

Patient-Derived Prostate Cancer: from Basic Science to the Clinic

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Abstract Systems that model cancer form the backbone of research discovery, and their accuracy and validity are a key determinant to ensure successful translation. In many tumour types, patient-derived specimens are an important model of choice for pre-clinical drug development. In this review, we consider why this has been such a challenge for prostate cancer, resulting in relatively few patient-derived xenografts (PDXs) of prostatic tumours compared to breast cancers, for example. Nevertheless, with only a few patient specimens and PDXs, we exemplify in three vignettes how important new clinical insights were obtained resulting in benefit for future men with prostate cancer.

While the overall universal goal is to cure cancer, the more immediate short-term objective is to control the disease, hinder progression and facilitate remission. Central to this approach is the imperative to reduce the high rate of failure of translating cancer discoveries to the clinic and patient benefit. As scientists, we strive for our discoveries in the laboratory to have an impact on patient management and care, but the pathway to realise this is long and the failure rate is high. Nevertheless, for solid tumours, and particularly hormone-sensitive tumours such as prostate cancer, the rate of development of new

therapies has closely aligned with discoveries in the laboratory. For example, recent research studies demonstrated that the androgen receptor (AR) remains the key driver of prostate cancer, even in advanced castrate-resistant disease (CRPC) [1, 2]. Consequently, targeting androgen biosynthesis and/or binding of ligand to the AR remains key to tumour control, with newer drugs such as abiraterone and enzalutamide becoming the standard of care for treatment of CRPC [3, 4]. In many laboratories, research is now focussing on understanding the mechanisms of resistance that develop to these second-generation AR targeting agents, to ultimately enable further control of tumour growth.

Model systems form the backbone of research discovery, with their accuracy and validity being a key determinant to ensure successful translation [5]. In many tumour types, patient-derived specimens are an important model of choice during pre-clinical drug development. However, there is great diversity in the models that are based on human specimens, including cell lines, spheroids and xenografts. Although cell lines provide a high-throughput system for testing, many cell lines, e.g. PC3, LNCaP and derivatives thereof, have been in circulation for years or decades, adapting and changing to their continual growth on plastic in laboratory cultures. These cell lines also do not reflect the biology of specimens available from men receiving contemporary treatments, such as enzalutamide or abiraterone, limiting their suitability to study mechanisms of resistance. Nowadays, we recognise that cell lines such as these only provide a crude means to screen potential agents of promise. Consequently, the development of procedures to make spheroids or organoids from human specimens has garnered enthusiasm over recent years and this effort is a significant advance to the field of pre-clinical testing and the reader is directed to reviews on this method [6, 7].

The method of xenografting is a more conservative approach for which there has been a resurgence of interest. Although patient-derived xenografts (PDXs) are more time

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consuming and laborious with less throughput than other models, they provide significant advantages due to their inherent ability to accurately recapitulate the tumour of origin. As a result, there has been a concerted worldwide effort to establish banks of PDXs and members of consortia have built collections of these models of human cancer for pre-clinical studies [8–10] (see www.europdx.eu project website; [10]). Whilst successful for hormone-dependent cancers such as breast cancer, PDXs from prostate cancer patients have been more difficult. Indeed, the minimal number of prostate cancer PDXs available in biobanks is particularly notable (e.g. the entire JAX labs PDX catalogue contains only 6 prostate cancer PDXs [of 463 total, equaling ~1 % of total]). The paucity of prostate cancer PDXs, however, is not surprising, primarily due to the well-known challenges of establishing them [11].

This has been a substantial roadblock to the field [11], not only in the development of PDXs of prostate cancer but also for organoid cultures, and hence, these methodologies have lagged behind other tumour types. The reasons for this delay are many and varied and include several key factors (see summary in Table 1).

Most men are diagnosed with localised disease, and some patients with tumours that have a low risk of progressing opt for treatment (versus active surveillance). The localised tumours removed at radical prostatectomy are taken to the laboratory to establish PDX, but it is not surprising that the take rate is low given that many of these tumours are *slow growing*, with typical tissue proliferation rates of 2–10 % in our hands. Hence, few laboratories can successfully establish PDX with low/moderate-grade localised tumour specimens. In part, we were able to improve the take rate for localised specimens by *including stroma* and grafting to the kidney capsule (a technique many of us learnt whilst training in the Cunha laboratories and routinely used by other leaders in PDXs, e.g. YZ Wang [15–17]). Other groups have used the orthotopic *site for xenografting* with success [18, 19]. Our standard protocols were summarised recently but are constantly updated [20]. For example, we incorporated the approach of Peehl and others to include the use of precision

slices for PDXs [21]. There are several advantages to this, including the opportunity to systematically engraft and fix alternate slices, so that pathologies in the engrafted specimens can be accurately recorded and examined.

For advanced or metastatic tumours, the issue of slow growth rate is not as limiting. However, even for these samples, the take rate is still only 25–30 % [22]. The sources of tissue vary considerably with this subsequently affecting the quality of the specimens used for engraftment. However, even the poor specimens obtained at TURP can be grafted sub-renally with moderate success [23]. Dr. Robert Vessella's group at the University of Washington was one of the first to establish serially transplantable PDX models from prostate cancer metastases, namely the LuCaP series [24]. LuCaP 23.1 and LuCaP 35 were lymph node metastases established at the sub-cutaneous xenografts, with tumour doubling times of 11 and 18 days and take rates of 100 and 87 % [25, 26]. When injected into the tibia of SCID mice, LuCaP 23.1 produces osteoblastic reactions, whereas LuCaP 35 produces osteolytic responses [27]. LuCaP PDXs have also been passaged through spheroid cultures and back into mice, whilst faithfully retaining the original phenotype [28, 29]. Prostate cancer bone metastases offer another potential source of tissue for prostate cancer PDXs. Two particular lines, MDA PCa 118a and MDA PCa 118b, were established as sub-cutaneous PDXs, providing a unique model of androgen-independent prostate cancer that induces a robust osteoblastic reaction in bone-like matrix and soft tissue [30]. Much more progress has been achieved through the recent utilisation of warm autopsy specimens, although the take rate from post-mortem specimens is less than that of palliative metastatic lesions, such as those used by the Vessella group [22]. In Australia, an oncology collaboration established a warm autopsy programme to collect specimens from men who failed contemporary treatments. Called CASCADE (*Cancer tissue collection after death*), this is a carefully crafted programme of autopsy of cancer patients, involving senior pathologists, palliative care, medical oncologists, familial cancer clinicians and scientists [22]. The

Table 1 Summary of some of the challenges of the prostate PDX cancer model system

Problem	Solution	References
Slow proliferative activity and lack of transplantability	Select more rapidly proliferating tumours, e.g. high Gleason grade, mCRPC.	[12–14]
Low testosterone levels in host mice when tumours are hormone responsive	Supplement with testosterone implants.	[13]
Tissue pathology is not uniform and includes benign glands.	Use precision-cut tissue slices to establish pathology in adjacent alternate grafted slices.	
Lack of stroma or microenvironment	Add mouse stroma at initial engraftment.	[15]
Site of engraftment	Commence engraftment at sub-renal site then transfer to sub-cutaneous site.	[11, 15, 16]

resource provides an exceptional opportunity to generate pre-clinical models for functional testing of tissues from patients who become treatment resistant, resulting in death.

In summary, in order to overcome the hurdles of growing human prostate cancer PDXs, we adapted protocols that have improved our ability to engraft prostate cancer specimens from different stages of disease progression. Our protocols allow us to engraft most tumour types with reasonable success although there is always room for improvements. The challenges and limitations that remain are those inherent to the PDX system, including the lack of immunological contribution because current protocols are conducted in immune-compromised host mice. The humanisation of our models will be necessary to address this obstacle to our knowledge. Other barriers include the use of single passage PDXs and the need to develop reliable methods to enable the PDX to be serially passaged, providing an enduring resource for research. Although this is possible for metastatic lesions, it is far less common for localised specimens. As improvements are made to overcome these challenges, it is important that investigators provide good analyses of their take rates. Reporting the take rate should include the efficiency of engrafting per patient and per graft/specimen, and will ensure better accuracy and reproducibility across different groups.

Despite these limitations to the grafting of prostate cancer specimens as PDXs, significant discoveries from these methods have provided important new clinical insights. As we now discuss, the potential for discovery remains exciting and rewarding as we exemplify in three vignettes.

Identification of High-Risk Features in Familial Prostate Cancers

It is estimated that 40 % of prostate cancers are inherited and influenced by germline genetics. Mutations in high-penetrant genes such as *BRCA2* significantly increase the risk of developing prostate cancer as well as having aggressive rather than indolent disease [31]. Whilst *BRCA2* mutation carriers often present with normal diagnostic features, they typically show an aggressive clinical course with higher tumour grades and distant metastases, with poor overall and prostate cancer-specific survival [31]. Using our optimised PDX approach to engraft localised prostate cancer specimens, we examined the biology of prostate cancers from men with germline *BRCA2* mutations. Only four patients were used, but the phenotype of *BRCA2* PDXs was consistent and strikingly different to age- and stage-matched sporadic prostate cancer cases. In particular, in *BRCA2* PDXs, we observed a specific and predominant pathology known as intraductal carcinoma of the prostate (IDC-P) [32]. IDC-P is a distinct pathological entity that is

associated with an aggressive prostate cancer phenotype that predicts poor treatment response [33, 34]. Arising from this scientific observation in the PDXs, a retrospective analysis of clinical biopsies and radical specimens was conducted on a larger cohort of men for whom there was follow-up and data on 10-year survival. The pathology reports determined the absence or presence of IDC-P in *BRCA2* mutation carriers, and the association with patient outcome (survival) was calculated. The analyses showed that a combination of *BRCA2* mutation and the presence of IDC-P is a strong prognostic factor for aggressive prostate cancer; the hazard ratio is 16.9. Put simply, the presence of IDC-P in *BRCA2* mutation carriers makes them 16.9× more likely to fail to survive, compared to those without any detectable IDC-P. There are two fundamental messages from this new information. Firstly, the original observations, derived from PDX studies, were made using only four patients and, secondly, this was sufficient to enable testing of the hypothesis that IDC-P may predict sub-groups of *BRCA2* mutation carriers who had aggressive disease. Altogether, the results are potentially practice changing, because a simple change to the pathology reporting might allow the treating urologists to identify patients with familial prostate cancer who are at greater risk of progressing and are likely to benefit from earlier and multi-modal treatment of their prostate cancer.

Androgen Deprivation Therapy (ADT) Induces Neuroendocrine Differentiation

In addition to IDC-P, other pathologies have been linked to poor outcome from prostate cancer, including neuroendocrine carcinoma. Research of neuroendocrine prostate cancer has been particularly hampered by a lack of clinically relevant *in vivo* models. One laboratory dedicated to improving models of prostate cancer is YZ Wang's at BC Cancer Agency, Vancouver. The team has established a Living Tumor Laboratory (<http://www.livingtumorcentre.com>; [35, 36]) that holds more than 200 transplantable human cancer tissue lines in SCID mice from a wide range of primary tumours. Of these, 45 lines represent prostate adenocarcinoma, neuroendocrine carcinoma and CRPC. A recent report described the first-in-field PDX model of complete neuroendocrine transdifferentiation of prostate adenocarcinoma [37]. This is a unique model (LTL331), because the original specimen was a typical adenocarcinoma, but after host castration and long-term propagation, a neuroendocrine subline arose (LTL331R). The emergence of this subline may mimic the clinical progression occurring in patients with advanced disease where there is divergent clonal evolution of CRPC tumours that have neuroendocrine features and are treatment resistant [38]. The investigators used this model to study the gene expression profiles of the original PDX compared to the

neuroendocrine PDX to map the progression of prostate cancer, especially to a neuroendocrine state. These data revealed a potential biomarker that may be useful in identifying high-risk tumours, as well as providing a novel therapeutic target for neuroendocrine tumours, for which there are currently limited treatment options. Altogether this is another translatable outcome involving a study of the transition of a single PDX, which might inform other patients with this tumour type.

Autopsy Cases and Genome/Growth Potential

With the advent of contemporary chemotherapy and androgen-targeted therapies comes the occurrence of novel resistance mechanisms as the tumour adapts to survive. In an effort to understand the mechanisms of therapy resistance, several large teams have undertaken whole-exome sequencing studies of metastatic biopsies from patients with prostate cancer, before and after drug treatments such as abiraterone or enzalutamide [38–40]. In addition, Kohli and colleagues established PDXs from pre- and post-enzalutamide treatments and comprehensively demonstrated that the genome and transcriptome landscapes of xenografts and the original patient tumour tissues were preserved in the PDXs with high fidelity, supporting their use in pre-clinical studies in the future [39].

Metastatic tumours can be obtained during palliative care for symptom relief or at autopsy for research purposes. As more genomic information from these precious samples becomes available, our understanding of the molecular changes that drive therapy resistance will improve. However, it was the pairing of genomic data to PDXs that was established from the same metastatic samples that is likely to result in significant advances. Serially transplantable PDXs will allow

researchers to pair the genomics with the biology of the original metastatic tumour, as well as to observe clonal evolution of the tumours in real time. PDXs could also be used to show heterogeneity in treatment response between individual metastatic sites from the same patient which is often difficult to assess with clinical monitoring. Although it is currently not feasible to establish metastatic prostate cancer PDXs from men with advancing disease and perform pre-clinical testing that will influence their individual treatment within their lifetime, the establishment of such PDXs will generate a valuable and ongoing resource, extending beyond the life of the patient to improve care for other men in the future.

Summary

There are significant advantages to prostate cancer PDX, and importantly, the technical limitations and challenges of efficacy that have prevented its use in the past are now being overcome. While some limitations remain, this platform has been proven to provide research discoveries that translate to the clinic and patient benefit (Fig. 1). Prostate cancer treatments are rapidly changing to combat and overcome the resistance that emerges to current therapies, and PDXs have the unique potential to unravel this process. The ability to individualise treatments is also a hope for the future, and pairing genomic information with biological and pre-clinical data obtained from PDX may offer this opportunity. Although the PDX model is unlikely to alter the clinical course for the individual patient from whom they are derived, they will provide novel insight to emerging mechanisms of resistance and benefit patients who subsequently receive treatment.

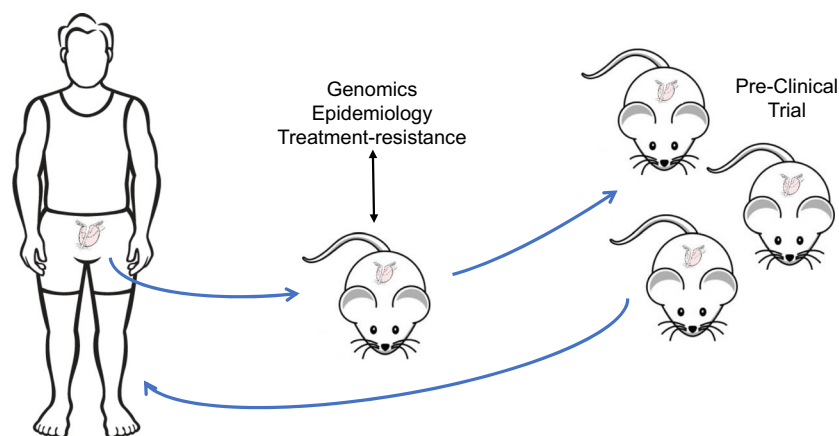


Fig. 1 Utility of prostate cancer patient-derived xenografts (PDXs). Prostate cancer PDXs can be derived from localised or metastatic tumours. Establishing them in mice has led to several important discoveries about tumour biology and the importance of different pathologies. Pairing the biology from PDXs with emerging genomic data, epidemiology and

treatment-resistance mechanisms will facilitate new discoveries in the laboratory that will translate into important new clinical applications. Performing pre-clinical testing of these novel therapeutics in PDX models will enable clinical translation of promising agents, improving outcomes for prostate cancer patients

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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