PhD Project Proposal

Funder details

Studentship funded by:	MRC DTP

Project details

Project title:	Overcoming oncogenic androgen receptor signalling to transform the care of lethal prostate cancer

Supervisory team

Primary Supervisor:	Dr. Adam Sharp
Associate Supervisor(s):	Prof. Joanna Loizou, Dr. Jimenez-Vacas
Secondary Supervisor:	Prof. Johann de Bono

Divisional affiliation

Primary Division:	Clinical Studies
Primary Team:	Translational Therapeutics Team
Site:	Institute of Cancer Research; Sutton

Project background

Prostate cancer (PCa) is a leading cause of male cancer-related death globally (1). The androgen receptor (AR) remains the main therapeutic target for lethal PCa, and although AR pathway inhibitors (ARPIs) remain the cornerstone of treatment, resistance is inevitable and advanced PCa remains fatal (2). We, and others, have shown that most ARPI resistant castration-resistant prostate cancers (CRPCs) have ongoing addiction to AR signalling (3-7). This can be driven by AR amplifications, AR activating point mutations and constitutively active AR splice variants (3, 6). **Critically, all currently available ARPIs have limited anti-tumour activity against these mechanisms of resistance and innovative therapeutic strategies that overcome persistent AR signalling are urgently required.**

We have identified that targeting a specific phosphatase (not named for confidentiality; referred to as phosphatase throughout) represents an attractive approach to overcome persistent AR signalling. We have shown that genomic knockdown of the phosphatase preferentially inhibits the growth of AR-driven cell lines when compared to AR-independent cell lines. Next, we have demonstrated that genomic knockdown of the phosphatase dramatically decreases PSA levels, ablating AR signalling, suggesting that this phosphatase is a novel critical regulator of AR signalling. Critically, genomic knockdown of the phosphatase reduced the growth of 22Rv1 PCa cell lines that are resistant to FDA-approved ARPIs, and novel ARPIs being clinically evaluated, suggesting that targeting the phosphatase will provide greater patient benefit than current therapeutic strategies in development. Furthermore, we have demonstrated preferential expression of this phosphatase in cells of prostate lineage providing clear evidence that therapies targeting this phosphatase will deliver PCa specific effects and spare normal tissues. Finally, the phosphatase is predicted to be both degradable and ligandable confirming its tractability and supporting rapid clinical translation of this approach (8). **Taken together, targeting this phosphatase, identifies an innovative tractable therapeutic target to completely ablate persistent AR signalling, through a novel mechanism of**

action, to deliver cancer specific kill to tumours from patients with limited treatment options, helping them live longer and better lives.

Project aims

Overarching hypothesis
Identification of a specific phosphatase as a therapeutic target to inhibit persistent AR signalling through a novel mechanism of action will support the development of an innovative treatment that delivers robust anti-cancer activity in ARPI resistant tumours to improve the outcome for patients with lethal PCa.

Project aims

1) We will evaluate the RNA and protein expression of the phosphatase, and develop and quantify phosphatase-specific activity signatures, across multiple PCa patient cohorts, to demonstrate that levels/activity signatures of the phosphatase are higher in prostate tumours, compared to other advanced cancers, and that they associate with persistent AR signalling to drive resistance to current standard of care treatments and worse clinical outcome for men suffering from lethal PCa.
2) We will utilise clinically relevant PCa patient-derived and cell line models to confirm that the phosphatase is essential for the growth of lethal PCas with persistent androgen receptor signalling *in vitro*, determining the mechanism through which the phosphatase regulates AR signalling, to support that targeting the phosphatase represents an attractive therapeutic strategy for lethal PCa.
3) We will elucidate predictive biomarkers, that will include expression of the phosphatase, to identify advanced PCa that respond robustly to targeting the phosphatase, confirming these findings in PCa patient-derived and cell line models *in vivo* to support the clinical development of therapeutic strategies targeting the phosphatase in lethal PCa.

Research proposal

Aim 1
Clinical qualification of phosphatase RNA expression and novel phosphatase activity signatures in pan-cancer and PCa patient whole-exome and transcriptome cohorts. Firstly, following genomic knockdown/out (inducible small hairpin RNA (shRNA) and/or CRISPR/Cas9) of the phosphatase in multiple PCa cell line models we will perform RNA-seq and develop novel phosphatase activity signatures (developed in Aim 2). Following which, we will utilise our established institutional and publicly available pan-cancer and localised/lethal PCa patient cohorts to interrogate the clinical relevance of phosphatase RNA expression and activity signatures confirming that phosphatase RNA levels/activity signatures demonstrate prostate tumour/tissue specificity and associate with AR expression/signalling signatures. Following this, we will focus on phosphatase RNA expression and activity signatures in localized and lethal PCa cohorts to determine the association with: **(1)** the development of castration resistance, **(2)** common PCa molecular subtypes, **(3)** gene expression and signalling pathways including AR signalling signatures, and **(4)** clinical outcome data. ***Clinical qualification of phosphatase protein expression in pan-cancer and PCa patient tissue biopsy cohorts.*** We will utilise established tissue biopsy cohorts to provide further insights into the clinical significance of phosphatase protein expression in prostate and other cancers. Having analytically validated an immunohistochemistry (IHC) assay that specifically detects the phosphatase protein expression in tissue we will confirm that the phosphatase protein levels are higher, and specific to, prostate tumours when compared to other commonly occurring cancers. Following this, we will evaluate the association between the phosphatase at protein level and those characteristics described above (for RNA analyses).

Aim 2
Development of PCa phosphatase activity signatures. Genes consistently upregulated upon phosphatase overexpression (inducible plasmids) and downregulated upon knockdown (KD; small interfering RNAs (siRNAs) and/or inducible shRNAs) in PCa cell line models will be selected to generate a phosphatase-Activated signature. Conversely, genes downregulated upon phosphatase overexpression and upregulated upon KD will define a phosphatase-Repressed signature. These signatures will be utilised in Aim 1. ***Molecular characterisation of our PCa patient-derived and cell line models.*** We will quantify phosphatase RNA (RNA-seq), protein (western blot, WB/IHC), and activity signatures, in all our models and compare this to AR expression/signalling signatures to further validate our clinical studies in Aim 1. ***Validation of the phosphatase as a critical factor for the growth of PCa models with persistent AR signalling.*** We will expand our preliminary data and assess the impact of phosphatase KD (siRNAs and inducible shRNAs) on cell growth (CellTiter-Glo) in PCa cell lines and patient-derived xenograft-organoids (PDX-Os), both with and without persistent AR signalling. We will also address the mechanism

of cell growth inhibition, analysing cell cycle arrest (fluorescence activated cell sorting, FACS and quantitative polymerase chain reaction, qPCR/WB), apoptosis induction (Caspase-Glo 3/7 and qPCR/WB) and senescence (β-Galactosidase detection, and qPCR/WB). ***Comparison of the effectiveness of phosphatase KD and ARPIs in PCa models with persistent AR signalling.*** We will assess the impact of phosphatase KD in comparison to FDA-approved ARPIs and those in clinical development by evaluating RNA and protein levels of AR-FL, AR-V7, and AR-regulated genes (qPCR and WB), as well as cell growth (CellTiter-Glo) and mechanism of cell growth inhibition in PCa cell lines, PDX-Os, and LuCaP 2D models, with high phosphatase expression and persistent AR signalling. ***Confirmation that phosphatase controls AR activity.*** We will perform RNA-seq following phosphatase KD in PCa cell lines with persistent AR signalling, analysing AR signalling signatures. Additionally, we will assess whether phosphatase KD affects the transactivation of a PSA ARE3-driven luciferase reporter in AR-negative PC3 cells transfected with AR-FL or AR-V7. **Determination of the molecular mechanism underlying AR regulation by phosphatase**. We will analyse the effects of phosphatase KD (in AR positive PCa cell line models with high levels of phosphatase) on: **(1)** AR localisation (Immunofluorescence, IF/IHC and cytoplasmic vs. nuclear fractionation), **(2)** AR chromatin binding and transcriptional activity efficiency (self-transcribing active regulatory region sequencing, chromatin immunoprecipitation (ChIP) sequencing and HiChIP), **(3)** phosphatase interactome (phosphatase co-immunoprecipitation/mass spectrometry), and **(4)** phosphatase-dependent phosphoproteome (phosphoproteomics).

Aim 3

Identification of genomic aberrations modulating PCa model response to phosphatase KD. We will first assess the response to phosphatase KD in a panel of PCa cell lines, PDX-O, and LuCaP 2D models, representing the most common CRPC genomic aberrations. Next, we will perform a genome-wide CRISPR/Cas9 dropout screen in PCa cell line models with high phosphatase levels and persistent AR signalling to: **(1)** cross-validate the genomic aberrations influencing the response to phosphatase KD identified in our LuCaP 2D and PDX-O panel, **(2)** identify genomic alterations affecting phosphatase KD response that are not represented in our set of PCa cell lines, PDX-O, and LuCaP 2D models, **(3)** discover potential synergistic therapeutic combinations by identifying genes whose loss/KO enhances the antiproliferative effects of phosphatase KD and for which targeted drugs are available. ***Validation of biomarkers predicting response to phosphatase KD.*** We will first develop novel PCa cell line models that harbour genomic aberrations identified as predictive biomarkers for response to phosphatase KD. To achieve this, we will generate long-term KD (stable shRNAs) and/or KO (CRISPR/Cas9) models using PCa cell lines with high phosphatase levels and persistent AR signalling. Subsequently, we will evaluate the effects of phosphatase KD on cell viability (CellTiter-Glo) and AR signalling (qPCR and WB analysis of AR-regulated genes) in these genetically modified PCa cell lines, aiming to assess whether phosphatase KD is more effective in the presence of these aberrations, thereby validating their predictive role. ***In vivo validation of the most promising single agent predictive biomarker approach.*** The most promising predictive biomarkers will be validated *in vivo* using our PDX models with high phosphatase levels, persistent AR signalling, and the identified genomic aberrations influencing phosphatase KD response. Once the tumours are established, phosphatase will be silenced with doxycycline treatment (inducible shRNAs). If these models are unavailable, PCa cell lines harbouring the relevant genomic aberrations (or genetically modified to harbour them) will be used. *In vivo* antitumor efficacy will be assessed through tumour growth measurement, and pharmacodynamic modulation will be evaluated via qPCR/RNA-seq and WB/IHC.

Literature references

1. Culp MB, Soerjomataram I, Efstathiou JA, Bray F, Jemal A. Recent Global Patterns in Prostate Cancer Incidence and Mortality Rates. Eur Urol. 2020;77(1):38-52.
2. Westaby D, Fenor de La Maza MLD, Paschalis A, Jimenez-Vacas JM, Welti J, de Bono J, Sharp A. A New Old Target: Androgen Receptor Signaling and Advanced Prostate Cancer. Annu Rev Pharmacol Toxicol. 2022;62:131-53.
3. Armstrong AJ, Halabi S, Luo J, Nanus DM, Giannakakou P, Szmulewitz RZ, et al. Prospective Multicenter Validation of Androgen Receptor Splice Variant 7 and Hormone Therapy Resistance in High-Risk Castration-Resistant Prostate Cancer: The PROPHECY Study. J Clin Oncol. 2019;37(13):1120-9.
4. Guo C, Sharp A, Gurel B, Crespo M, Figueiredo I, Jain S, et al. Targeting myeloid chemotaxis to reverse prostate cancer therapy resistance. Nature. 2023;623(7989):1053-61.
5. Pernigoni N, Zagato E, Calcinotto A, Troiani M, Mestre RP, Cali B, et al. Commensal bacteria promote endocrine resistance in prostate cancer through androgen biosynthesis. Science. 2021;374(6564):216-24.
6. Sharp A, Coleman I, Yuan W, Sprenger C, Dolling D, Rodrigues DN, et al. Androgen receptor splice variant-7 expression emerges with castration resistance in prostate cancer. J Clin Invest. 2019;129(1):192-208.
7. Welti J, Sharp A, Brooks N, Yuan W, McNair C, Chand SN, et al. Targeting the p300/CBP Axis in Lethal Prostate Cancer. Cancer Discov. 2021;11(5):1118-37.

8. Mitsopoulos C, Di Micco P, Fernandez EV, Dolciami D, Holt E, Mica IL, et al. canSAR: update to the cancer translational research and drug discovery knowledgebase. Nucleic Acids Res. 2021;49(D1):D1074-D82.

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:	First/2:1 in biological/medical sciences or related subject area
Intended learning outcomes:	Knowledge: Prostate cancer biology and medicine Knowledge: AR and calcium signalling/phosphatase biology Skills: Interdisciplinary working/skills Skills: Bioinformatics and clinical analyses Skills: Immunohistochemistry/immunofluorescence Skills: Patient-derived models Skills: Molecular biology

Advertising details

Project suitable for a student with a background in:

- ☒ Biological Sciences
- ☐ Physics or Engineering
- ☐ Chemistry
- ☐ Maths, Statistics or Epidemiology
- ☐ Computer Science