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REVIEW ARTICLE

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Defining the challenges and opportunities for using patient-derived models in prostate cancer research

W. Nathaniel Brennen PhD[1,2,3] | Clémentine Le Magnen PhD[4,5,6] | Sofia Karkampouna PhD[7] | Nicolas Anselmino PhD[8] | Nathalie Bock PhD[9,10,11] | Nicholas Choo PhD[12] | Ashlee K. Clark PhD[12] | Ilsa M. Coleman MS[13] | Robin Dolgos[4,5,6] | Alison M. Ferguson PhD[14,15] | David L. Goode PhD[16,17] | Marianna Krutihof-de Julio PhD[7,18,19] | Nora M. Navone MD, PhD[8] | Peter S. Nelson MD[13,20] | Edward O'Neill PhD[21] | Laura H. Porter PhD[12] | Weranja Ranasinghe[12,22,23,24] | Takuro Sunada MD[25] | Elizabeth D. Williams PhD[9,26,27] | Lisa M. Butler PhD[28,29] | Eva Corey PhD[30] | Wytske M. van Weerden PhD[31] | Renea A. Taylor PhD[16,17,32,33,34] | Gail P. Risbridger PhD[12,16,17,33,34] | Mitchell G. Lawrence PhD[12,16,17,33,34]

Correspondence
Mitchell G. Lawrence, 19 Innovation Walk, Monash University, Clayton, Victoria, Australia, 3800.
Email: mitchell.lawrence@monash.edu

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Abstract

Background: There are relatively few widely used models of prostate cancer compared to other common malignancies. This impedes translational prostate cancer research because the range of models does not reflect the diversity of disease seen in clinical practice. In response to this challenge, research laboratories around the world have been developing new patient-derived models of prostate cancer, including xenografts, organoids, and tumor explants.

Methods: In May 2023, we held a workshop at the Monash University Prato Campus for researchers with expertise in establishing and using a variety of patient-derived models of prostate cancer. This review summarizes our collective ideas on how patient-derived models are currently being used, the common challenges, and future opportunities for maximizing their usefulness in prostate cancer research.

Results: An increasing number of patient-derived models for prostate cancer are being developed. Despite their individual limitations and varying success rates, these models are valuable resources for exploring new concepts in prostate cancer biology and for preclinical testing of potential treatments. Here we focus on the need for

For affiliations refer to page 632.

W. Nathaniel Brennen and Clémentine Le Magnen contributed equally to this article.

This article is dedicated to our colleague and friend, Professor Nora Navone who inspired us all with her outstanding contributions to science, including the creation and use of prostate cancer models.

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larger collections of models that represent the changing treatment landscape of prostate cancer, robust readouts for preclinical testing, improved in vitro culture conditions, and integration of the tumor microenvironment. Additional priorities include ensuring model reproducibility, standardization, and replication, and streamlining the exchange of models and data sets among research groups.

Conclusions: There are several opportunities to maximize the impact of patient-derived models on prostate cancer research. We must develop large, diverse and accessible cohorts of models and more sophisticated methods for emulating the intricacy of patient tumors. In this way, we can use the samples that are generously donated by patients to advance the outcomes of patients in the future.

KEYWORDS

explants, models, organoids, tumor microenvironment, xenografts

1 | INTRODUCTION

Prostate cancer is a global health issue with ~1,400,000 diagnoses and 375,000 deaths each year.[1] The scale of this challenge requires international research efforts to uncover the underlying biology of prostate cancer across the disease trajectory. The new insights in prostate cancer biology can then be used to develop novel and more effective treatments for patients. Experimental models of prostate cancer are a critical part of this process, because they provide functional tools to test scientific hypotheses, to screen new therapies, and to identify mechanisms of drug response and resistance.

Unfortunately, prostate cancer research is hampered by an insufficient number of experimental models that adequately reflect the molecular and cellular complexity of prostate cancer.[2] The reliance on a relatively small number of immortalized cell lines available to study prostate cancer means there is limited scope to study the diversity of prostate cancer. The small collection of widely available cell lines of prostate cancer also means that it is underrepresented in pan-cancer analyses, such as DepMap, compared to other common malignancies.[3]

Over several decades, many laboratories have attempted to develop new models of prostate cancer derived from patient tissue.[4,5] Technical advances have enabled the establishment of more physiologically and clinically relevant models such as patient-derived xenografts (PDXs), organoids (PDOs), and explants (PDEs). Collectively, these contemporary patient-derived models provide an opportunity to study human tumor biology in vitro and in vivo, and are often used to complement other experimental models, such as immortalized cell lines and genetically engineered mice, to provide a range of models for different experimental applications.

To identify current state-of-the-art techniques for patient-derived models of prostate cancer, ongoing challenges, and emerging opportunities, we convened a 3-day workshop at the Monash University campus in Prato, Italy, in May 2023. The workshop was

attended by researchers from Australia, Japan, the Netherlands, Switzerland, the United Kingdom, and the United States, who have extensive and varied expertise in developing and using patient-derived models of prostate cancer. In this article, we summarize the participants' views on novelties in the field, limitations, and proposed solutions for these challenges as a research community on a global scale.

1.1 | The current state of patient-derived models of prostate cancer

1.1.1 | PDXs

PDXs are important models for translational oncology research because their characteristics closely resemble patients' tumors.[6] In most instances, PDXs retain the genomic features, histopathology and heterogeneity of the original patient tumors, although there is evidence of clonal selection or evolution in some cases.[7,8] Cohorts of PDXs offer a diverse platform to explore tumor biology, disease progression, and therapeutic responses in a manner that closely reflects the clinical scenario. Accordingly, several groups have dedicated considerable resources to generating prostate cancer PDXs.

The efforts to establish PDXs of prostate cancer began 50 years ago and increased over the last two decades. So far, over 330 prostate PDXs have been generated by individual laboratories.[9] Yet, only a few prostate cancer PDXs are available in large international PDX collections. Consequently, there is a disparity between the global incidence of prostate cancer (7.3% of all cancer diagnoses) and its representation in large collections of PDXs (0.17% of models).[9] This reflects the multiple challenges associated with establishing, maintaining, and sharing prostate cancer PDXs. For example, samples of primary prostate cancer that are readily accessible have relatively low engraftment rates and it can be difficult to access patient samples of metastases, which have more aggressive phenotypes. Two recent articles synthesize the information on available prostate cancer PDXs and the challenges associated with developing and using these models.[9,10]

As a greater number of prostate cancer PDXs are being generated, the focus is shifting towards developing PDXs that cover less common phenotypes and reflect the emerging treatment landscape. A survey of the workshop participants documented a need to establish new PDXs and that this should involve a strategic focus on underrepresented subsets of tumors (Figure 1A). These models should span castration-sensitive and castration-resistant prostate cancer, given the differences in molecular features between these stages of disease. Several laboratories have established PDXs of castration-sensitive prostate cancer from samples of treatment-naive disease; however, they are less common than PDXs of castration-resistant prostate cancer.[10–13] Some PDXs that respond to castration in mice were originally grown from patient samples with castration-resistant prostate cancer, so it is important to note the source of patient tissue.[10] An additional consideration for the growth of castration-sensitive PDXs is that adult male mice have lower testosterone levels than adult male humans. Based on serum testosterone levels, intact mice are similar to hypogonadal humans, while castrated mice approximate patients on androgen deprivation therapy plus the CYP17A1 inhibitor abiraterone acetate.[14] For these reasons, some PDXs of castration-sensitive prostate cancer are established and maintained in host mice with testosterone implants.[12,13]

Another priority is to develop PDXs that better reflect genomic and transcriptomic tumor heterogeneity to understand the intricate interplay between the molecular features of tumors, their microenvironment, and therapeutic sensitivity. This includes establishing PDXs from patients treated with new generation treatments, including androgen receptor (AR) signaling inhibitors, poly (ADP-ribose) polymerase inhibitors and prostate-specific membrane antigen directed therapies, to capture the dynamic evolution of prostate cancer under selective pressure. This can be achieved by consenting patients with advanced prostate cancer who are undergoing biopsies or metastasectomy and through rapid autopsy programs. Finally, efforts should also encompass PDXs that better reflect patient diversity, particularly models from patients with nonwhite ancestries.

Although PDXs are useful models for preclinical testing, researchers need to be aware of limitations that can confound the interpretation of experimental findings. (1) PDXs are generated in immunocompromised hosts, so they provide limited information on the interactions between tumor cells and the immune system and its impact on therapy responses. (2) Differences in pharmacokinetics between humans and mice also warrant careful consideration, necessitating the administration of agents to achieve clinically relevant exposure. (3) There are also differences in steroidogenic environments between species.[14] (4) Finally, the intricacy of the tumor microenvironment (TME), comprising host stroma and vasculature, underscores the need for a greater understanding of the interactions of the different species-specific cell types within PDXs. Efforts are being made to address these issues by adapting protocols to humanize immune-deficient mice. There is no universal method that is widely accepted by the field, so this is an area of extensive ongoing investigations.[15] Regardless of these experimental caveats, PDXs offer a useful platform to study cell biology and therapy responses of tumors with varied genotypes and phenotypes that are relevant to the clinic.

1.1.2 | PDOs and PDEs

PDOs and PDEs have emerged as complementary models to PDXs and established cell lines. PDOs are three-dimensional (3D) multicellular in vitro models grown from patient material. They are typically cultured in basement membrane extracts, such as Matrigel or Cultrex, or in ultra-low attachment plates.[16] PDOs can overcome some of the limitations of PDXs, because they are more suitable for genetic engineering and high-throughput drug testing.[17] However, PDO are usually grown as monocultures lacking interaction between

different cell types. PDEs are pieces of patient tissue that are maintained ex vivo, often suspended above culture media with filters or gelatin sponges.[18,19] As PDEs use intact tissue, they retain the architecture and cell types present in the original sample. However, PDEs require ongoing recruitment of patient samples and testing is limited to ~1 week duration. PDOs and PDEs both address the strong societal voice worldwide to reduce the use of animals in research, but require further optimization and standardization to reach their full potential.[20]

In vitro culturing of prostate cancer directly from patient tissue is not trivial. Attempts have been ongoing for decades and have produced a small number of cell lines. Seminal studies have reported the successful establishment of prostate organoids from benign tissue and prostate cancer.[21–23] Although efforts to grow organoids from primary and low-grade prostate cancer have been largely unsuccessful,[24] several laboratories have cultured organoids from advanced prostate cancer.[21,23–27] These PDOs recapitulate the molecular and phenotypic traits commonly observed in patients and include rare aggressive phenotypes such as neuroendocrine prostate cancer where therapeutic options are limited. Additional studies have aimed to improve the generation of PDOs of prostate cancer by using alternative sources of cells, such as circulating tumor cells, and by optimizing medium composition for drug screening and basic research applications.[11,24,28–30]

Similar to PDXs, PDOs have major drawbacks. This includes low success rates and suboptimal culture conditions that favor the expansion of benign cells.[24] Notably, the definition of "success" for organoids may depend on the scientific question and the desired applications. For example, short-term cultures may represent promising preclinical models of therapy response for personalized medicine.[29] However, the establishment of stable, long-term PDO lines remains a challenge, which hinders their use for studying mechanisms driving the disease. Reflecting this challenge, the international collection of expandable prostate cancer PDOs comprises fewer than 30 shareable models and is characterized by an over-representation of specific molecular subtypes.[21,23–26] This is greater than the number of immortalized cell lines of prostate cancer that are available. However, it illustrates the lack of in vitro models emulating specific disease states, such as primary prostate cancer, castration-sensitive prostate cancer, rare subtypes, and tumors that are resistant to emerging therapies. This may be due to differences in the ability of certain subtypes to grow in vitro and the potential enrichment of phenotypes of prostate cancer based on the composition of growth factors in organoid culture medium. Acknowledging this issue, all workshop attendees agreed that there is a need to establish new organoid models of prostate cancer, as they offer a tangible model system for high-throughput screening and genetic manipulation (Figure 1B).

As an alternative to fresh patient tissue, PDXs provide an indefinite source of tumor material to generate organoids (PDXOs). Yet, the success rate of growing organoids from PDXs also remains a challenge. Most PDXs can be grown as organoids for a few passages, but only 20%–50% of tumors grow as organoids for several passages.[12,28,30] It is not clear why some tumors are more efficient at growing as organoids than others. Thus, future efforts should focus on improving culture conditions by modulating a range of variables, including the type of extracellular matrix, medium composition, and oxygen levels.

Regardless of their origin, from fresh patient tissue or PDXs, organoids do not capture the complex TME. Improving current PDXOs and PDOs by the addition of stromal and immune cells will be a significant advance for the field. In the meantime, PDEs are being exploited to preserve the unique tumor context.[19,31,32] PDEs arguably come closest to retaining the original structure and cellular composition of the TME, as the tumor samples undergo minimal disruption in the establishment of the explants, and even in culture for up to 1 week, the major structural features of the TME are retained, including immune cell infiltrates.[33] Thus, PDEs are well-

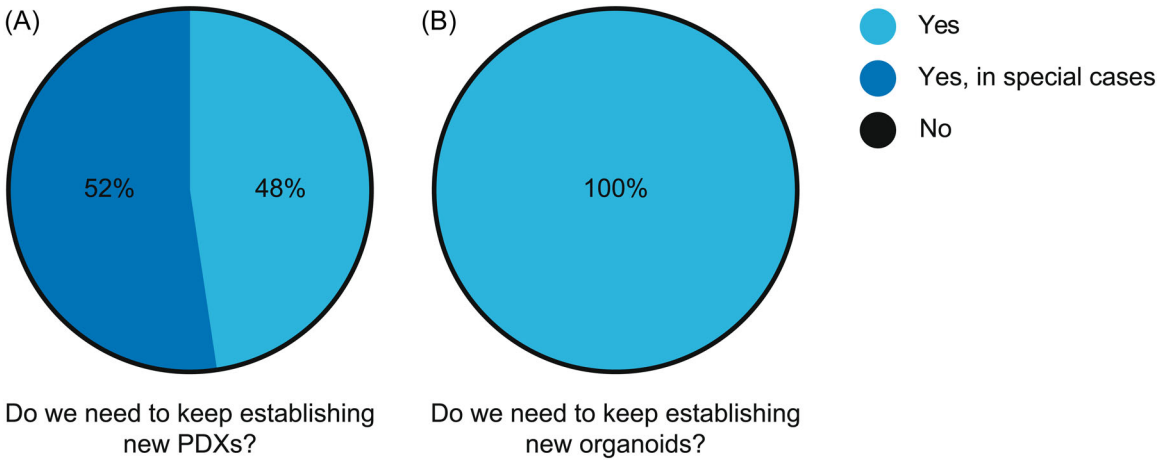

FIGURE 1 Summary of poll results on the need for additional patient-derived models of prostate cancer. Participants were able to select the one response for each question. 'No' was not selected by any participant for either question. PDXs, patient-derived xenografts. [Color figure can be viewed at wileyonlinelibrary.com]

suited to perform patient-matched analysis of various TME component cell types via immunohistochemical or, increasingly, spatial omic approaches.

There are also limitations in modeling the TME with PDEs. It is still uncertain the extent to which the original functions and behavior of each TME cell type, beyond viability, are maintained throughout explant culture. In addition, heterogeneous tissue structure or cellularity differences between individual PDEs complicate comparative analyses, as does the variable incidence of necrosis detected in some specimens.[34] This heterogeneity can be at least partly addressed by incorporation of systematic image analysis to quantify differences in immunostaining across PDEs. For example, statistical modeling of Ki67 staining in prostate cancer PDEs demonstrated that the number of fields of view that are counted across each PDE has a greater impact on the power to detect treatment-induced effects compared to the number of cells that are counted in each field.[34]

2 | COMMON CHALLENGES AND OPPORTUNITIES

2.1 | Preclinical testing and drug development

Drug discovery and development requires a deep understanding of disease biology and the specific molecular mechanisms involved in therapeutic responses. Understanding how each drug is absorbed, distributed, metabolized, and excreted (pharmacokinetics), and how it interacts with the target to produce a therapeutic effect (pharmacodynamics) is essential. Rigorous preclinical studies are necessary to ensure sufficient exposure and to assess potential toxicities, including organ toxicity, genotoxicity, and off-target effects. However, the translatability of results from patient-derived models is not straightforward. Differences in ligand and cognate receptor affinities, pharmacokinetics, pharmacodynamics, interactions with the TME, and tumor heterogeneity all pose challenges when extrapolating preclinical findings to humans.[30,35–37]

Given the complexities of patient-derived models, it is important to carefully evaluate the results of preclinical studies arising from them during drug development. In other tumor types, the responses of PDXs and PDOs to treatment have been correlated with the outcomes of the original patients through retrospective analyses or co-clinical trials.[38–40] This has not been done comprehensively for prostate cancer, in part due to the long timeframes for establishing patient-derived models and collecting patient follow-up. Nevertheless, it would provide additional confidence that the outcomes of preclinical studies may predict the efficacy of emerging treatments in the clinic. The concern is that a statistically significant change in tumor viability in response to drug treatment in a PDX, PDO, or PDE may not necessarily translate to a clinically relevant effect in patients. Some experimental endpoints may be more informative than others. For example, a variety of statistical approaches can be used to analyze PDX experiments including the percentage change in tumor volume, the area under the curve, tumor growth inhibition, and modified RECIST (Response Evaluation Criteria in Solid Tumors) categories. Encouragingly, the National Cancer Institute PDXNet Consortium found that the outcomes of PDX studies were reproducible across laboratories, but they also noted that the outcomes of statistical testing need to be accompanied by an assessment of the magnitude of response to judge whether it is also biologically relevant.[41]

Robust experimental measures are also an important consideration for drug testing using PDOs or PDXOs. Currently, the most common approach is to use cell viability assays that measure overall cell responses only at the endpoint of drug treatment. Experiments are not well standardized and lack quality assessments, which complicates comparisons across experiments and research groups. Standardized quality measures for each organoid culture before drug screening should include viability scores across the entire experiment. It is also important to measure the tumor cell content, because PDOs may be contaminated with benign epithelial cells if they are from primary tumors, and PDOs and PDXOs may have stromal contamination. Another shortcoming of current methods is the use of viability assays that provide bulk measurements of whole wells of organoids rather than separate clusters of cells. This limits information of the impact of a drug on different organoid colonies within the culture and individual tumor cells within an organoid, which is determined by the penetration of the drug across the matrix and into the organoid. To overcome this limitation, high-content live-cell imaging can offer a range of other metrics to address the drug response in the context of a 3D culture, but these measures will also need to be validated for their reproducibility across different assays and laboratories.[30,42]

Another important consideration for developing new therapies is to anticipate and address the mechanisms of drug resistance that may arise. Drug resistance can emerge due to surviving subclones, target mutations or compensation by alternative signaling pathways,[43] and this may not be captured by relatively short-term assays with patient-derived models. For PDOs, better assessment of drug resistance demands extended cultures beyond the current short-term applied drug regimens, preferably with dedicated imaging-based readouts for individual cell responses. Alternatively, PDXs can be challenged with drug treatments for weeks to months in vivo, to allow for regrowth of cells that are resistant to the drug. This process has already been done with castration for several prostate cancer PDXs to produce castration-resistant sublines.[12,44–47] Developing therapy-resistant models for other treatments, including standard-of-care agents and new compounds, will be resource-intensive and financially demanding, but will create greater opportunity to study emerging mechanisms of resistance. For each new treatment it will be important to develop multiple therapy-resistant models, because tumor heterogeneity may produce different mechanisms of resistance.

2.2 | Encompassing the TME

A major hurdle in developing patient-derived models of prostate cancer is the difficulty in accurately modeling the TME. This richly complex ecosystem includes supportive stroma, extracellular matrix, immune infiltrate, blood and lymphatic vessels, and it varies between the primary tumor and metastatic niches.[5,31] The difficulty in replicating the TME reflects the inevitable changes that occur when patient tumor tissues are excised from their in situ location, cut up or disaggregated, grown in culture or passaged through immunocompromised mice, and disconnected from their original circulatory nutrient supply and cross-talk between cell types.

There is now overwhelming evidence that the aggressiveness of prostate cancer, and its responsiveness or resistance to therapy, is influenced not only by the molecular features of the tumor epithelial cells, but also by the TME.[48–51] Removing or altering the "native" TME—for example, by generating organoids, integration of mouse stroma, or prolonged ex vivo culture—limits the insights that can be gained from studying existing patient-derived models. It has also limited the types of therapeutic interventions that can be evaluated, including those that target key aspects of the cancer-related stroma and most immunotherapies.[52]

Despite these limitations, recent work suggests that patient-derived models still provide valuable preclinical data regarding tumor-stromal cross-talk, drug accessibility in solid tumors, and the optimization of certain types of immunotherapy.[53] Profiling of stromal cells isolated from a range of PDXs has shown model-specific responses to androgen manipulation at the gene and protein level, which may provide clinically relevant insights into tumor-stromal cell interactions and extracellular matrix remodeling despite the reduced complement of immune cells.[29] For example, certain gene signatures induced by androgen deprivation in stroma of bone metastatic PDXs, even when implanted in a different microenvironment, are not only expressed but are prognostic in clinical prostate cancer.[29] However, in a similar fashion to ex vivo cultured PDEs, the stromal component of PDXs changes over time.[54] Moreover, this may be exacerbated by methodological differences between laboratories in terms of how passaging occurs, particularly for early passages, the use of cryopreservation, and the specific mouse strains used.

Moving forward, there is interest in performing spatial transcriptomics and comparing existing RNA sequencing data sets from patient samples and PDXs normalized for stromal content. These analyses would provide insight into the proportions of mouse-derived transcriptomes in these data sets and generate an atlas of common stromal elements that could be used to design tumor-stromal targeting strategies.

A compelling new application for PDXs is the evaluation and optimisation of chimeric antigen receptor (CAR) T cell immunotherapy for prostate cancer. While commonly overlooked, PDXs provide the opportunity to target components of the TME and study interactions between the tumor and human CAR T cells. A study comparing various pharmacological approaches to modulate the TME in a panel of prostate PDXs showed modification of the TME by the chemotherapeutic agent carboplatin, which enhanced both entry of the CAR T cells into the PDXs and their persistence in vivo.[53] Carboplatin caused a pro-inflammatory shift within the TME, inducing alterations in multiple cell types, including myeloid and fibroblastic cells, which was required for improved efficacy of CAR T cell therapy in the tumor. While the authors of this study acknowledged the necessarily reductionist setting for evaluating this form of therapy, which lacks interactions between immune cells within the TME, it offers the unique potential to assess the impact of multiple types of TME-directed interventions across a range of clinically relevant tumor phenotypes. This could then enable validation in a more targeted way using immunocompetent or humanized model settings.

Although PDEs retain the original structure and cellular composition of the TME, the architecture of the stroma often changes with increasing time in culture, and the function and activation of the resident immune cell population is not routinely analyzed.[34] Despite this issue, a recent report testing PD-1 blockade in ex vivo cultured PDEs from five different tumor types, not including prostate cancer, showed that the immunological response in the resident T cells of these PDEs was indicative of the subsequent clinical response of the donor patients.[55] The heterogeneity of the TME within a single lesion in a patient, at tumor invasion fronts, or between different metastatic sites, is also difficult to capture in any preclinical models, although increasing use of spatial multi-omics is likely to provide some insights.[56,57] Spatial mass spectrometry imaging is also useful to evaluate drug penetration into PDEs.[58]

Efforts to better maintain or mimic the TME are gaining traction in model systems. For example, incorporating bone-like scaffolds in PDX, PDO, or PDE cultures provides important structural signals that may improve modeling of the bone metastatic niche of prostate cancer.[59] There is also increasing interest in minimizing the dramatic change from a hypoxic, nutrient-depleted TME in the patient, to an environment with supraphysiological nutrient levels in the case of PDOs and PDEs, by using a range of contemporary physiological culture media with restricted nutrient levels.[60] Overall, this means that there is still broad scope for modifying patient-derived models of prostate cancer to mimic the cellular composition and physiological conditions of tumors in patients.

2.3 | Combining models across different cohorts

Simply establishing a greater number and variety of patient-derived models of prostate cancer will not be sufficient to overcome all the barriers for effective translational research. It is also important to thoroughly characterize all models and provide unconstrained access to them for the research community. Collaborative initiatives are pivotal, including using public repositories to house patient-derived models and the extensive data sets arising from them (Figure 2A). This will expand the capacity of more laboratories to use patient-derived models and improve the coordination between groups with existing collections.

There are several impediments to widespread sharing of PDXs (Figure 2B). This includes practical issues around resourcing and infrastructure. There are also technical issues, such as the difficulty in cryopreserving some PDXs, which limits their transferability. Ethical considerations surrounding patient consent, data privacy, and animal welfare also demand careful navigation. Establishing partnerships between research institutions and regulatory bodies is pivotal to ensure responsible and transparent utilization of PDXs. Collaboration among research institutions is essential to address these challenges effectively. Sharing PDX data, tissues, and expertise can accelerate discoveries and enhance the reliability of research outcomes. To enable more extensive collaborations, we must overcome institutional barriers, align interests, streamline material transfer agreements, adopt common standards for recording and describing data, utilize shared databases, and replicate protocols between laboratories. This is a major focus as we aim to maximize the use of patient-derived models beyond the laboratories that established and maintain them. Indeed, despite the challenges in sharing patient-derived models (Figure 2B), in the poll at the Prato workshop, none of the attendees thought that there are too many barriers to collaboration between laboratories (Figure 2C).

Similar to PDXs, legal barriers need to be overcome so that in vitro patient-based models can be shared across the globe for optimal impact. Despite this hurdle, the ease of growing PDXOs and PDOs has facilitated more accessible transfer of models and sharing of protocols across research groups. Our limited understanding of the essential medium requirements for prostate epithelial tumor cells to survive and proliferate in vitro, while retaining the features of the donor tumors, remains a significant challenge for safe storage and recovery of the original PDX, PDXO, and PDO. Culture media lacks numerous factors that are present in vivo and has higher concentrations of others, which may impact the features of patient-derived cells. For example, several growth factors added to organoid media can modulate AR expression in benign prostate organoids. Omitting FGF10, noggin, R-spondin, or nicotinamide from organoid media, decreases AR expression, whereas removing EGF increases AR signaling.[22] It is possible that changes in the relative concentrations of these additives could enrich different subsets of cells based on their dependency on the AR and other signaling pathways. To safeguard intratumoral heterogeneity at PDO initiation, the parallel use of different media may capture the full repertoire of heterogeneity of the original tumor.

3 | FUTURE DIRECTIONS

3.1 | Advanced modeling

Preclinical drug discovery and development requires extensive effort over many years, owing in part to the use of animal models, like PDXs, which are costly for large-scale drug testing. Increasingly sophisticated in vitro models may enable more efficient and accurate preclinical pipelines. As microfluidic, organ-on-a-chip and 3D coculture models develop, they will provide physiologically relevant platforms for testing candidate compounds by integrating multiple cell and tissue types.[20] These models aim to mimic the complexity of human tissues, allowing for better prediction of drug efficacy, toxicity, and metabolism before advancing to clinical trials. For example, microfluidic chips can support the viability of microdissected samples of benign and malignant human prostate tissue and slices of prostate cancer PDX tissue for several days.[61,62] These systems could also model biological interfaces and mechanical forces to mimic the TME and dynamic changes in various factors, including drugs, over time.[63] There are many potential uses of advanced in vitro models of prostate cancer, including testing drug activity and toxicity, evaluating pharmacodynamics/pharmacokinetics, and disease modeling with small samples of patient tissue. The limitations of these assays will be addressed over time including their expense, the need for reproducible and stable sources of primary cells or tissue, and engineering challenges.[63]

Currently, organoids are usually grown in Matrigel. This growth factor-rich extracellular matrix preparation from the Engelbreth–Holm–Swarm mouse sarcoma contains a mixture of defined and undefined components.[64] An emerging alternative is to use synthetic hydrogels as matrices for growing organoids in more controlled and reproducible conditions.[30,65,66] For instance, cells from prostate cancer PDXs have been cultured in polyethylene glycol, an inert polymer widely used for 3D cell culture.[67] Polyethylene glycol can also be modified with specific peptides, adhesive elements, and degradable ligands to mimic components found in the TME. Semi-synthetic hydrogels, like methacrylated or thiolated gelatin, combine the advantages of natural matrices—such as low immunogenicity and integrin-binding motifs—with the tailored characteristics of synthetic hydrogels, including their customizable chemical and physical properties.[68] Methacrylated gelatin (GelMA) has been successfully used for co-cultures of prostate cancer cells lines with cells from the TME, and for automated protocols with bioprinting.[69–71] Therefore, this approach holds great potential for tackling the next step of introducing patient-derived cells.

Specialized matrices for organoids could also be developed based on the insights from ex vivo explant models of metastases. To replicate the bone metastatic niche, scaffolds might offer greater potential than hydrogels. This stems from their increased stiffness, more closely resembling the specific biophysical characteristics of bone. For example, mineralized polycaprolactone scaffolds have been used to co-cultures cells from prostate cancer PDXs with osteoblasts.[72,73] Collectively, the advances in creating more sophisticated in vitro models of prostate cancer will address some of the limitations of current patient-derived models and provide researchers with new tools to mimic the complex characteristics of patient tumors.

Although cell lines are not usually considered to be advanced in vitro models, it would be a major advance for the field to overcome the shortage of prostate cancer cell lines, because they are relatively easy to grow, manipulate, and disseminate. The derivation of 22Rv1 cells from the CWR22 xenograft

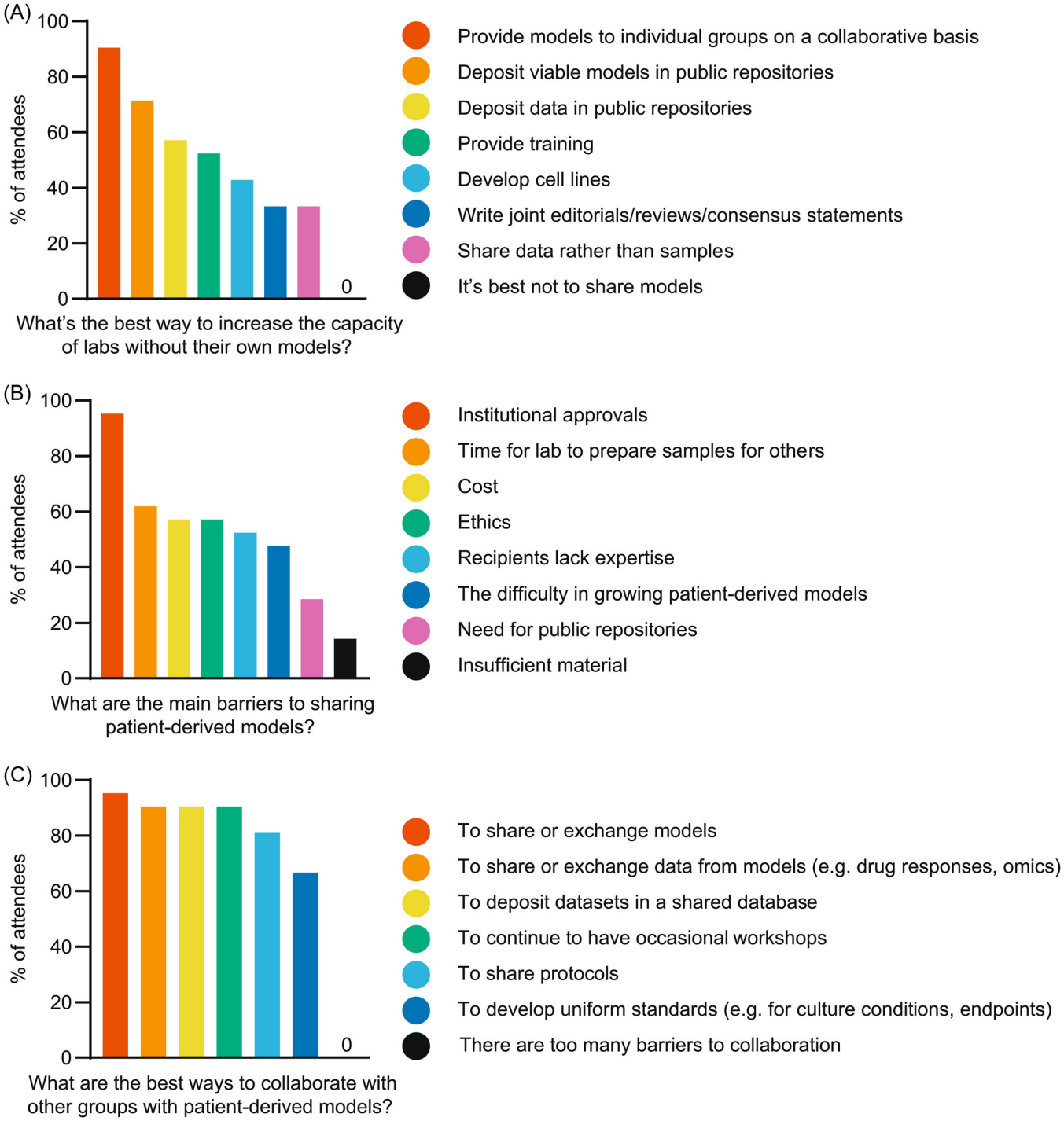

FIGURE 2 Summary of poll results on the practicalities of sharing patient-derived models or prostate cancer. (A–C) Graphs show the percentage of attendees who selected each response to questions about sharing patient-derived models. Participants were able to select multiple options for each question. [Color figure can be viewed at wileyonlinelibrary.com]

demonstrated the possibility of establishing cell lines from prostate cancer PDXs in some cases.[74] A cell line has also been established from the LTL331R PDX using the conditional reprogramming methodology.[75] Concerted efforts are underway to establish cell lines from other prostate cancer PDXs with the goal of expanding the variety of available models of prostate cancer.

3.2 | Artificial intelligence (AI)

AI is becoming increasingly essential in drug development due to its capability to handle vast and intricate data sets.[76,77] AI has the potential to expedite drug development by reducing the need for numerous rounds of lead optimization. It is already successfully

employed in various applications, such as predicting protein structures, virtual screening, aiding in synthesis planning, and developing algorithms for predicting bioactivity, pharmacokinetic/pharmacodynamic parameters, toxicity, and physicochemical properties.[76] Nevertheless, there remain significant challenges to AI implementation, including the requirement for extensive high-quality training data sets that ideally do not rely on proxy measures, standardizing and integrating existing data into compatible formats, handling uncertainty and noise resulting from incomplete data matrices, addressing issues of transparency in algorithms, and educating new generations of scientists to embrace and comprehend these models.[76]

As patient-derived models of prostate cancer become more sophisticated and are used in higher throughput settings, it will become necessary to use AI to help analyze the intricate multi-parameter data sets they generate. Presently, high-context live cell imaging and endpoint analyses are used to assess changes in the size, composition, and viability of prostate organoid cultures.[30,42] These experiments produce an abundance of images capturing organoids from various focal planes, over time, under different treatments, and through various microscopy techniques, including brightfield and fluorescence microscopy targeting cell nuclei or viability stains. Despite the richness of these data sets, their full potential is underutilized, as there are many more parameters that could be measured to identify patterns in how different organoids respond to distinct treatments.

AI is already starting to be used to analyze the features of organoids in different disease contexts, such as investigating the differentiation of retinal organoids or characterizing the phenotype of brain organoids.[78–80] Therefore, a promising future direction is to apply these AI techniques to the study of prostate cancer organoids. Another advantageous application of AI in the context of patient-derived models of prostate cancer would involve spatial analysis of multiple cell populations. This could provide insights into how these populations change in response to drug treatments and whether these changes vary based on the spatial distribution of different cell types. By doing so, we can ensure that the sophistication of our analysis pipelines keeps pace with the growing complexity of patient-derived models and the evolving methodologies used to profile them.

4 | CONCLUSIONS

There are no simple solutions to the complex clinical challenges of prostate cancer. All experimental models have their benefits and shortcomings, making them appropriate to address some research questions but unsuitable for others. These considerations highlight the need to use a combination of multiple models rather than rely on single models. All of these models must be deeply characterized and their therapy responses carefully validated using robust readouts. Yet, as more patient-derived models are established, treated with different drugs, and profiled with multiple omics techniques, the task of interpreting the results becomes a growing challenge for individual laboratories. Therefore, greater use of patient-derived models is part of the solution to advancing our understanding of prostate cancer biology and improving preclinical drug testing, but it comes with inherent challenges. We have distilled the challenges into three main areas for our laboratories and others to address.

4.1 | Developing diverse and accessible cohorts of models

There is a need to make a global effort to establish patient-derived models that represent the spectrum of prostate cancer subtypes, patient ancestries, and therapy-resistant phenotypes. This will require wider inclusion of diverse patient populations to ensure comprehensive representation. Sharing these models requires clear ethical guidelines that address patient consent, data privacy, and animal welfare. Engaging with regulatory bodies and ethics committees will ensure responsible and transparent research practices.

4.2 | Collaboration, data integration, and standardization

Open science initiatives that promote sharing of methodologies, data and results will accelerate the pace of discovery and improve the reproducibility of results. Compiling data from different collections of patient-derived models will provide a holistic view of tumor biology, treatment responses, and genetic alterations. To drive collaboration and accelerate discoveries, we need to combine molecular and functional data sets and disseminate them to the research community using standardized, centralized and publicly available data repositories. These collaborative efforts should span academia, pharmaceutical companies, and government organizations to pool resources, knowledge, and expertise. Current efforts to standardize patient-derived models include the PDX models Minimal Information standard, which lists essential and desirable features to report, spanning patient and tumor features, model creation, and quality assurance.[81] This standard could be applied to prostate cancer PDXs and adapted for PDOs and PDEs.

4.3 | Innovations in modelling

Advanced techniques, such as humanized mouse models and organ-on-chip systems, will enhance the accuracy of modeling tumor-immune interactions and therapy responses. As the models become more sophisticated, technologies with single-cell and spatial resolution will increasingly be required to unravel complex cellular dynamics within them. AI may facilitate many of these processes given the volume of data that will be produced. These advances may also decrease the need for animals as in vitro and ex vivo alternatives are developed; however, it is currently premature to eliminate animal testing until these technologies are more thoroughly validated.

4.4 | Summary

In conclusion, patient-derived models are enabling researchers to study prostate cancer in ways that mirror its clinical complexity. By learning from the limitations of current models, we can improve the development of new tools rather than perpetuating the same issues. Although challenges abound, the solutions are within reach through technological advancements, collaborative efforts, and ethical and legal frameworks that enable sharing worldwide. As the field evolves, we need to embrace diversity, data integration, and shared resources, and outreach to different fields such as engineering and AI. This will propel prostate cancer research toward novel insights, refined treatments, and improved patient outcomes.

AFFILIATIONS

[1]Department of Oncology, Sidney Kimmel Comprehensive Cancer Center (SKCCC), Johns Hopkins University, Baltimore, Maryland, USA

[2]Department of Urology, James Buchanan Brady Urological Institute, Johns Hopkins University, Baltimore, Maryland, USA

[3]Department of Pharmacology & Molecular Sciences, Johns Hopkins University, Baltimore, Maryland, USA

[4]Institute of Medical Genetics and Pathology, University Hospital Basel, University of Basel, Basel, Switzerland

[5]Department of Urology, University Hospital Basel, Basel, Switzerland

[6]Department of Biomedicine, University Hospital Basel, University of Basel, Basel, Switzerland

[7]Urology Research Laboratory, Department for BioMedical Research, University of Bern, Bern, Switzerland

[8]Department of Genitourinary Medical Oncology and the David H. Koch Center for Applied Research of Genitourinary Cancers, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

[9]School of Biomedical Sciences at Translational Research Institute, Faculty of Health, Queensland University of Technology (QUT), Brisbane, QLD, Australia

[10]Max Planck Queensland Centre for the Materials Science of Extracellular Matrices, Queensland University of Technology (QUT), Brisbane, QLD, Australia

[11]Centre for Biomedical Technologies, Queensland University of Technology, Brisbane, QLD, Australia

[12]Department of Anatomy and Developmental Biology, Biomedicine Discovery Institute Cancer Program, Monash University, Clayton, VIC, Australia

[13]Division of Human Biology, Fred Hutchinson Cancer Center, Seattle, Washington, USA

[14]Department for BioMedical Research, University of Bern, Bern, Switzerland

[15]Katharina Gaus Light Microscopy Facility, Mark Wainwright Analytical Centre, Division of Research and Enterprise, University of New South Wales, Sydney, NSW, Australia

[16]Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

[17]Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, VIC, Australia

[18]Department of Urology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

[19]Department for BioMedical Research, Translational Organoid Resource, University of Bern, Bern, Switzerland

[20]Division of Clinical Research, Fred Hutchinson Cancer Center, Seattle, Washington, USA

[21]Nuffield Department of Surgical Sciences, University of Oxford, Oxford, UK

[22]Department of Surgery, Monash University, Melbourne, VIC, Australia

[23]Department of Urology, Monash Health, Melbourne, VIC, Australia

[24]Department of Urology, Austin Health, Melbourne, VIC, Australia

[25]Department of Urology, Kyoto University Graduate School of Medicine, Kyoto, Japan

[26]Australian Prostate Cancer Research Centre—Queensland, Brisbane, QLD, Australia

[27]Centre for Genomics and Personalised Health, Queensland University of Technology (QUT), Brisbane, QLD, Australia

[28]South Australian Immunogenomics Cancer Institute, University of Adelaide, Adelaide, SA, Australia

[29]Precision Cancer Medicine Theme, South Australian Health and Medical Research Institute, Adelaide, SA, Australia

[30]Department of Urology, University of Washington, Seattle, Washington, USA

[31]Department of Urology, Erasmus Medical Center, Rotterdam, The Netherlands

[32]Department of Physiology, Biomedicine Discovery Institute Cancer Program, Monash University, Clayton, VIC, Australia

[33]Cabrini Institute, Cabrini Health, Malvern, VIC, Australia

[34]Melbourne Urological Research Alliance, Biomedicine Discovery Institute, Monash University, Melbourne, VIC, Australia

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

W. Nathaniel Brennen http://orcid.org/0000-0001-9807-7433
Clémentine Le Magnen http://orcid.org/0000-0002-6084-6054
Robin Dolgos http://orcid.org/0009-0008-4699-3331
Edward O'Neill http://orcid.org/0000-0002-7987-8160
Elizabeth D. Williams http://orcid.org/0000-0002-3364-6655
Lisa M. Butler http://orcid.org/0000-0003-2698-3220
Eva Corey http://orcid.org/0000-0002-9244-3807
Wytske M. van Weerden http://orcid.org/0000-0001-9916-5347
Renea A. Taylor http://orcid.org/0000-0003-2609-2380
Gail P. Risbridger http://orcid.org/0000-0003-3089-4028
Mitchell G. Lawrence http://orcid.org/0000-0001-9520-3000

REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209-249. doi:10.3322/caac.21660
2. Pienta KJ, Abate-Shen C, Agus DB, et al. The current state of preclinical prostate cancer animal models. *Prostate*. 2008;68(6):629-639. doi:10.1002/pros.20726
3. Ghandi M, Huang FW, Jané-Valbuena J, et al. Next-generation characterization of the cancer cell line encyclopedia. *Nature*. 2019;569(7757):503-508. doi:10.1038/s41586-019-1186-3
4. Brennen WN, Isaacs JT. The what, when, and why of human prostate cancer xenografts. *Prostate*. 2018;78(9):646-654. doi:10.1002/pros.23510
5. Sailer V, von Amsberg G, Duensing S, et al. Experimental in vitro, ex vivo and in vivo models in prostate cancer research. *Nat Rev Urol*. 2023;20(3):158-178. doi:10.1038/s41585-022-00677-z
6. Gao H, Korn JM, Ferretti S, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. *Nat Med*. 2015;21(11):1318-1325. doi:10.1038/nm.3954
7. Sun H, Cao S, Mashl RJ, et al. Comprehensive characterization of 536 patient-derived xenograft models prioritizes candidatesfor targeted treatment. *Nat Commun*. 2021;12(1):5086. doi:10.1038/s41467-021-25177-3
8. Woo XY, Giordano J, Srivastava A, et al. Conservation of copy number profiles during engraftment and passaging of patient-derived cancer xenografts. *Nat Genet*. 2021;53(1):86-99. doi:10.1038/s41588-020-00750-6
9. Lawrence MG, Taylor RA, Cuffe GB, et al. The future of patient-derived xenografts in prostate cancer research. *Nat Rev Urol*. 2023;20(6):371-384. doi:10.1038/s41585-022-00706-x
10. Navone NM, van Weerden WM, Vessella RL, et al. Movember GAP1 PDX project: an international collection of serially transplantable prostate cancer patient-derived xenograft (PDX) models. *Prostate*. 2018;78(16):1262-1282. doi:10.1002/pros.23701
11. Germain L, Lafront C, Paquette V, et al. Preclinical models of prostate cancer - modelling androgen dependency and castration resistance in vitro, ex vivo and in vivo. *Nat Rev Urol*. 2023;20(8):480-493. doi:10.1038/s41585-023-00726-1
12. Risbridger GP, Clark AK, Porter LH, et al. The MURAL collection of prostate cancer patient-derived xenografts enables discovery through preclinical models of uro-oncology. *Nat Commun*. 2021;12(1):5049. doi:10.1038/s41467-021-25175-5
13. Lin D, Wyatt AW, Xue H, et al. High fidelity patient-derived xenografts for accelerating prostate cancer discovery and drug development. *Cancer Res*. 2014;74(4):1272-1283. doi:10.1158/0008-5472.CAN-13-2921-T

14. Sedelaar JPM, Dalrymple SS, Isaacs JT. Of mice and men-warning: intact versus castrated adult male mice as xenograft hosts are equivalent to hypogonadal versus abiraterone treated aging human males, respectively. *Prostate*. 2013;73(12):1316-1325. doi:10.1002/pros.22677
15. Jin KT, Du WL, Lan HR, et al. Development of humanized mouse with patient-derived xenografts for cancer immunotherapy studies: a comprehensive review. *Cancer Sci*. 2021;112(7):2592-2606. doi:10.1111/cas.14934
16. Linxweiler J, Hammer M, Muhs S, et al. Patient-derived, three-dimensional spheroid cultures provide a versatile translational model for the study of organ-confined prostate cancer. *J Cancer Res Clin Oncol*. 2019;145(3):551-559. doi:10.1007/s00432-018-2803-5
17. Zhou L, Zhang C, Zhang Y, Shi C. Application of organoid models in prostate cancer research. *Front Oncol*. 2021;11:736431. doi:10.3389/fonc.2021.736431
18. Centenera MM, Raj GV, Knudsen KE, Tilley WD, Butler LM. Ex vivo culture of human prostate tissue and drug development. *Nat Rev Urol*. 2013;10(8):483-487. doi:10.1038/nrurol.2013.126
19. Zhang W, van Weerden WM, de Ridder CMA, et al. Ex vivo treatment of prostate tumor tissue recapitulates in vivo therapy response. *Prostate*. 2019;79(4):390-402. doi:10.1002/pros.23745
20. Buskin A, Scott E, Nelson R, et al. Engineering prostate cancer in vitro: what does it take? *Oncogene*. 2023;42(32):2417-2427. doi:10.1038/s41388-023-02776-6
21. Gao D, Vela I, Sboner A, et al. Organoid cultures derived from patients with advanced prostate cancer. *Cell*. 2014;159(1):176-187. doi:10.1016/j.cell.2014.08.016
22. Karthaus WR, Iaquinta PJ, Drost J, et al. Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell*. 2014;159(1):163-175. doi:10.1016/j.cell.2014.08.017
23. Puca L, Bareja R, Prandi D, et al. Patient derived organoids to model rare prostate cancer phenotypes. *Nat Commun*. 2018;9(1):2404. doi:10.1038/s41467-018-04495-z
24. Servant R, Garioni M, Vlajnic T, et al. Prostate cancer patient-derived organoids: detailed outcome from a prospective cohort of 81 clinical specimens. *J Pathol*. 2021;254(5):543-555. doi:10.1002/path.5698
25. Tang F, Xu D, Wang S, et al. Chromatin profiles classify castration-resistant prostate cancers suggesting therapeutic targets. *Science*. 2022;376(6596):eabe1505. doi:10.1126/science.abe1505
26. Mout L, van Dessel LF, Kraan J, et al. Generating human prostate cancer organoids from leukapheresis enriched circulating tumour cells. *Eur J Cancer*. 2021;150:179-189. doi:10.1016/j.ejca.2021.03.023
27. Pauli C, Hopkins BD, Prandi D, et al. Personalized in vitro and in vivo cancer models to guide precision medicine. *Cancer Discov*. 2017;7(5):462-477. doi:10.1158/2159-8290.CD-16-1154
28. Beshiri ML, Tice CM, Tran C, et al. A PDX/organoid biobank of advanced prostate cancers captures genomic and phenotypic heterogeneity for disease modeling and therapeutic screening. *Clin Cancer Res*. 2018;24(17):4332-4345. doi:10.1158/1078-0432.CCR-18-0409
29. Karkampouna S, La Manna F, Benjak A, et al. Patient-derived xenografts and organoids model therapy response in prostate cancer. *Nat Commun*. 2021;12(1):1117. doi:10.1038/s41467-021-21300-6
30. Van Hemelryk A, Tomljanovic I, de Ridder CMA, et al. Patient-derived xenografts and organoids recapitulate castration-resistant prostate cancer with sustained androgen receptor signaling. *Cells*. 2022;11(22):3632. doi:10.3390/cells11223632
31. Mackenzie NJ, Nicholls C, Templeton AR, et al. Modelling the tumor immune microenvironment for precision immunotherapy. *Clin Transl Immunol*. 2022;11(6):e1400. doi:10.1002/cti2.1400
32. Davies EJ, Dong M, Gutekunst M, et al. Capturing complex tumour biology in vitro: histological and molecular characterisation of precision cut slices. *Sci Rep*. 2015;5:17187. doi:10.1038/srep17187
33. Centenera MM, Hickey TE, Jindal S, et al. A patient-derived explant (PDE) model of hormone-dependent cancer. *Mol Oncol*. 2018;12(9):1608-1622. doi:10.1002/1878-0261.12354
34. Centenera MM, Vincent AD, Moldovan M, et al. Harnessing the heterogeneity of prostate cancer for target discovery using patient-derived explants. *Cancers*. 2022;14(7):1708. doi:10.3390/cancers14071708
35. Marshall LJ, Bailey J, Cassotta M, Herrmann K, Pistollato F. Poor translatability of biomedical research using animals - a narrative review. *ATLA: Altern Lab Anim*. 2023;51(2):102-135. doi:10.1177/02611929231157756
36. Pound P, Ritskes-Hoitinga M. Is it possible to overcome issues of external validity in preclinical animal research? Why most animal models are bound to fail. *J Transl Med*. 2018;16(1):304. doi:10.1186/s12967-018-1678-1
37. Marine JC, Dawson SJ, Dawson MA. Non-genetic mechanisms of therapeutic resistance in cancer. *Nat Rev Cancer*. 2020;20(12):743-756. doi:10.1038/s41568-020-00302-4
38. Izumchenko E, Paz K, Ciznadija D, et al. Patient-derived xenografts effectively capture responses to oncology therapy in a heterogeneous cohort of patients with solid tumors. *Ann Oncol*. 2017;28(10):2595-2605. doi:10.1093/annonc/mdx416
39. Millen R, De Kort WWB, Koomen M, et al. Patient-derived head and neck cancer organoids allow treatment stratification and serve as a tool for biomarker validation and identification. *Med*. 2023;4(5):290-310.e12. doi:10.1016/j.medj.2023.04.003
40. Guillen KP, Fujita M, Butterfield AJ, et al. A human breast cancer-derived xenograft and organoid platform for drug discovery and precision oncology. *Nature Cancer*. 2022;3(2):232-250. doi:10.1038/s43018-022-00337-6
41. Evrard YA, Srivastava A, Randjelovic J, et al. Systematic establishment of robustness and standards in patient-derived xenograft experiments and analysis. *Cancer Res*. 2020;80(11):2286-2297. doi:10.1158/0008-5472.CAN-19-3101
42. Choo N, Ramm S, Luu J, et al. High-throughput imaging assay for drug screening of 3D prostate cancer organoids. *SLAS Discov*. 2021;26(9):1107-1124. doi:10.1177/24725552211020668
43. Vasan N, Baselga J, Hyman DM. A view on drug resistance in cancer. *Nature*. 2019;575(7782):299-309. doi:10.1038/s41586-019-1730-1
44. Hao J, Ci X, Xue H, et al. Patient-derived hormone-naive prostate cancer xenograft models reveal growth factor receptor bound protein 10 as an androgen receptor-repressed gene driving the development of castration-resistant prostate cancer. *Eur Urol*. 2018;73(6):949-960. doi:10.1016/j.eururo.2018.02.019
45. Palanisamy N, Yang J, Shepherd PDA, et al. The MD Anderson prostate cancer patient-derived xenograft series (MDA PCa PDX) captures the molecular landscape of prostate cancer and facilitates marker-driven therapy development. *Clin Cancer Res*. 2020;26(18):4933-4946. doi:10.1158/1078-0432.CCR-20-0479
46. Marques RB, van Weerden WM, Erkens-Schulze S, et al. The human PC346 xenograft and cell line panel: a model system for prostate cancer progression. *Eur Urol*. 2006;49(2):245-257. doi:10.1016/j.eururo.2005.12.035
47. Nguyen HM, Vessella RL, Morrissey C, et al. LuCaP prostate cancer patient-derived xenografts reflect the molecular heterogeneity of advanced disease and serve as models for evaluating cancer therapeutics. *Prostate*. 2017;77(6):654-671. doi:10.1002/pros.23313
48. Pederzoli F, Raffo M, Pakula H, Ravera F, Nuzzo PV, Loda M. Stromal cells in prostate cancer pathobiology: friends or foes? *Br J Cancer*. 2023;128(6):930-939. doi:10.1038/s41416-022-02085-x
49. Kang J, La Manna F, Bonollo F, et al. Tumor microenvironment mechanisms and bone metastatic disease progression of prostate cancer. *Cancer Lett*. 2022;530:156-169. doi:10.1016/j.canlet.2022.01.015

50. Clark KC, Wu Y, Taylor RA, Daly RJ. Novel therapeutic targets and biomarkers associated with prostate cancer-associated fibroblasts (CAFs). *Crit Rev Oncogenesis*. 2022;27(1):1-24. doi:10.1615/CritRevOncog.2022043478
51. Shafi AA, Schiewer MJ, de Leeuw R, et al. Patient-derived models reveal impact of the tumor microenvironment on therapeutic response. *Eur Urol Oncol*. 2018;1(4):325-337. doi:10.1016/j.euo.2018.04.019
52. Votanopoulos KI, Forsythe S, Sivakumar H, et al. Model of Patient-Specific immune-enhanced organoids for immunotherapy screening: feasibility study. *Ann Surg Oncol*. 2020;27(6):1956-1967. doi:10.1245/s10434-019-08143-8
53. Porter LH, Zhu JJ, Lister NL, et al. Low-dose carboplatin modifies the tumor microenvironment to augment CAR T cell efficacy in human prostate cancer models. *Nat Commun*. 2023;14(1):5346. doi:10.1038/s41467-023-40852-3
54. Toivanen R, Berman DM, Wang H, et al. Brief report: a bioassay to identify primary human prostate cancer repopulating cells. *Stem Cells*. 2011;29(8):1310-1314. doi:10.1002/stem.668
55. Voabil P, de Bruijn M, Roelofsen LM, et al. An ex vivo tumor fragment platform to dissect response to PD-1 blockade in cancer. *Nat Med*. 2021;27(7):1250-1261. doi:10.1038/s41591-021-01398-3
56. Mutuku SM, Spotbeen X, Trim PJ, Snel MF, Butler LM, Swinnen JV. Unravelling prostate cancer heterogeneity using spatial approaches to lipidomics and transcriptomics. *Cancers*. 2022;14(7):1702. doi:10.3390/cancers14071702
57. Salachan PV, Rasmussen M, Ulhøi BP, Jensen JB, Borre M, Sørensen KD. Spatial whole transcriptome profiling of primary tumor from patients with metastatic prostate cancer. *Int J Cancer*. 2023;153(12):2055-2067. doi:10.1002/ijc.34708
58. Mutuku SM, Trim PJ, Prabhala BK, et al. Evaluation of small molecule drug uptake in patient-derived prostate cancer explants by mass spectrometry. *Sci Rep*. 2019;9(1):15008. doi:10.1038/s41598-019-51549-3
59. Bray LJ, Hutmacher DW, Bock N. Addressing patient specificity in the engineering of tumor models. *Front Bioeng Biotechnol*. 2019;7:217. doi:10.3389/fbioe.2019.00217
60. Lagziel S, Gottlieb E, Shlomi T. Mind your media. *Nat Metab*. 2020;2(12):1369-1372. doi:10.1038/s42255-020-00299-y
61. Astolfi M, Péant B, Lateef MA, et al. Micro-dissected tumor tissues on chip: an ex vivo method for drug testing and personalized therapy. *Lab Chip*. 2016;16(2):312-325. doi:10.1039/c5lc01108f
62. Chakrabarty S, Quiros-Solano WF, Kuijten MMP, et al. A microfluidic cancer-on-chip platform predicts drug response using organotypic tumor slice culture. *Cancer Res*. 2022;82(3):510-520. doi:10.1158/0008-5472.CAN-21-0799
63. Ingber DE. Human organs-on-chips for disease modelling, drug development and personalized medicine. *Nat Rev Genet*. 2022;23(8):467-491. doi:10.1038/s41576-022-00466-9
64. Hughes CS, Postovit LM, Lajoie GA. Matrigel: a complex protein mixture required for optimal growth of cell culture. *Proteomics*. 2010;10(9):1886-1890. doi:10.1002/pmic.200900758
65. Aisenbrey EA, Murphy WL. Synthetic alternatives to Matrigel. *Nat Rev Mater*. 2020;5(7):539-551. doi:10.1038/s41578-020-0199-8
66. Loessner D, Holzapfel BM, Clements JA. Engineered microenvironments provide new insights into ovarian and prostate cancer progression and drug responses. *Adv Drug Deliv Rev*. 2014;79-80:193-213. doi:10.1016/j.addr.2014.06.001
67. Fong ELS, Martinez M, Yang J, et al. Hydrogel-based 3D model of patient-derived prostate xenograft tumors suitable for drug screening. *Mol Pharmaceutics*. 2014;11(7):2040-2050. doi:10.1021/mp500085p
68. Loessner D, Meinert C, Kaemmerer E, et al. Functionalization, preparation and use of cell-laden gelatin methacryloyl-based hydrogels as modular tissue culture platforms. *Nat Protoc*. 2016;11(4):727-746. doi:10.1038/nprot.2016.037
69. Bessot A, Gunter J, Waugh D, et al. GelMA and biomimetic culture allow the engineering of mineralized, adipose, and tumor tissue human microenvironments for the study of advanced prostate cancer in vitro and in vivo. *Adv Healthcare Mater*. 2023;12(14):e2201701. doi:10.1002/adhm.202201701
70. Xu K, Huang Y, Wu M, Yin J, Wei P. 3D bioprinting of multi-cellular tumor microenvironment for prostate cancer metastasis. *Biofabrication*. 2023;15(3):035020. doi:10.1088/1758-5090/acd960
71. Kahl M, Schneidereit D, Meinert C, Bock N, Hutmacher DW, Friedrich O. A fluorescence-based opto-mechatronic screening module (OMSM) for automated 3D cell culture workflows. *Biosens Bioelectr X*. 2023;14:100372. doi:10.1016/j.biosx.2023.100372
72. Paindelli C, Navone N, Logothetis CJ, Friedl P, Dondossola E. Engineered bone for probing organotypic growth and therapy response of prostate cancer tumoroids in vitro. *Biomaterials*. 2019;197:296-304. doi:10.1016/j.biomaterials.2019.01.027
73. Shokoohmand A, Ren J, Baldwin J, et al. Microenvironment engineering of osteoblastic bone metastases reveals osteomimicry of patient-derived prostate cancer xenografts. *Biomaterials*. 2019;220:119402. doi:10.1016/j.biomaterials.2019.119402
74. Sramkoski RM, Pretlow TG, Giaconia JM, et al. A new human prostate carcinoma cell line, 22Rv1. *In Vitro Cell Dev Biol Animal*. 1999;35(7):403-409. doi:10.1007/s11626-999-0115-4
75. Ci X, Hao J, Dong X, et al. Conditionally reprogrammed cells from patient-derived xenograft to model neuroendocrine prostate cancer development. *Cells*. 2020;9(6):1398. doi:10.3390/cells9061398
76. Vijayan RSK, Kihlberg J, Cross JB, Poongavanam V. Enhancing preclinical drug discovery with artificial intelligence. *Drug Discov Today*. 2022;27(4):967-984. doi:10.1016/j.drudis.2021.11.023
77. Subbiah V. The next generation of evidence-based medicine. *Nat Med*. 2023;29(1):49-58. doi:10.1038/s41591-022-02160-z
78. Albanese A, Swaney JM, Yun DH, et al. Multiscale 3D phenotyping of human cerebral organoids. *Sci Rep*. 2020;10(1):21487. doi:10.1038/s41598-020-78130-7
79. Kegeles E, Naumov A, Karpulevich EA, Volchkov P, Baranov P. Convolutional neural networks can predict retinal differentiation in retinal organoids. *Front Cell Neurosci*. 2020;14:171. doi:10.3389/fncel.2020.00171
80. Renner H, Schöler HR, Bruder JM. Combining automated organoid workflows with artificial intelligence-based analyses: opportunities to build a new generation of interdisciplinary high-throughput screens for Parkinson's disease and beyond. *Mov Disord*. 2021;36(12):2745-2762. doi:10.1002/mds.28775
81. Meehan TF, Conte N, Goldstein T, et al. PDX-MI: minimal information for patient-derived tumor xenograft models. *Cancer Res*. 2017;77(21):e62-e66. doi:10.1158/0008-5472.CAN-17-0582

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