EPSRC Centre for Doctoral Training (CDT) in Molecular Sciences for Medicine (MoSMed)

Development of DNA-PK-targeting PROTACs to maximise DNA-PK blockage in advanced prostate cancer

Newcastle University (Chemistry), Durham University

Supervisory Team

- **Dr Celine Cano (Lead), Newcastle University**
- **Dr Luke Gaughan, Newcastle University**
- **Professor Ehmke Pohl, Durham University**

Project Overview

Prostate cancer accounts for 375,000 deaths per year worldwide. Targeting the androgen receptor (AR) using hormone therapy is the mainstay treatment for advanced prostate cancer. Unfortunately, not all patients show durable responses; with many becoming resistant to radiotherapy or acquiring mechanisms that overcome AR blockade. Hence, there is an urgent need to provide more effective and durable treatments for metastatic disease.

In advanced prostate cancer, DNA-dependent protein kinase (DNA-PK) expression is elevated and correlates with metastatic spread.
The project has two main objectives:
1) to develop DNA-PKcs PROTACs to destabilise DNA-PKcs in prostate cancer;
2) to demonstrate unequivocal levels of DNA-PKcs inhibition in a range of *in vitro* assays across a range of prostate cancer cell lines and provide the means to evaluate DNA-PK degradation as a potential treatment in advanced prostate cancer.

Research Project

Background

Targeting the androgen receptor (AR) using hormone therapy is the main treatment for advanced prostate cancer and is often employed with radiotherapy in metastatic disease for both, local control in the prostate and also, targeting of oligometastic disease. Unfortunately, not all patients show durable responses; with many resistant to radiotherapy or acquiring mechanisms that overcome AR blockade. Hence, there is an urgent need to provide more effective and durable treatments for metastatic disease.

The DNA-dependent protein kinase catalytic subunit (DNA-PKcs) is a key component of the error-prone non-homologous end-joining pathway required to repair double-stranded DNA breaks. In advanced prostate cancer, DNA-PKcs expression is elevated and correlates with metastatic spread, and its kinase inhibition diminishes prostate cancer growth *in vitro* and *in vivo*. Importantly, the Newcastle/Oxford Team have demonstrated a kinase-independent pro-proliferative role of DNA-PKcs in prostate cancer which suggests that complete ablation of DNA-PKcs function would enhance anti-tumour efficacy.
These findings provide a robust rationale for developing DNA-PKcs PROTACs to destabilise DNA-PKcs in prostate cancer and afford maximal levels of DNA-PKcs inactivation; quelling both, kinase-dependent and -independent functions of DNA-PKcs for maximal treatment efficacy.

Research Plan

A proteolysis targeting chimeric molecule (PROTAC) will provide a proof of concept tool to test the hypothesis that DNA-PK can be targeted for degradation (Fig. 1).

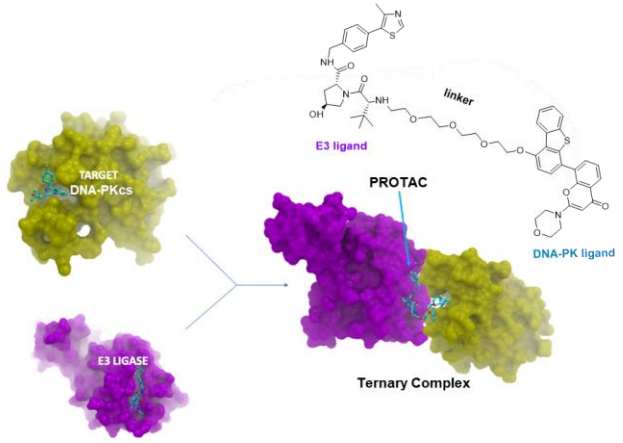

Fig. 1 Project strategy: design and synthesis of PROTAC degraders of DNA-PKcs to maximise DNA-PK blockage in prostate cancer cells.

The student will synthesise DNA-PK PROTACs that retain kinase domain binding capacity based on established DNA-PK inhibitors. These will comprise a ligand for an E3 ligase protein (Fig 2B) attached by an appropriate linker to a ligand for DNA-PK (Fig. 2A), including highly selective DNA-PK inhibitor NU5455 and AZD7648.

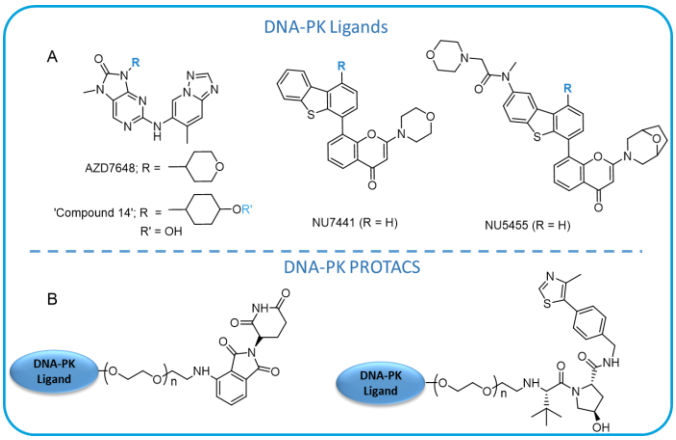

Fig. 2 A) DNA-PK inhibitors AZD7648 and 'Compound 14' (AstraZeneca inhibitors), NU7441 and NU5455 (Newcastle inhibitors), indicating attachment point for linker (in blue); B) PROTACS conjugated to cereblon (CRBN) and VHL ligands.

The initial PROTAC library will comprise a 'traditional' linker (alkyl, PEG, and extended glycol chains) to a ligand for DNA-PK (Fig. 2A) but we will also explore alternative linker strategies. Alkyne-containing linkers and heterocyclic scaffolds (e.g. piperazine/piperidines) will provide some rigidity but also, enable the modulation of the PROTAC physico-chemical properties in an attempt at reducing the gap between PROTACs and traditional drug-like chemical space. For example, introduction of an ionisable pyridine/di-piperidine motif has the potential to significantly improve aqueous solubility compared to parent PROTACs bearing hydrocarbon linkers. The triazole moiety also appears as an attractive replacement of traditional linkers. Triazole click chemistry will be used for the combinatorial PROTAC synthesis and rapid identification of anchor-linker-warhead combinations displaying optimal degradation efficiency.

The synthesised PROTACs will enter an immunofluorescent-based assay pipeline, utilising a prostate cancer cell line stably expressing a green fluorescent protein (GFP)-DNA-PKcs fusion protein, to test the capacity of each compound to destabilise DNA-PKcs by confocal microscopy (collaboration with Dr Gaughan, Newcastle Centre for Cancer). It is anticipated that functional DNA-PKcs PROTACs will diminish levels of intracellular GFP-DNA-PKcs while parent DNA-PKIs lacking the PROTAC component will not impact DNA-PKcs protein abundance. The most effective DNA-PKcs PROTACs, as determined by their capacity to degrade DNA-PKcs, will then enter a comprehensive validation pipeline involving *in vitro* efficacy testing across a spectrum of DNA-PKcs-expressing prostate cancer cell lines and iPSC-derived organoids in comparison to controls. Ultimately, we expect to develop DNA-PKcs PROTACs that have anti-tumour activity in multiple models of prostate cancer that are likely to be efficacious in other disease indications.

Training & Skills

The student will be based in a dynamic multidisciplinary drug discovery and translational research environment. The Newcastle Medicinal Chemistry Group is a fully integrated drug discovery group, consisting of 30 researchers. The group hosts regular group meetings to discuss progress, as well as medicinal and synthetic chemistry literature reviews. We also hold monthly multidisciplinary project reviews at which the student will be expected to present results to colleagues in Biosciences. Our laboratories house state of the art equipment, including a dedicated 500MHz NMR spectrometer, modern microwave synthesisers, automated chromatography, preparative HPLC and an Agilent 6550 iFunnel QTOF mass spectrometer.
Each year, the PhD student will be able to undertake short (circa 2 months) placements within the biology labs (Dr Gaughan, Newcastle Centre for Cancer) to provide further multidisciplinary training.

Further Information

Please contact the lead supervisor for project enquiries:
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How to Apply

Newcastle University · Durham University · UKRI Engineering and Physical Sciences Research Council

If applying to a **Newcastle project**, you must apply through the University's **Apply to Newcastle Portal**. Once registered select **'Create a Postgraduate Application'.**

Use 'Course Search' to identify your programme of study:

- search for the 'Course Title' using the programme code: **8207F**
- select **'PhD Molecular Sciences for Medicine (SNES)'** as the programme of study

You will then need to provide the following information in the 'Further Questions' section:

- a 'Personal Statement' (this is a mandatory field) - upload a document or write a statement directly into the application form. Please include the full title of the studentship, the studentship code, and how your interests and experience relate to the project.
- the relevant studentship code **(mos23_04)** in the 'Studentship/Partnership Reference' field. If you wish to apply for additional studentships, please make sure to add the relevant studentship reference each time, before submitting each separate application. For example, you may wish to apply for mos23_03 AND mos23_04. **You must include the relevant code for your application to be considered.**
- when prompted for how you are providing your research proposal - select 'Write Proposal'. You should then type in the title of the relevant research project. You do not need to upload a research proposal.
- An up to date CV.
- Please upload all documents in PDF format.

Equality, Diversity and Inclusion (EDI)
Within the MoSMed CDT we are committed to building a diverse community based on excellence and commitment. To that end in our recruitment of Doctoral Researchers we welcome applications from outstanding candidates of all backgrounds regardless of ethnicity, disability, gender identity, sexual orientation and will consider all applications equally based on merit.

Should you have any queries regarding the MoSMed application process to Newcastle University please contact Craig Hinds, the MoSMed CDT Manager: **mosmed.cdt@newcastle.ac.uk**

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