the upregulation of drug transporter pumps. The PhD candidate will use classical molecular biology approaches to assess whether novel mechanisms of PARPi resistance operate by any of these known processes. For example, using confocal and time lapse microscopy, the candidate will assess the localization of DNA repair proteins to the site of DNA damage in order to monitor DNA repair processes in cells with CRISPR-mediated mutations in “hit” genes. The candidate will also use classical biochemistry (e.g. protein immunoprecipitation) and more novel “split-GFP” based approaches to assess how “hit” proteins interact with and/or modulate proteins known to be involved in DNA repair or associated processes. If none of the known mechanisms of resistance explain the effects seen, the candidate will use unbiased approaches (e.g. synthetic rescue screens, generation of protein/protein interaction maps using mass-spec based proteomic profiling) to generate testable, mechanistic, hypotheses that could explain PARPi resistance.

3. Integrating functional genomics data with clinical response data to assess mechanisms of drug resistance

In addition to mechanistic insight, functional genomics can inform potential mechanisms of clinical PARPi resistance. Therefore, analysis of results from the screens in the context of available clinical data is important to ensure findings can be translated into patient benefit. The candidate will work with bioinformatics staff in the group and Breast Cancer Now core to obtain appropriate data and integrate this into the analysis of hits from the screen. This will include published outcome datasets from breast and ovarian cancer patients that included platinum treatment (e.g. Metabric, TCGA ovarian) as well as data available from sequencing of PARPi resistant tumours from investigator-led studies at ICR and collaborating institutes.

4. Identifying drug combination approaches for minimising the impact of PARP inhibitor resistance

A closely related clinical question, once mechanisms of drug resistance have been established, is how to design subsequent treatments to target any new vulnerabilities that the tumour cells have acquired as a result of the genetic changes leading to resistance. For example, for PARPi resistance that occurs via restoration of DNA resection could impart additional DNA repair-related vulnerabilities. The candidate will generate and test specific hypotheses based on published DNA repair literature and the resistance mechanisms in question. The candidate will also take an unbiased approach to investigate vulnerabilities in a high throughput screening format using our in-house drug libraries, screening patient derived organoid models of TNBC to identify therapeutic vulnerabilities. Where appropriate, hypotheses will also be tested *in vivo* using PDX models derived from resistant patients and/or xenograft or GEMM models of PARPi resistance.

During this work (2,3,4), the learning objectives for the PhD candidate will include:

- Techniques – Bioinformatics and Data Science approaches to integrate and analyse large datasets
- Techniques – Analysis of targeted capture sequencing and patient survival data
- Techniques – Use of statistics
- Techniques – DNA repair techniques including confocal microscopy, laser microirradiation studies
- Techniques – Protein biochemistry techniques, both classical and high-throughput
- Techniques – Drug synergy modelling
- Techniques – Design and use of animal models of cancer
- Research skills – pre-clinical translational research
- Research skills - Scientific writing and presentation
- Research skills – Grant and manuscript review

LITERATURE REFERENCES

1. Farmer, H., McCabe, N., Lord, C.J., Tutt, A. N., Johnson, D. A., Richardson, T. B., Santarosa, M., Dillon, K. J., Hickson, I., Knights, C., Martin, N. M., Jackson, S. P., Smith, G. C. & Ashworth, A. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. ***Nature*** 434, 917-921, (2005).

2. Lord, C.J. & Ashworth, A. The DNA damage response and cancer therapy. ***Nature*** 481, 287-294, (2012).

3. Edwards, S. L., Brough, R., Lord, C.J., Natrajan, R., Vatcheva, R., Levine, D. A., Boyd, J., Reis-Filho, J. S. & Ashworth, A. Resistance to therapy caused by intragenic deletion in BRCA2. ***Nature*** 451, 1111-1115, (2008).

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5. Lord, C.J. & Ashworth, A. BRCAness revisited. ***Nat Rev Cancer*** 16, 110-120, (2016).

6. Bajrami, I., Frankum, J. R., Konde, A., Miller, R. E., Rehman, F. L., Brough, R., Campbell, J., Sims, D., Rafiq, R., Hooper, S., Chen, L., Kozarewa, I., Assiotis, I., Fenwick, K., Natrajan, R., **Lord, C.J.** & Ashworth, A. Genome-wide profiling of genetic synthetic lethality identifies CDK12 as a novel determinant of PARP1/2 inhibitor sensitivity. ***Cancer Res*** 74, 287-297, (2014).

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8. Lord, C.J. & Ashworth, A. Mechanisms of resistance to therapies targeting BRCA-mutant cancers. ***Nat Med*** 19, 1381-1388, (2013).

9. Dréan, A., Williamson, C.T., Brough, R., Brandsma, I., Menon, M., Konde, A., Garcia-Murillas, I., Pemberton, H.N., Frankum, J. Rafiq, R., Badham, N., Campbell, J., Gulati, A., Turner, N.C., Pettitt, S., Ashworth, A. & Lord, C.J. Modelling therapy resistance in *BRCA1/2* mutant cancers. ***Mol. Cancer. Ther.*** (2017) In press.

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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. or equivalent in Biology, Biochemistry, Genetics, Medicine or other allied science

Intended learning outcomes:

- Broad experimental knowledge of cancer models and functional genomics techniques
- Understanding of the DNA damage response and techniques to investigate it
- Use and development of CRISPR-Cas9 technology
- Design of translational research projects
- Use of small molecule inhibitors as drugs and chemical probes
- Analysis of high throughput functional genomics data