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# MODELS OF ADVANCED PROSTATE CANCER


Group Leader

Imran Ahmad

CRUK Clinician Scientist
Clinical Senior Lecturer
(University of Glasgow)
Consultant Urological Surgeon
(NHS Greater Glasgow & Clyde)

Research Scientists
Amy Tibbo
Richa Vasan

Graduate Student
Andrew Hartley[1]

[1]CRUK Glasgow Centre


Prostate cancer is a leading cause of cancer mortality in men in the Western world. Identifying and understanding the pathways that drive advanced and treatment-resistant prostate cancer will provide important information that will allow prognostication and individualised patient treatments.

Our current research interest lies in understanding the mechanisms of treatment resistance in advanced prostate cancer. Work in our lab together with Hing Leung's group uses state-of-the-art *in vivo* models in conjunction with patient samples to interrogate the disease processes in advanced and treatment-resistant prostate cancer. This work will help to provide information on drivers of prostate cancer progression and to identify novel biomarkers of disease and/or drug targets to treat the disease.

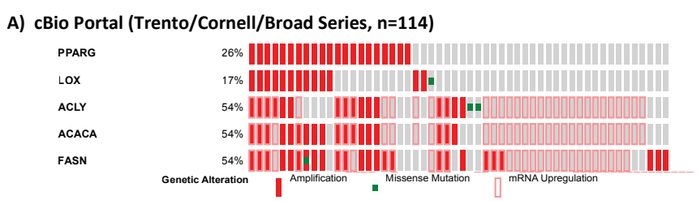
As an Honorary Consultant Urological Surgeon based at the Queen Elizabeth University Hospital in Glasgow, I have one of the highest-volume robotic prostatectomy practices in the UK for patients with aggressive and locally advanced prostate cancer, allowing me to keep my translational research clinically relevant.

## Sleeping Beauty screen reveals Pparγ activation in metastatic prostate cancer

Using a murine forward mutagenesis screen (Sleeping Beauty) in a PtenNull background, we were able to identify the gene peroxisome proliferator-activated receptor gamma (Pparγ, which encodes a ligand-activated transcription factor), as a promoter of metastatic prostate cancer. PPARγ is a critical regulator of fatty acid and glucose metabolism, influencing lipid uptake and adipogenesis. In our model, upregulation of PPARγ was associated with an activation of lipid signalling pathways, including upregulation of lipid synthesis enzymes (fatty acid synthase (FASN), acetyl-CoA carboxylase (ACC) and ATP citrate lyase (ACLY)), resulting in aggressive prostate cancer.

As a proof of principle, we were able to demonstrate that inhibition of PPARγ suppressed tumour growth *in vivo*, with downregulation of the lipid synthesis programme. We showed that elevated levels of PPARγ strongly correlated with elevation of FASN in human prostate cancer and that high levels of PPARγ/FASN and PI3K/pAKT pathway activation conferred a poor prognosis, with these patients succumbing to their disease up to five years earlier.

Figure 1
Data from cBio portal (www.cbioportal.org) demonstrating PPARγ gene amplification or its upregulated mRNA expression in 26% of clinical castrate-resistant prostate cancer specimens, with upregulation of one or more of the lipid synthesis genes (FASN, ACC, ACLY).




Our data suggested that prostate cancer patients could be stratified in terms of PPARγ/FASN and PTEN levels to identify patients with aggressive prostate cancer who might respond favourably to PPARγ/FASN inhibition (low PTEN/high pAKT expression); a finding that has potential to guide the design of future clinical trials. Ongoing research by our group has demonstrated that this lipid synthesis phenotype might be driven through alterations in mitochondrial function and AKT3 activations.

In addition, to our knowledge, we were the first to demonstrate the strength of the Sleeping Beauty transposon model system in successfully determining low-frequency somatic mutations that might drive prostate tumorigenesis. We are further investigating and validating other novel and clinically relevant 'hits' from this screen. (Ahmad *et al., PNAS 2016*; 113 (29) 8290-829; Galbraith *et al., Oncogene*. 2021;40:2355-66; Hartley *et al., BJC*. 2023; 128:940-945))

## Identification and validation of new therapeutic targets in castrate-resistant prostate cancer

Androgen receptor aside, current treatment for advanced prostate cancer remains non-targeted. The development of targeted therapies has been hampered by a paucity of genes and pathways identified to be responsible for prostate cancer progression.

We aim to identify novel genes and pathways in castrate- and enzalutamide-resistant prostate cancer (CRPC and ERPC, respectively). We are using an unbiased insertional transposon mutagenesis screen (PiggyBac) and then validating the top genes of interest in patient-derived samples. Validating these genes in mice and humans will allow us to discover new pathways that can be targeted in patients with CRPC and ERPC.

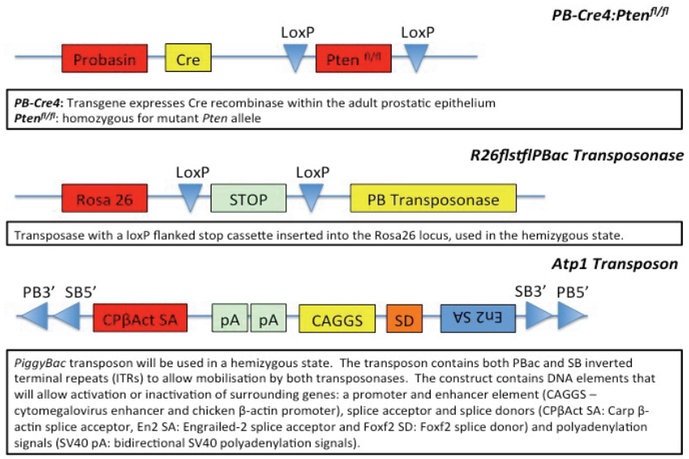



Figure 2
Genetic modifications of the PiggyBac mice.

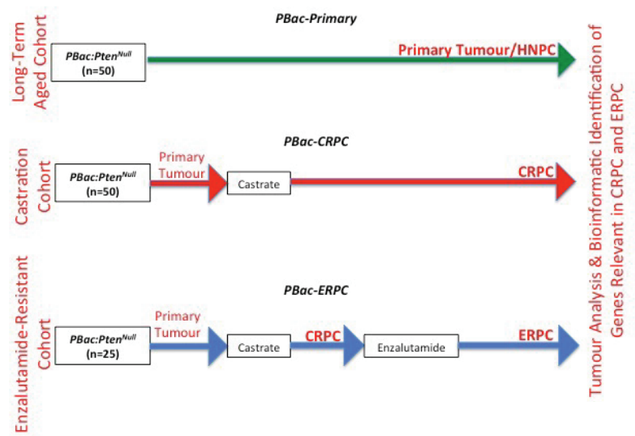



Figure 3
Experimental design for the ageing, castration and enzalutamide-treatment of the PiggyBac (PBac) mice.

## Role of Arid1a in prostate cancer

ARID1A was also identified as a potential driver in prostate cancer by the Sleeping Beauty screen. ARID1A is part of the BAF complex, and functions as a key regulator controlling DNA accessibility and organisation by chromatin remodelling. The BAF complex itself is highly mutated in metastatic prostate cancer. Including mRNA alterations, the BAF complex is mutated in 60-70% of metastatic prostate cancer cases (Figure 4). The potential for therapeutically targeting the BAF complex in prostate cancer was reviewed in our recent publication. (Hartley *et al., Expert Opin Drug Discov.* 2021; 16:173-181)

## Role of MBPTS2 in prostate cancer

Membrane-bound transcription factor site-2 protease *(Mbtps2)* was also identified from our Sleeping Beauty screen and demonstrated to be associated with metastatic prostate cancer *in vivo*. Regulated intramembrane proteolysis (RIP) plays an integral role in maintaining multiple cellular pathways. The most well described RIP pathway is carried out by serine proteases, S1P (site-1 protease) and MBTPS2. The sequential cleavage of membrane spanning proteins results in the release of a mature N-terminal fragments that can shuttle to the nucleus and function as transcription factors. Among reported S1P and MBTPS2 targets are the sterol regulatory element binding proteins (SREBPs) and the activating transcription factor 6 (ATF6).

Our group has been working on characterising its role in cholesterol uptake and synthesis along with regulation of fatty acid synthesis in metastatic prostate cancer.

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Figure 4
Mutations in the BAF complex in metastatic prostate cancer