The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL:

PROJECT DETAILS	
Project Title:	Investigation and characterisation of drug tolerant persister cells that arise in response to CHK1 and MPS1 inhibition

SUPERVISORY TEAM	
Primary Supervisor:	Olivia W Rossanese
Other members of the supervisory team:	Michael Bright and Jessica Downs

DIVISIONAL AFFILIATION	
Primary Division:	Cancer Therapeutics
Primary Team:	Target Evaluation and Molecular Therapeutics

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Drug resistance remains the key challenge to effective long-term cancer treatment. A number of mechanisms that lead to drug resistance have been reported, including increased drug efflux, mutations in the drug binding site of targets, and engagement of compensatory signalling pathways. More recently, it has become evident that the emergence of a subpopulation of drug-tolerant persister cells (DTPs) contributes to the generation of resistance [1]. DTPs are quiescent and arise in a number of cancer cell lines in response to a variety of cancer treatments. They are characterised by a reversible, altered chromatin state that allows for rapid adaptation to drug treatment. Importantly, emergence of DTPs does not preclude the development of drug resistance via other mechanisms; in fact, it has been demonstrated that DTPs give rise to cell lines with diverse mechanisms driving long-term resistance [2].

To date, investigation of DTPs has primarily been studied in response to treatment with tyrosine kinase inhibitors (erlotinib, lapatinib) and there are limited studies on the emergence of DTPs in response to combination therapy. This project aims to investigate the mechanisms driving emergence and progression of DTPs in response to treatment with CHK1 and MPS1 inhibitors, either alone or in clinically relevant combinations. CHK1 is a kinase involved in regulating the cell cycle and is a key mediator of the DNA damage response (DDR). MPS1 is a kinase involved in activation of the spindle assembly checkpoint (SAC) and is necessary in cancer cells with elevated levels of aneuploidy. Both CHK1 and MPS1 inhibitors are currently under investigation in the clinic for both single agent and combination trials. In this project, we will investigate the mechanisms leading to emergence and progression of DTPs in response to drug treatment, understand how those mechanisms vary in response to the specific drug used, and identify potential strategies for mitigating the emergence of drug resistance.

PROJECT AIMS

1. **Generate and characterise drug tolerant persister cells (DTPs) in response to CHK1 and MPS1 inhibitor treatment**

2. **Use pharmacologic approaches to identify small molecules / molecular targets that block the formation or progression of DTPs**
3. **Investigate the impact of combination therapy on the emergence and progression of DTPs**

RESEARCH PROPOSAL

We and others have demonstrated that treatment of cancer cell lines with high doses of small molecule therapeutics leads to the survival of a rare (0.3 – 5%) subpopulation of DTPs [1,3,4]. For example, we have isolated a population of A549 lung cancer cells that remains viable after 9 days treatment with a lethal dose (100X GI_{50}) of either gemcitabine, paclitaxel, or the CHK1 inhibitor tool compound CCT244747 [5]. Further, in the continued presence of drug, a subset of DTPs will resume proliferation and will demonstrate stable resistance to further drug treatment; these cells are termed drug tolerant expanded persisters (DTEPs) [1]. In previous studies, parental cells, DTPs, and DTEPs have proven distinct from one another, although altered chromatin states appear to play a role in generating the plasticity associated with DTPs [1,3].

We will examine whether agents that act via diverse mechanisms, such as inhibition of the cell cycle or disruption of the SAC, have unique pathways to the generation and progression of DTPs / DTEPs and investigate how this cellular plasticity contributes to long-term resistance.

Aim1: Generate and characterise drug tolerant persister cells (DTPs) in response to Chk1 and MPS1 inhibitor treatment. We will generate DTPs and DTEPs from cancer cell lines using high dose, long-term single agent treatment with Chk1 inhibitors, MPS1 inhibitors, gemcitabine, and paclitaxel. In the first instance, we will examine CHK1 inhibitors in non-small cell lung cancer cell lines and MPS1 inhibitors in triple negative breast cancer cell lines to align with current clinical hypotheses. Having obtained the relevant cell populations, we will use large scale –omics and hypothesis –driven experiments to characterise them, including:

- Gene expression and epigenetic profiling: we will use RNASeq and / or microarrays to determine gene expression changes among the cell populations to identify gene involved in the generation or maintenance of the altered cellular state. We will interrogate chromatin status, via measurement of histone and DNA modifications and ChIP-Seq, to determine epigenetic factors involved in the populations.
- Comparison of the growth rates of the populations, both in the absence and the presence of drug.
- Examination of cross-sensitivity of the populations to other inhibitors of the same target, inhibitors of other targets in the same pathway, and additional cytotoxics.
- Evaluation of the reversibility of the varying states by withdrawing drug at varying timepoints and monitoring the return of the population to a drug-sensitive state.

Results from these experiments will suggest genes and pathways that are involved in the transition between cell states. The relevance and mechanism of these genes will be examined using knockdown with RNAi / CRISPR or overexpression, as appropriate.

Aim 2: Use pharmacologic approaches to identify small molecules / molecular targets that block the formation or progression of DTPs. To facilitate the identification of proteins that, when inhibited, block the emergence or progression of DTPs / DTEPs, we will conduct a focused screen using the Cancer Therapeutics Unit Drugs & Tools small molecule library. The Drugs & Tools set is a collated collection of small molecules consisting of approved drugs, drug candidates, and probe molecules of known function. We will screen this library at 3 concentrations for the ability to inhibit the formation of DTPs (measured as no surviving cells at day 9) or the progression to DTEPs (measured as failure of colonies to grow out at day 33). Hits from this screen will be followed up with additional experiments using small molecules (concentration-response, timing, order of addition) and genetic ablation of

putative targets (RNAi / CRISPR), as appropriate.

Aim 3: Investigate the impact of combination therapy on the emergence and progression of DTPs

To date, most studies have looked at the emergence and progression of DTPs in response to an initial treatment with a single agent. While both Chk1 and MPS1 have potential as single agent therapeutics, there is mechanistic rationale for using them in combination. CHK1 inhibition potentiates the genotoxic effects of gemcitabine [5] while MPS1 inhibition is particularly effective in magnifying the effect of paclitaxel (ref); both these therapeutic hypotheses are under test in on-going clinical trials. We will examine how the use of combination strategies, including timing of treatment with the individual agents, impacts DTP formation and progression. As above, we will investigate how combination treatment affects time to emergence of DTPs, progression to DTEPs, plasticity of the cellular state, reversibility of the cellular state, and cross-sensitivity.

Drug tolerant persister cells have been identified in a number of cancer cell lines, in response to a variety of cancer therapeutics. This slow-growing subpopulation of cells can survive drug treatment long enough to support the emergence of cells with long-term drug resistance, obtained through a number of diverse mechanisms [2]. It is less clear whether DTPs, derived from treatment with diverse cancer therapeutics, arise through the same or unique mechanisms, depending on the drug treatment. Successful completion of the aims in this project are therefore expected to uncover:

- Specific information about mechanisms of resistance to CHK1 and MPS1 inhibitors that could inform clinical practice
- Common mechanisms of DTP formation and progression that could suggest strategies to abrogate plasticity and prevent resistance
- New targets for further drug discovery efforts to target resistance

LITERATURE REFERENCES

1. Sharma SV *et al* (2010). *A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations.* Cell 141(1):69-80
2. Ramirez M *et al* (2016). *Diverse drug-resistance mechanisms can emerge from drug-tolerant cancer persister cells.* Nat Comm. DOI: 10.1038/ncomms10690
3. Guler GD *et al* (2017). *Repression of stress-induced LINE-1 expression protects cancer cell subpopulations from lethal drug exposure.* Cancer Cell 32: 221-237
4. Hangauer MJ *et al* (2017). *Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition.* Nature 551: 247-250
5. Walton MI *et al* (2012). *CCT244747 is a novel potent and selective CHK1 inhibitor with oral efficacy alone and in combination with genotoxic anticancer drugs.* Clin Cancer Res. 18(20):5650-61
6. Maia AR *et al* (2015). *Inhibition of the spindle assembly checkpoint kinase TTK enhances the efficacy of docetaxel in a triple-negative breast cancer model.* Ann Oncol. 26(10):2180-92

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 or related
Intended learning outcomes: Please provide a bullet point list (maximum of seven) of the knowledge and skills you expect the student to have attained on completion of the	• Experience and training in modern cell and molecular biology techniques • In-depth knowledge of cancer biology, including an understanding of signalling pathways contributing to

project.	disease progression • Training and experience in the principles and application of cellular and molecular pharmacology • An understanding of the pathways leading to the emergence of resistance to clinically relevant anti-cancer agents • Familiarity with the principles and practice of modern drug discovery in oncology • Facility in formulating testable scientific hypotheses and planning & executing appropriate experiments to interrogate the hypotheses • Good scientific communication and presentation skills, including clear scientific writing skills.