Preclinical and 'near-patient' models for the evaluation of experimental therapy in prostate and bladder cancer

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Patient-Derived Tumour Models for Personalized Therapeutics in Urological Cancers

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Abstract

Preclinical knowledge of dysregulated pathways and potential biomarkers for urological cancers has undergone limited translation into the clinic. Moreover, the low approval rate of new anticancer drugs and the heterogeneous drug responses in patients indicate that current preclinical models do not always reflect the complexity of malignant disease. Patient-derived tumour models used in preclinical uro-oncology research include 3D culture systems, organotypic tissue slices and patient-derived xenograft models. Technological innovations have enabled major improvements in the capacity of these tumour models to reproduce the clinical complexity of urological cancers. Each type of patient-derived model has inherent advantages and limitations that can be exploited, either alone or in combination, to gather specific knowledge on clinical challenges and address unmet clinical needs. Nevertheless, few opportunities exist for patients with urological cancers to benefit from personalized therapeutic approaches. Clinical validation of experimental data is needed to facilitate the translation and implementation of preclinical knowledge into treatment decision making.

Introduction

Globally, urological malignancies contribute to >750,000 deaths each year and >2 million patients are diagnosed with a urological cancer annually (1,2). Prostate, bladder and kidney cancer are the second, tenth and fourteenth most commonly diagnosed cancer types and the fifth, ninth and sixteenth leading causes of cancer-related death worldwide (2). Important unmet clinical needs for patients with urological cancers include approaches that target therapy resistance, development of anti-metastatic therapy with increased potency, and identification of robust prognostic and predictive biomarkers. Advances in 'omics' research (that is, genomics, transcriptomics, proteomics and metabolomics) have increased understanding of key cancer-promoting genomic alterations, pro-tumorigenic pathways and the role of the supportive cellular and non-cellular tumour microenvironment (3,4). Moreover, potential biomarkers derived from circulating tumour cells and extracellular vesicles have been identified in blood and urine samples from patients with urological cancer (5). However, the effect of these preclinical findings on the management of patients with urological cancers is limited. Current European Association of Urology and American Urological Association guidelines still recommend measurement of serum PSA levels and digital rectal examination for risk assessment in prostate cancer (6,7). Detection of elevated PSA levels is not specifically associated with the presence of prostate cancer as other prostatic diseases such as inflammation, trauma or benign hyperplasia are also known to increase PSA levels. In addition to being uncomfortable for patients, digital rectal examination is relatively inaccurate: only 40–50% of all abnormal prostates identified during digital rectal examination eventually contained cancer (8). Despite the availability of additional tests for prostate cancer, such as the PCA3, SelectMDX and ExoDx Prostate Intelliscore tests, these assays have not yet been implemented in daily clinical practice (6) as clinical effectiveness of PCA3 testing in prostate cancer diagnostics remains unknown and the remaining tests are still in the investigational phase (9-14). Diagnostic tools for bladder cancer currently include cystoscopy and urine cytology. The ongoing search for urinary biomarkers of bladder cancer has not yet resulted in their utilization in daily clinical practice (15). Imaging techniques (for example, CT and MRI) are still the gold-standard methods for diagnosis of renal cell carcinoma (RCC) according to the European Association of Urology guidelines (16) and no screening tests are recommended for RCC, despite preclinical identification of promising candidate biomarkers in serum and urine samples and the availability of experimental tests for RCC (17-19).

In addition to preclinical findings not being effectively implemented in clinical practice, observations in preclinical models are not always representative of clinical treatment responses (20). Different preclinical model systems exist, and each model system has its own inherent advantages and limitations. Preclinical models can be exploited independently or in combination with other models to gather specific knowledge on clinical challenges, but are not able to fully reproduce the in vivo environment. As elegantly summarized in 1979 by the statistician George E.P. Box, "All models are wrong; the practical question is how wrong they have to be to not be useful" (21). To improve the clinical value of preclinical model systems, innovative patient-derived models have been developed. Many preclinical tumour models rely on previously established immortalized cell lines and/or xenografting of tumour cells in immunodeficient hosts. Patient-derived models in uro-oncology encompass multiple model systems, including 3D cell cultures, organotypic tumour tissue slice cultures, patient-derived xenograft (PDX) models, microchamber cultures (22-24) and conditionally reprogrammed cell cultures, and rely on the use of freshly obtained primary biopsy material (25-27). In this review, we describe the strengths and limitations of 3D culture systems, ex vivo cultured tumour tissue slices and PDX models. In particular, we discuss their capacity to improve diagnosis and treatment of urological cancers and thereby to strengthen the field of personalized medicine in uro-oncology.

Key points

- Personalized therapeutic approaches currently have limited use in uro-oncology clinics.
- Discrepancies between preclinical data and clinical outcomes, high drug attrition rates and heterogeneous drug responses indicate the need for additional clinically relevant patient-derived tumour models including 3D cultures, organotypic tissue slices and patient-derived xenograft models.
- Each patient-derived model has advantages and limitations and can be used alone or in combination to gather knowledge on clinical challenges in uro-oncology.
- Co-clinical trials and cross-validation of preclinical results with patient outcomes are expected to advance the implementation of patient-derived models in treatment decision-making.

Personalized therapeutics

Personalizing therapeutics involves tailoring treatments and/or therapeutic interventions to an individual patient. In addition, personalized medicine is dedicated to identifying predictors of therapy response and unravelling the mechanisms that drive tumorigenesis and disease progression in individual patients (28). Combining data from preclinical disease models, including the culture of immortalized cell lines, xenografting of immortalized cell lines in immunodeficient mice and 'omics' platforms, has been crucial to the elucidation of disturbed pathways and disease mechanisms in various diseases. For example, the *TMPRSS2-ERG* fusion gene in prostate cancer and *FGFR3* mutations in bladder cancer have been identified by using the omics platform in combination with preclinical disease models (29-30). Successful translation of experimental findings to patient care requires the routine implementation of clinically relevant patient-derived models. Current preclinical models do not fully reflect clinical reality, as indicated by the discordance between clinical responses observed in patients and the outcomes from preclinical models (20, 31). For instance, in the TGN1412 trial, a dose 500-fold smaller than that tested in animal studies caused severe adverse effects in patients (32). In addition, of the 23 phase II/III trials investigating the efficacy of antitumour vaccines, 18 studies failed during clinical testing and only 4 were successful (33). The high failure rate of drug efficacy trials and the approval of only a few new anticancer drugs by the FDA and EMA in uro-oncology seem to be further indicative of this discrepancy (34-35). Between 2015 and 2020, only four new prostate cancer drugs, six new bladder cancer drugs and four new RCC drugs have been approved by the FDA (36). Furthermore, patients' responses to FDA-approved and EMA-approved compounds are notoriously variable, exemplified by inter-patient differences in clinical responses to chemotherapeutic agents such as docetaxel and gemcitabine (37). The plasticity and genetic instability of urological tumours is challenging to capture in a clinically relevant model system. Important requirements of such systems include the ability to study inter-patient and intra-patient tumour heterogeneity and to examine interactions between cancer cells, the immune system and the supportive tumour microenvironment, including cellular and non-cellular heterotypic tumour–stroma interactions. Additional tumour model requirements are a high success rate, a short generation time and validation of preclinical outcomes with clinical responses.

Table 1 overview of the patient-derived tumour models that are used in uro-oncology research.

The translational value of each model depends on their intrinsic advantages and limitations. PDX, patient-derived xenograft.

Model	Applications	Advantages	Limitations
3D cultures	Drug screening; biomarker discovery; biobank repository	Short culture protocol; suitable for high-throughput screening; small amount of material required	Varying success rate; missing cellular complexity; unknown correlation with clinical response
Organotypic tissue slices	Drug screening; biomarker discovery	Short culture protocol; intact original tumour–stroma interactions; more representative of clinical diversity	No intact vasculature; time-consuming analysis of treatment response; unknown correlation with clinical response
PDX models	Drug screening; biomarker discovery; biobank repository; expansion of tumour tissue	Intact organism; intact tumour–stroma interactions; known correlation with clinical response	Varying success rate; long generation time; replacement by murine stroma; risk of lymphoma development

Patient-derived models

Innovative patient-derived preclinical models have been developed that closely reflect the clinical complexity and diversity of malignant disease. These models use freshly obtained patient-derived tumour tissue from liquid or solid biopsy samples and can include circulating or disseminated cancer cells.

3D culture models

3D cell culture systems employed for urological cancers include organoid, spheroid and sphere cultures (**Figure 1**). Unfortunately, the terminology of the different 3D model systems is not always used consistently, making it difficult to accurately compare the different systems (38). An organoid is defined as a multicellular miniature 3D organ derived from single cells and/or stem cells after tissue dissociation, which in turn can be propagated in vitro. A spheroid consists of a cluster of cells grown in a non-adherent way, whereas a sphere is the culture of cancer stem(-like) cells (38). Organoids have a higher order of complexity than spheroid and sphere cultures and, unlike the other two systems, are composed of multiple heterotypic cell types (for example, epithelial and stromal cell compartments) (38). 3D cultures can be rapidly generated from patient-derived tissues, -normal tissue (39-41), primary tumour tissue (40-44), metastases (43, 45, 46) or circulating tumour cells (from blood or urine) (43) - and other sources such as cell lines or tumours from PDX models (47-48) over timescales ranging from a couple of days to several weeks.

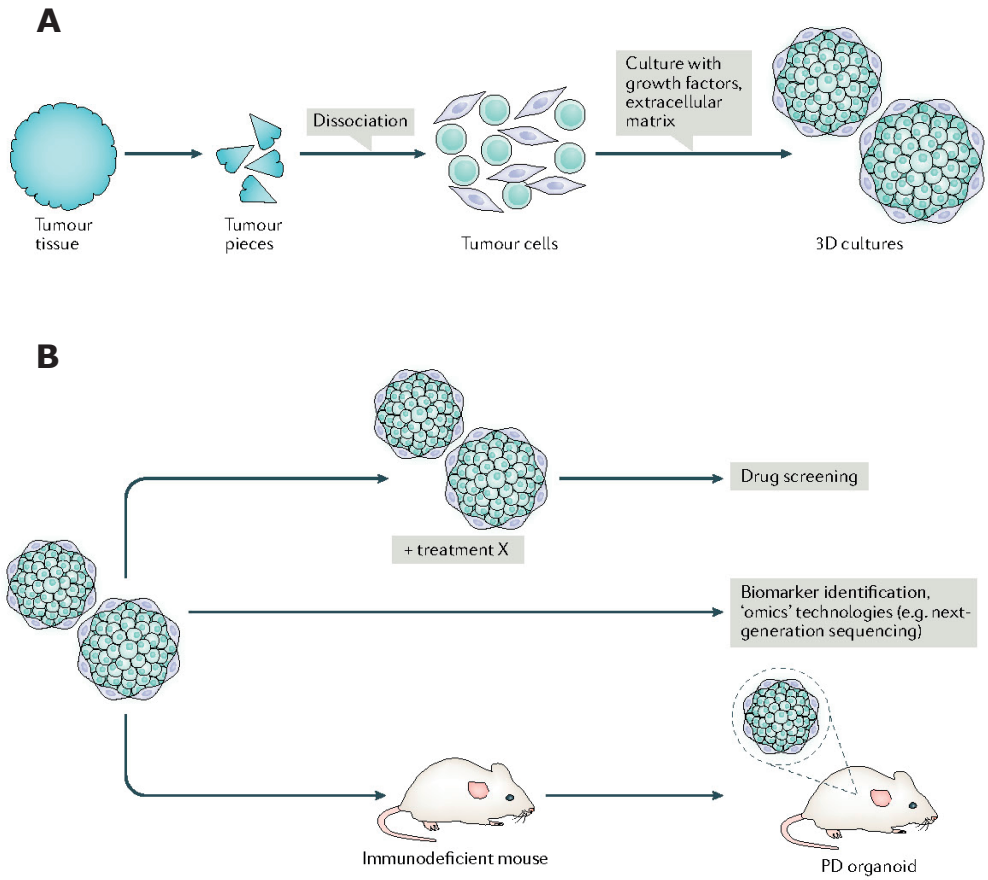


Figure 1 3D culture systems.

*(**A**) Patient-derived tumour tissue is minced and dissociated to obtain a single-cell suspension. Subsequently, the cell suspension is cultured in the presence of specific combinations of growth factors and/or extracellular matrices, resulting in the assembly of 3D cellular cultures. (**B**) Established 3D cultures can be used for drug screening, biomarker identification, molecular profiling ('omics' technologies) or the generation of patient-derived (PD) organoids upon inoculation in immunodeficient mice.*

Multiple protocols for generating 3D cultures are currently in use for urological cancers (39, 40, 42-44, 48-52). In general, tumour tissue is minced and dissociated into a single-cell suspension by incubation of the tumour tissue with collagenase and/or trypsin. After enzymatic digestion, the cell suspension is plated in a culture medium supplemented with specific growth factors and inhibitors.

3D culture protocols differ in the specific composition of culture medium, particularly with regard to growth factors and inhibitors such as R-spondin, Noggin, ROCK inhibitor, epithelial growth factor and fibroblast growth factor and the presence or absence of extracellular matrices composed of Matrigel or synthetic hydrogel (53-55), or 3D scaffolds. To date, the effects of different 3D culture protocols on model generation have not been investigated.

Applications

Owing to the short generation time and the small amount of material required to generate 3D cultures, these models are widely used for screening (56-57). Prior to high-throughput screening of various candidate drugs, 3D models should be validated by assessing the extent to which cultured tumour cells correspond to the original patient tumour. Whether molecular characteristics, such as gene expression patterns and driver mutations, and/or drug responses of the cultured cells are comparable with those of the patient tumour prior to collection of tumour tissue for generation of 3D cultures should be assessed.

High-throughput anticancer drug screening using organoid cultures was initially developed in colorectal cancer (58). Colorectal organoids were exposed to a compound library of different drugs and drug sensitivity was assessed by an automated robotic drug sensitivity screen. A similar high-throughput screening approach with a 127-compound drug library was performed in neuro-endocrine prostate cancer organoids in 2018 and demonstrated heterogenous antitumour responses between the different organoid lines (45). Interestingly, these drug screening efforts indicated that adenocarcinoma prostate cancer organoids were primarily sensitive to enzalutamide, cabazitaxel and docetaxel, whereas neuroendocrine prostate cancer organoids were mainly sensitive to inhibitors of epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor 2 (VEGFR2). This unresponsiveness of neuroendocrine prostate cancer organoids to enzalutamide, cabazitaxel and docetaxel supports the notion that adenocarcinoma prostate cancer can progress towards neuroendocrine prostate cancer after treatment with hormonal therapy as a mechanism of acquired treatment resistance in a clinical setting (59).

In the past 5 years, the generation of ‘living bio- banks’ comprising organoid cultures generated from biopsy samples from a large number of patients with different tumours represents a novel development in patient-derived models. This promising development might complement other approaches such as engrafting 3D cultures in immunodeficient mice.

However, despite including a large number of patients, living biobanks still might not accurately represent the entire population of patients with a given type of cancer, as not all tumours can be successfully cultured. To date, organoid biobanks have been generated for bladder cancer, prostate cancer and RCC (42, 44, 60).The living biobank approach enables drug screening strategies that are based on the genetic profile of the tumour. In a 2018 study, an organoid biobank was generated, using samples from 16 patients with bladder cancer, that reproduced a common mutational status found in bladder cancers, including mutations in *TERT*, *KDM6A*, *CDKN2A* and *FGFR3* (42). However, of the 22 organoid lines only 36% displayed phenotypic stability and showed similar marker expression to the primary tumour. Of the 64% of organoid lines that displayed differences in marker expression compared with the primary tumour, the majority of these organoids (86%) gained a basal phenotype and expressed keratin 5, whereas the parent tumours expressed the luminal marker keratin 8 (42). These observations are indicative of a phenotypic shift in bladder cancer organoid cultures from the original clinical luminal phenotype towards a more basal phenotype in vitro. This shift suggests that epithelial plasticity exists in 3D cultures, a notion that is further substantiated by the disappearance of basal markers and reappearance of the original luminal phenotype when cancer cells from the organoids were grown in vivo (42). Alternatively, these observations might represent genomic instability, which can lead to clonal expansion and has been previously reported in organoid cultures (61). This potential epithelial plasticity and genomic instability raises the question of whether organoid cultures that display this phenotypic switch or clonal expansion remain representative of the parent tumour and whether the drug sensitivity observed in organoid cultures is representative of the parent tumour. The researchers applied 50 different agents, including chemotherapeutic agents, EGFR inhibitors and FGFR inhibitors, to the bladder cancer organoids, and the results suggested a partial correlation between the mutational status of the organoid and therapy response (42). As this biobank included tissue samples from some patients with bladder cancer who underwent multiple biopsies at different time points, organoid cultures could also be generated from chronologically different lesions from the same patient. Organoid cultures generated from recurrent disease displayed stronger resistance to both MEK (mitogen-activated protein kinase kinase) inhibition and EGFR inhibition than organoid lines derived from the same patient before recurrence (42). These findings demonstrate that organoid cultures can be applied to studying tumour recurrence and monitoring the development of therapy resistance.

A separate 2014 study reported on the generation of organoid cultures from metastatic lesions and circulating tumour cells from seven patients with prostate cancer (46). Sequencing of the established organoid lines revealed mutations in genes that are frequently dysregulated in patients with aggressive prostate cancer, including *TMPRSS2–ERG* fusions, *SPOP* mutation, *SPINK1* over-expression and *CHD1* loss. Androgen receptor (*AR*) gene amplification was also observed in the MSK-PCa2 prostate cancer organoid line. Strikingly, this organoid line was extremely sensitive to enzalutamide, compared with the *AR*-negative organoid line MSK-PCa1. However, to date, whether *AR* amplification predicts response to enzalutamide in the clinic is not entirely clear (62-63). Moreover, whether the mutational profile of prostate cancer 3D cultures can be predictive of therapy response in the clinic remains to be established. For example, key components of the tumour microenvironment that are important in the regulation of therapy resistance and tumour progression might be lacking in 3D cultures (52). In a 2018 study, organoid lines generated from neuroendocrine prostate cancer tumours (45) were representative of the matched patient prostate cancer neuroendocrine cohort, as indicated by overexpression of neuroendocrine genes (for example, *MYCN13*, *PEG1014* and *SRRM415*) and low expression of *AR*. Another organoid biobank included multiple tumour types, including prostate cancer, bladder cancer and RCC (60). However, experimental data on high-throughput screening of urological cancers were not presented in this paper. Unfortunately, organoid biobanks have not yet been developed for penile and testicular cancers, likely owing to the lower incidence of these cancer types than bladder and prostate cancer and the corresponding limited availability of clinical tissues for establishing organoid cultures.

Advantages and disadvantages

In general, 3D cultures are structurally more complex than two-dimensional (2D) cell cultures. 3D cultures display different sensitivity to drug treatments and have an altered gene expression profile compared with monolayer cultures of the same cells (38, 64). A 2014 study reported that 3D spheroid cultures of human prostate cancer cell lines are more resistant than monolayer cultures of the same cell line to docetaxel treatment (65). Similar findings were reported in a 2018 study in which 3D prostate cancer cultures were less sensitive than monolayer cultures to abiraterone (66).

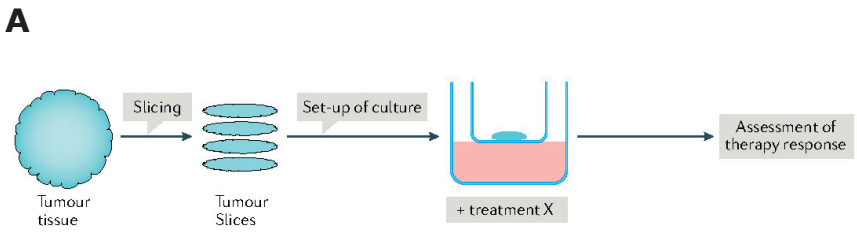
However, in earlier studies, prostate spheroids were more sensitive than 2D cultures to therapeutic intervention with protease inhibitor PS-341 and PI3K-inhibitor NU7026 (67-68), although whether these observed differences in drug sensitivity are due to differences in methodology remains unclear.

Thus, whether 3D cultures are superior predictors of individual therapy responses remains to be determined. Co-clinical trials are warranted to investigate whether 2D or 3D cultures are better predictors of therapy responses.

Additionally, gene expression is often altered in 3D cultures compared with monolayer cultures (64, 68). In a study that compared gene expression in prostate cancer spheroid cultures with gene expression in monolayer cultures of the same cell line, decreased levels of E-cadherin and keratin 18 expression were observed in the prostate cancer spheroids (64). Despite improvement and optimization of culture protocols, 3D cultures often lack the complexity of clinical tumours. Key tumour–stroma interactions are missing in 3D cultures: for example, the heterotypic interactions of cancer cells with the cellular or acellular microenvironment, including mesenchymal stem cells, fibroblasts, immune cells and/or endothelial cells. The generation of hybrid organoids, which are established by co-culturing tumour cells with other cell types including osteoblasts (54-55), (myo)fibroblasts (64, 69), lymphatic cells (70) and endothelial cells (70), is a promising strategy for overcoming these limitations. Owing to the limited number of published studies on 3D cultures that link preclinical results with clinical responses, the exact translational value of 3D culture systems for urological tumours is not yet clear. Whether the observed treatment responses in 3D models can adequately reproduce the sensitivity of patients' tumours to candidate drugs is not yet determined. In this respect, co-clinical trials (studies in which preclinical testing is performed in parallel with human phase I/II trials) can be of clear added value (70-71).

Organotypic tissue slice cultures

Organotypic tissue slice cultures involve the ex vivo culture of explanted pieces of patient-derived tissue. These models maintain the inter-tumour and intra-tumour heterogeneity, cellular or acellular tumour microenvironment, tissue architecture and complexity of the original tumour (73-75) (**Figure 2**).


A
Slicing
Set-up of culture
Assessment of therapy response
Tumour tissue
Tumour Slices
+ treatment X
B
Normal prostate tissue
Normal bladder tissue
Primary prostate cancer
Primary bladder cancer
Bone metastasis (PCa)
C
Pancytokeratin
AR
KRT18
KRT5/6
KRT14
D
Vimentin
CD31
αSMA
FSP1
Type I collagen
E
Non-cultured day 0
Vehicle day 5
Docetaxel 3 nM day 5

◄ Figure 2 Organotypic tissue slice models.

*(**A**) Tumour biopsy samples are sliced and the slices cultured, typically for drug screening purposes.*

*(**B**) Organotypic tissue slices can include both normal and neoplastic tissue types (that is, primary tumours, affected lymph nodes and/or metastases). Epithelial cells are stained in red for pan-cytokeratin, type I collagen is stained in white and nuclei are stained in blue. The effect of specific treatments can be examined on tumour cells, subsets of epithelial cells, stromal cells and extracellular matrix molecules (scale bar: 25 μm). (**C**) Examples of ex vivo cultured tumour slices from prostate and bladder cancers depicting immunolocalization of nuclear androgen receptor (AR)-positive prostate cancer cells with active AR-mediated signalling, luminal epithelial prostate tumour cells (which express keratin 18 (KRT18)), basal epithelial bladder tumour cells (which express KRT5 and KRT6) as well as bladder cancer cells that express KRT14 and pan-cytokeratin (scale bar: 25 μm).*

*(**D**) Moreover, the stromal compartment includes multiple cell types and extracellular matrix molecules, including mesenchymal cells (which express vimentin), endothelial cells (which express CD31), myofibroblasts and smooth muscle cells (which express α-smooth muscle actin (αSMA)), fibroblasts (which express fibroblast-specific protein 1 (FSP1)) and type I collagen. Stromal compartment of prostate cancer tissue (scale bar: 25 μm). (**E**) Apoptosis induction and cell death in ex vivo cultured human prostate cancer tissue in the presence or absence of docetaxel after 5 days of culture. Apoptosis and cleaved caspase 3 activity are depicted in green, luminal epithelial cells (KRT18) are displayed in red and type I collagen is shown in white (scale bar: 25 μm).*

Culture conditions for organotypic tissue slices include static filter inserts (74, 76, 77), rocking platforms (78), sponges (79), or submerging the tissue slices in culture medium (76, 77, 80). Additionally, specialized culture media and differences in oxygen tension (74, 77) are used to maintain tissue integrity, ensure efficient uptake of nutrients and oxygen and facilitate elimination of waste products by the cultured tissue. As cultured tumour tissue lacks a functional vasculature, efficient uptake of nutrients and oxygen and elimination of waste products largely rely on diffusion. To help to ensure adequate diffusion, thin tissue slices of consistent thickness can be generated using a vibratome (75, 76), precision tissue-slicing machines (78, 81) or simply by manual slicing (74, 79, 80). Manual slicing is especially useful for fragile and delicate tumour types such as bladder cancer.

Applications

Organotypic tissue slice cultures are predominantly used to assess drug sensitivity (73, 74, 77) and to determine drug accumulation, uptake (82) and toxicity (83). Research by our group (74) on optimizing ex vivo culture of prostate and bladder cancer tissue involved tissue slices from primary prostate and bladder cancer material, which were cultured on filter inserts in a sealed and oxygenated system.

Tissue slices from five prostate cancer and five bladder cancer tumours were maintained for up to 10 days, providing a therapeutic window sufficient for ex vivo treatment. Treatment of prostate and bladder cancer tissue slices with docetaxel and gemcitabine resulted in an antitumour response indicated by increased levels of cleaved caspase 3 and decreased levels of keratin 18. Subsequently, this model was used in a separate study to investigate the antitumour effects of the antipsychotic drug penfluridol in a panel of bladder cancer tissue slices (84). Penfluridol induced antitumour effects in 35 of 39 bladder cancer tissue slices after 3 days of exposure. In a different study, a heterogeneous response was observed in cultured prostate cancer tissue slices treated with bicalutamide (85). Of the 23 prostate cancer biopsies tested, decreased cellular proliferation occurred in 44% of the treated tissue slices, whereas increased cellular proliferation occurred in 26% and no change was observed in the remaining 30%. Similarly heterogeneous responses were observed in PSA levels secreted by the cultured slices upon bicalutamide treatment. In a subset of 12 tissue slices, decreased PSA levels were observed in 58% of the samples, whereas 42% of the samples displayed increased PSA levels upon bicalutamide treatment. These results highlight the interpatient heterogeneity in tissue slice cultures, which correspond with interpatient heterogeneity in clinical responses to therapy.

In another study that investigated the links between *AR* expression and response to enzalutamide, and between *BRCA2* mutational status and the effect of poly(ADP-ribose) polymerase (PARP) inhibition, prostate cancer tissue slices were generated from PDX models with different *AR* and *BRCA* status and subsequently treated with enzalutamide or the PARP inhibitor Olaparib (76). AR-positive PDX models responded to ex vivo treatment with enzalutamide, as shown by a decrease in proliferation and a concomitant induction of apoptosis. By contrast, AR-negative PDX models did not respond to enzalutamide treatment. Similarly, PARP inhibition had a beneficial effect on tissue slices carrying *BRCA2* mutations but not on *BRCA* wildtype tissue slices, as indicated by stronger reduction in proliferation and an induction in apoptosis in *BRCA*-mutated tissue slices.

Tissue slice cultures can also be exploited to determine the efficacy of oncolytic virotherapy. Bladder cancer tissue slices treated with oncolytic coxsackie virus in combination with mitomycin C showed increased viral replication and increased induction of apoptosis compared with either type of monotherapy (75). In addition to studying the therapeutic effects of compounds on tumour cells, tissue slice cultures can be used to examine the interplay between tumour cells and the cellular or acellular tumour microenvironment, including the immune system. Immune cells are preserved in pancreatic ductal adenocarcinoma tissue slices after ex vivo culture (86, 87).

However, to date, no studies have specifically investigated the interactions between urological tumours and the tumour microenvironment in tissue slices. When co-cultured with pancreatic ductal adenocarcinoma tissue slices, autologous patient-derived immune cells or peripheral monocytic blood cells migrated towards the organotypic tumour tissue slices (86, 87). This strategy might facilitate studies to identify the most appropriate personalized immune therapy (for example, immune checkpoint inhibitors) in urological cancers.

Advantages and disadvantages

Tumor-stroma interactions are of pivotal importance for the progression and therapy response of urological cancers (88-89). In organotypic tissue slice culture systems, tumour cells are studied in their original multicellular and extracellular tissue context with minimal manipulation and isolation steps (74). Thus, organotypic tissue slices have a major advantage over other preclinical model systems, including 2D and 3D culture systems (which require tumour tissue to be dissociated before culture) and PDX models (in which the human stroma is gradually replaced by mouse stroma) (**Table 1**). In organotypic tissue slice cultures, key homotypic and heterotypic cell-cell and cell-matrix interactions in the tumour tissue are minimally disrupted during ex vivo culture. Tissue slice cultures have been reported to maintain tissue architecture and viability for 10 days for prostate and bladder cancer (74-76), thus providing a window of opportunity for experimentation. Additionally, if sufficient tissue is available, multiple regions of the same tumour and/or different tumour foci can be studied, and occasionally, primary tumours and matched metastases can be studied (74). Furthermore, tissue slices obtained from the same patient before and after recurrence can be compared to investigate the potential link between the response observed in tissue slices and clinical outcome in patients. Unfortunately, no such studies have been completed to date for urological cancers.

The tumour tissue slice model represents a straightforward and convenient option as ex vivo cultures are relatively easy to generate. However, obtaining sufficient tumour tissue for tissue slice models is not always possible. This problem can limit the application of the tissue slice model for studying metastatic disease. Additionally, histological characterization of the potential response to anticancer therapy is time-consuming. New automated analysis platforms are available that combine spatial and molecular profiling of multiple targets in a sample (90). These automated analysis tools are crucial to enable high-throughput screening and might facilitate implementation of this model in clinical decision-making. As with 3D cultures, whether the observed preclinical responses in urological tumour tissue slices can predict clinical outcomes remains unclear. Co-clinical trials have already been performed for head and neck squamous-cell carcinoma and colorectal cancer (91), and could similarly improve the reliability of predictions of antitumour responses in individual patients with urological cancers.

Patient-derived xenograft models

PDX models rely on the implantation of tumour tissue or the inoculation of a cell suspension into immunodeficient mice (**Figure 3**). PDX models are used to propagate and maintain patient-derived tumours to generate 'avatar' mice (92). In addition, tumour tissue can be can be expanded in PDX models to study functional genomics, biomarker development, experimental therapeutics and precision medicine (93). A landmark study (94) generated 1,075 PDX models for several solid tumour types, including gastrointestinal, soft tissue, ovary, breast, lung skin, kidney, endometrium, CNS, head and neck tumours and lymphoma. Subsequently, in vivo screens of multiple compounds were performed in the PDX models. Associations between genotype and responses to these agents were observed and several mechanisms of therapy resistance were confirmed, indicating that PDX models could potentially facilitate the prediction of treatment responses.

Applications

Success rates for establishing stable PDX models vary between cancer types and protocols (95). The successful generation of a stable PDX model depends on multiple factors, including the quality of the tumour tissue, the site of engraftment, the choice of immunodeficient mouse strain and the characteristics of the biopsy material.

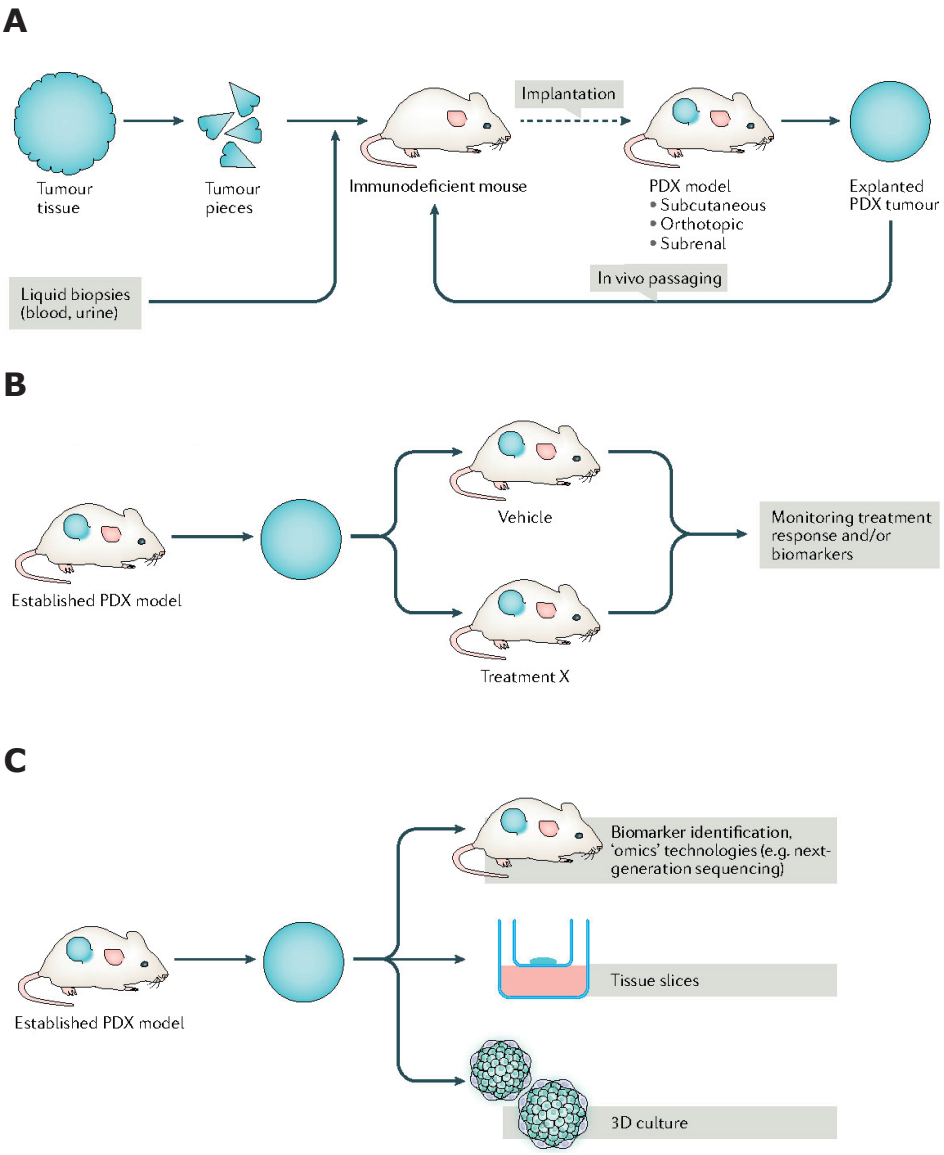


Figure 3 PDX models.

Patient-derived xenograft (PDX) models can be generated from both tumour tissue and cancer cells obtained from liquid biopsy samples. Tumour cells or pieces of tumour tissue can be inoculated or implanted subcutaneously, orthotopically or subrenally in immunodeficient mice (part a). Xenografts can be serially transplanted and used for compound screening in vivo (part b) or expanded for use in other preclinical models, molecular profiling and/or 'omics' platforms (part c).

In a study of PDX models generated from transurethral resection of the prostate biopsy samples, the presence of >50% viable cancer cells, the absence of physical tissue damage and the presence of proliferating cells in the tissue sample were highlighted as important for successful generation of a PDX model (96). Unsurprisingly, engraftment rates correlate with tumour aggressiveness: highly aggressive tumours engraft more successfully than indolent tumours (97). Despite lower success rates than orthotopic and subrenal engraftment, subcutaneous engraftment is commonly used for establishing PDX models as implantation and monitoring tumour growth can easily be performed using calliper measurements or optical imaging. Orthotopic engraftment is used to preserve the original tissue context of the tumour.

Finally, subrenal grafting is frequently used in tumour types with a low engraftment rate as the renal capsule is highly vascularized. However, monitoring orthotopic and subrenal tumour growth is more challenging than monitoring subcutaneous tumour growth. In a study that directly compared different prostate cancer xenograft engraftment sites, subrenal engraftment had the highest success rate (93.4%), compared with orthotopic implantation (71.9%) and subcutaneous implantation (58.1%) (98). However, this study did not report the proportion of engrafted tumours that could be serially passaged over time, which is necessary for the successful generation of a stable PDX model.

Various immunodeficient mouse strains are suitable for the generation of stable PDX models. Each mouse strain is characterized by a different degree of immune deficiency, ranging from moderately immunodeficient T cell-deficient BALB/c-*Foxn1*$^{nu/nu}$ mice to profoundly immunodeficient NSG (non-obese diabetic/ severe combined immunodeficient (NOD/SCID) gamma) mice. For example, the success rate of prostate cancer engraftment was reported to be tenfold higher in immunodeficient NMRI-*Foxn1*$^{nu/nu}$ mice than in immunodeficient BALB/c-*Foxn1*$^{nu/nu}$ mice (99). Thus, the immune status of the host animal is a key determinant of the success of the tumour engraftment (95, 100, 101).

PDX models have been established for human prostate, bladder, renal and testicular cancer (93, 102-107). The collaborative effort of the Movember consortium has resulted in the generation of 98 PDX models for prostate cancer (103). These models represent several clinical disease stages, including androgen-sensitive, castration-resistant, primary and metastatic tumours.

Moreover, validated prostate cancer, bladder cancer and RCC PDX models can now be obtained from several open-access sources, including the National Cancer Institute Patient-Derived Models Repository and the Jackson Laboratory. A 2019 study assessing the effect of supraphysiological levels of testosterone in 13 different prostate cancer PDX models demonstrated that 31% of the tested PDX models showed an antitumour response to supraphysiological levels of testosterone (108). Furthermore, treatment of four enzalutamide-resistant PDX models with supraphysiological levels of testosterone resulted in inhibition of tumour growth in 75% of the models. Pathway analysis of these enzalutamide-resistant PDX models indicated that supraphysiological levels of testosterone reduced ARv7 transcription and decreased Myc-E2F signalling and the DNA damage response. In a separate study of bladder cancer PDX models, the PI3K inhibitor pictilisib had an antitumour effect across multiple models (109). Interestingly, pictilisib had a stronger antitumour effect in PDX models with amplifications in the PI3K pathway than in models without such amplifications. In another study, actionable mutations identified in a panel of 22 bladder cancer PDX models were used to develop a mutational profile, from which FGFR3, EphB4, SRC, HER2, HER3 and PIK3CA were identified as druggable targets (110). However, treatment with an EGFR and HER2 dual inhibitor in two $HER2^{+}$ PDX lines achieved a response in only one PDX model, which indicates that HER2 status is not a reliable marker for predicting a favourable response to EGFR/HER2 inhibition in bladder cancer. Despite the increasing number of studies describing PDX models of prostate cancer, bladder cancer and RCC, only one study has reported the generation of PDX models for testicular cancer (107) and, to date, no PDX models for penile cancer have been reported. This lack of PDX models for penile cancer might be related to the low incidence of penile cancer and the corresponding poor availability of penile tumour tissue for preclinical research, as well as the (potential) low engraftment rates of penile cancer tissue.

Advantages and disadvantages

Tumor tissue from PDX models can be used directly for compound screening and/or biomarker identification. Furthermore, tumour material from PDX models can also be used to generate 3D cultures and ex vivo PDX-derived tumour tissue slice models. As spontaneous formation of lung, liver, brain and bone metastases has been observed in several prostate cancer and RCC PDX models (111-113), these models can also be used to study tumour progression, growth and metastasis.

A major advantage of PDX models is that tumour cells are studied in an intact organism and cell–cell and cell–matrix interactions are better maintained than in other patient-derived models. However, mouse stroma gradually replaces the human parenchyma of the original patient-derived tissue during serial in vivo passaging (114), which is likely to affect tumour–stroma interactions owing to species differences, such as differences in the immune system, between humans and mice, for example (115).

Successfully engrafting a patient tumour in an immunodeficient host is challenging and, therefore, available PDX models do not yet represent the full spectrum of interpatient heterogeneity and clinical disease stages. Prostate cancer PDX models have primarily been established from advanced prostate cancer — that is, from metastatic castration-resistant prostate cancer with Gleason score 9. Similarly, the majority of bladder cancer and RCC PDX models have been generated from high-grade primary tumours (**Supplementary figure 1**). Despite multiple collaborative efforts, low-grade PDX models are still lacking in preclinical urological research. Whether this absence is solely due to the intrinsic properties of low-grade cancer cells, which might lack specific properties that enable these foreign cells to survive and/or thrive in the mouse, or because more patients with aggressive than indolent disease are being sampled, is yet to be determined. The absence of low-grade PDX models hinders the study of processes related to disease progression and therapeutic responses in low-grade tumours.

Owing to the fairly low engraftment rate and slow growth of certain cancer types, especially prostate cancer, the validation and implementation of PDX models are time consuming. Thus, their translational value (for example, for personalized therapeutics and clinical decision making) for patients who donate tumour material could be limited: for example, the tumour could have progressed in the time taken to generate a PDX model. Moreover, it has been estimated that >90% of all people have been exposed to Epstein–Barr virus (EBV) during their lifetime (116). The use of EBV-positive tumour tissue for generating PDX models frequently results in the clonal expansion of EBV-infected lymphocytes in immunodeficient mice (117-118). In patients, numbers of EBV-infected lymphocytes are kept under control by the immune system. However, EBV-positive lymphocytes can expand when explanted into SCID mice.

In these mice, human B cell or T cell lymphomas can develop and overgrow solid tumours (117), as reported in several PDX models of RCC, neuroblastoma, and breast, gastric, colon, pancreatic, testicular, prostate and bladder cancers (117-123). This clonal expansion of EBV-infected lymphocytes has been reported in up to 80% of all PDX models (117-118) and results in the loss of the models and a decreased success rate of establishing PDX models. Thus, screening new PDX tumour models for human lymphoma overgrowth is of critical importance, particularly during the initial in vivo passages in SCID mice.

As the antitumour components of the immune system cannot be studied in SCID mice, humanized mice have been generated for studies in PDX models. Humanized mice are immunodeficient mice that have been engrafted with components of the human immune system such as peripheral blood mononuclear cells or $CD34^+$ haematopoietic stem cells (124-127). Currently, humanized mouse models are increasingly used for engrafting a variety of tumour types, including breast cancer, colorectal cancer, lung cancer, sarcoma and bladder cancer (125-128). A 2018 study generated humanized mice by engrafting $CD34^+$ cells, haematopoietic progenitor cells and stem cells (127). An established bladder cancer PDX model was then subcutaneously implanted in these humanized mice. Treatment of the allogenic human bladder cancer grafts with the PDL1 inhibitor pembrolizumab showed different responses depending on which donors supplied the haematopoietic progenitor cells and stem cells. The results of this study emphasize the importance of the immune system in mediating responses to cancer therapy. Humanized PDX models of other urological cancers, including prostate cancer, are still lacking, despite the large volume of studies aiming to improve the responses of urological cancer to immune therapy. Once established and validated, PDX models are useful for studying drug sensitivity in vivo, either alone or combined with 'omics' approaches and molecular profiling. To date, the predictive value of drug responses seen in PDX models and their clinical utility remain to be established for urological malignancies. Co-clinical trials that compared treatment responses in PDX models with outcomes in patients have already proven useful for other tumour types, such as pancreatic ductal adenocarcinoma (129).

Co-clinical trials

Patient-derived tumour models are predominantly used to identify molecular and cellular mechanisms in tumour biology and drug sensitivity screening before performing clinical trials. Thus, whether the responses observed in patient-derived tumour models correspond with clinical responses in patients has not yet been determined. Co-clinical trials comprise simultaneous preclinical investigations and clinical trials in patients (71, 130-132). In one of the first co-clinical trials (129), drug responses in patients with different types of advanced cancers (including pancreatic cancer, lung cancer, colon cancer, breast cancer, sarcoma and melanoma) were compared with drug responses in a corresponding PDX model. A correlation was observed between the responses in PDX models and clinical outcomes. In 12 of 14 patients, effective treatment regimens were identified based on the outcomes of PDX studies, as indicated by partial remissions. The majority of co-clinical trials use PDX models as the patient-derived model system (130), although some studies have used organoids (133-134). A 2018 study generated organoids from patients with gastrointestinal cancer who were enrolled in a phase I/II clinical trial (133). Patients were treated with anti-EGFR therapy, and the clinical responses of the patients were compared with the responses to anti-EGFR treatment observed in organoids. Despite the presence of EGFR amplification in both the patients' tumours and patient-derived organoids, no response to anti-EGFR therapy was observed in either the patients or the organoids. This result indicates that the patients' mutational profiles were not a good predictor of clinical response, whereas treatment responses in organoids offered improved predictions of clinical responses. In the same study, organoids were generated from sequential tumour samples obtained from patients before and after treatment with paclitaxel. One patient acquired resistance to paclitaxel during treatment. Paclitaxel resistance was observed in organoids derived from the paclitaxel-resistant tumour, and not observed in organoids generated from the paclitaxel-sensitive tumour. Thus, 3D culture models have the potential to reflect patient responses observed in clinical trials, which, once these observations have been replicated in large numbers of patients, might facilitate the study of disease recurrence and therapy resistance. However, additional studies are required to confirm this finding in other tumour types, including urological tumours.

In a phase II trial that examined the effect of alisertib in patients with neuro-endocrine prostate cancer (134), organoids were generated using tumour tissue from one responding and one non-responding patient. Responses of the organoids to alisertib matched those of the responding patient. Despite the small number of patient-derived organoids studied, this trial provides the first indication that patient-derived organoids can be used to predict drug responses in urological cancers. A co-clinical trial involving a PDX model of RCC has also been reported (135). Tumour tissue from patients with clear cell RCC (ccRCC) enrolled in a clinical study was used to generate cell lines and PDX models. Two patients showed a sustained response to sunitinib, which was also observed in the corresponding PDX models. Unresponsiveness to sunitinib was also confirmed in PDX ccRCC models derived from two patients who showed disease progression after sunitinib treatment. Importantly, all cell lines generated from both responsive and unresponsive patients showed similar EC50 values after in vitro treatment with sunitinib. Thus, PDX models of human ccRCC, but not the corresponding in vitro cell lines, reproduce clinical patient responses of ccRCC to sunitinib. To the best of our knowledge, no co-clinical trials have been performed using organotypic tissue slice systems to date. Strikingly, no co-clinical trial studies have yet been reported for other urological cancers, despite the large number of clinical trials being performed for these cancer types. Validation of preclinical responses in co-clinical trials is expected to accelerate the implementation of personalized medicine in urological cancers.

Conclusions

Clinical implementation of patient-derived tumour models is expected to further strengthen the field of personalized medicine in uro-oncology. Each model system has its own intrinsic advantages and restrictions and no single model will be able to address all questions or accurately predict therapy response and drug resistance in all urological cancers. Each of the different patient-derived models has the potential to address a specific combination of unmet clinical and preclinical needs in urological cancers (**Figure 4**).

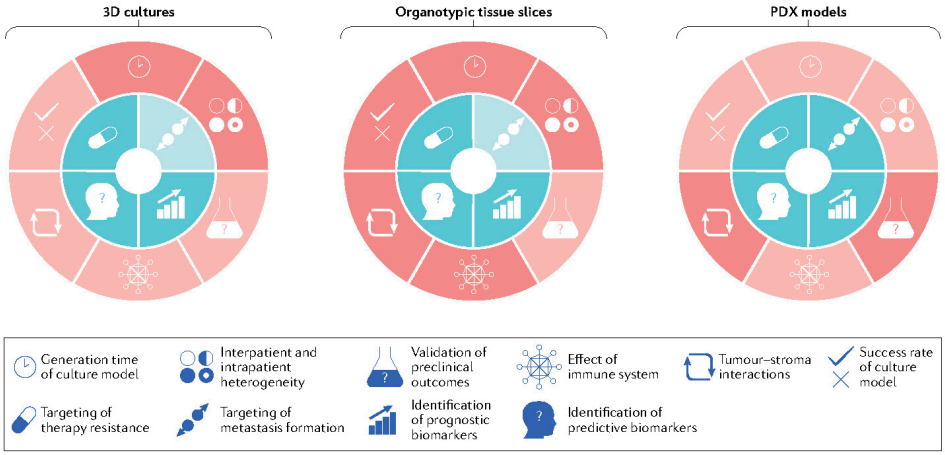


Figure 4 Contribution and translational value of preclinical patient-derived models.

Addressing the unmet clinical needs for patients with urological cancers (blue shading) depends in part on meeting the key challenges facing preclinical tumour models (red shading). Different experimental models can be applied to address specific clinical and preclinical unmet needs and can potentially contribute to improved management of patients with urological cancers. The ideal preclinical model would address all clinical needs and preclinical prerequisites. The characteristics of the three main types of preclinical models are depicted with their intrinsic advantages for addressing specific clinical issues (dark blue) and preclinical questions (dark red). Limitations on the utility of each model to address clinical and preclinical questions are depicted as light-blue and light-red shading, respectively. PDX, patient-derived xenograft.

Thus, combinations of multiple validated patient-derived models are required to address these unmet needs, enabling improved clinical translation of preclinical findings. Increasing the availability and quality of clinical material for preclinical research is of pivotal importance. Technological advances in 3D co-culturing of tumour cells with stromal cells, the generation of humanized mouse models and the development of automated analysis tools enabling high-throughput screening are adding crucial complexity to preclinical urological tumour models, improving their capacity to mimic the clinical environment. The implementation of advanced automated analysis tools will also facilitate clinical translation of experimental findings. The same holds true for the use of preclinical data in the clinical management of patients with urological cancers.

Co-clinical trials involving the generation of patient-derived models in parallel with patient clinical trials are expected to enable the cross-validation of preclinical results with clinical outcomes and facilitate the identification of new biomarkers and treatment strategies. The validation of preclinical data in a clinical setting will pave the way for implementation of validated patient-derived models in future clinical decision-making.

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Author contributions

A.F.v.d.M researched data for the manuscript, A.F.v.d.M, G.v.d.H and G.v.d.P. wrote the manuscript, and all authors made substantial contributions to discussions of content and reviewed and edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

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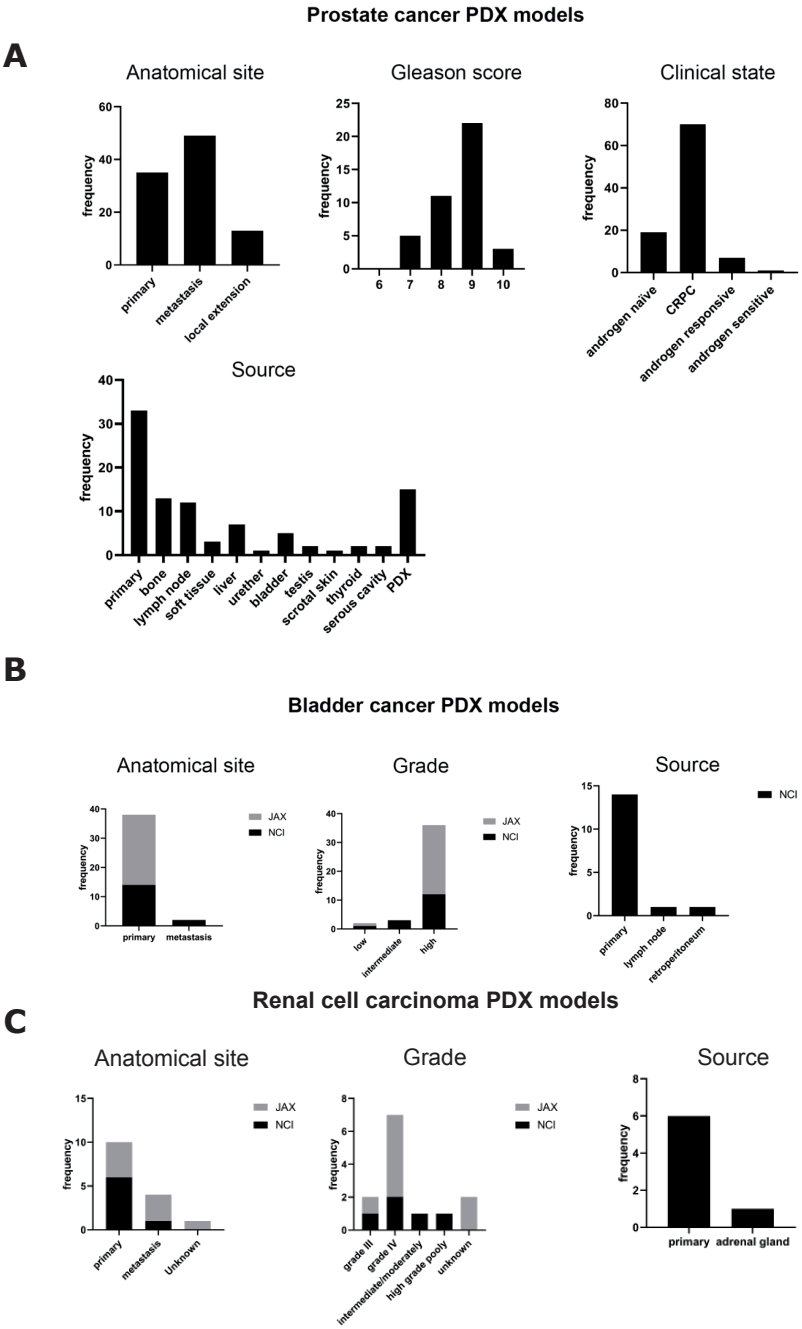

Supplementary figure 1 Clinical characteristics and origin of publicly available, validated PDX models of human prostate bladder and renal cell carcinoma.