Review

Novel patient-derived 3D culture models to guide clinical decision-making in prostate cancer

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Abstract

Castration-resistant prostate cancer remains an incurable disease. The unmet clinical need to optimally select individual treatment options, and thereby maximize survival benefit, can be addressed by patient-specific preclinical models. Patient-derived organoids preserve original tumor characteristics and have shown potential for high-throughput assessments and coclinical drug testing, as highlighted for several cancer types in this review. This new patient-derived 3D culture technique and its downstream applications are the subjects of intense investigation in prostate cancer. Although challenges are not trivial, we expect a major impact on prostate cancer research, with a window of opportunities for early bench-to-bedside translation of new drug discoveries and guidance of patient-tailored disease management.

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Introduction

In recent years substantial advances have been made in local and systemic therapies for prostate cancer (PC), but nonetheless it remains a lethal disease. Although a majority of patients can be treated with curative intent, those presenting with advanced, metastatic, recurrent or progressive disease eventually develop incurable castration-resistant prostate cancer (CRPC). Several new agents for treating CRPC have been introduced recently and additional novel agents are under investigation [1]. Despite these successes in PC drug development, mortality rates are only slowly declining in Western countries [2]. To date, the optimal treatment sequence and/or optimal drug combination(s) to achieve maximal survival benefit in this subset of patients remains unclear [1,3]. Moreover, cross-resistance between different drugs has been described, influencing the potential benefit of subsequent treatments [1,4,5]. The ability to select the optimal (next line) treatment for individual CRPC patients is an unmet clinical need that could potentially be tackled by representative patient-specific preclinical models. Such experimental models will help our understanding in the development of CRPC, the mechanisms of cross-resistance and the optimal sequential or combinational use of available agents.

The establishment of *in vitro* and *in vivo* patient-derived PC models has proven to be very difficult, with long latency times and very low success rates [6−8]. Hence, preclinical PC research has predominantly been founded on a limited number of *in vitro* PC cell lines, with three patient-derived lines (PC-3, DU-145, and LNCaP) dominating the field [6,7]. Although these cell lines have been instrumental for many developments in PC research, they are limited in their reflection of the actual clinical disease, with two of the cell lines lacking androgen receptor (AR) expression, the key driver of PC development and progression [6]. Despite obvious advantages of 2D model systems (e.g., ease in handling, propagation, and maintenance, limited costs), they are compromised by serious limitations, such as aberrant cell behavior regarding cell morphology, polarity, proliferation and migration as a consequence of artificial growth as a monolayer on plastic, absent 3D structure, lack of extracellular matrix, cell-matrix, and cell−cell interactions [7].

Our group and others worldwide have taken the effort to generate human PC patient-derived xenografts (PDXs), with an overall take rate of 10−40% (for a complete overview of internationally available PC PDXs we refer to Navone et al. [8]). These preclinical mouse models more accurately retain the histologic, molecular, and genetic characteristics of the primary patient tumor, and predict patient drug responses more precisely compared to 2D cell lines [9−11]. Although the relevance of PDXs

in PC research is well established, their use is limited to low-throughput assessments and is associated with significant ethical, regulatory, facility, financial, and inter-species concerns.

In recognition of these limitations, novel patient-derived 3D culture technologies are under investigation, aiming to fill the gap between or even replace traditional cancer cell lines and PDXs. Organoids have been shown to maintain patient-specific phenotypic and genetic characteristics [12,13]. Combined with their suitability for high-throughput screening approaches [13], these new techniques can pave the way to early bench-to-bedside translation and personalized medicine. Although a clear consensus is lacking on what to call a spheroid or an organoid, we here define organoids as 3D self-organizing organotypic structures cultured in a 3D matrix [13].

In this review, we explore the possibilities for applying current scaffold-based organoid culture techniques specifically in human prostate cancer, a notoriously difficult cancer to culture *in vitro*. We discuss current insights, applications and challenges of primary patient-derived PC organoids in preclinical research and for early bench-to-bedside translation, as well as the use of PC PDXs, as an intermediate step to generate human PC organoids. These PDXs constitute an indefinite source of human tumor tissue that allows for the necessary optimization of PC organoid culture methods and downstream processing protocols.

In vitro prostate cancer patient-derived organoid cultures

A major advance in the organoid culture technology was achieved by Sato et al., in 2009 [14]. In groundbreaking research, they were able to grow villus-like intestine epithelial organoids by seeding Lgr5-positive mouse intestinal stem cells in Matrigel overlaid with medium supplemented with epidermal growth factor (EGF), R-spondin 1 and Noggin [14]. Since then, the organoid research field has dramatically evolved, with the establishment of long-term patient-derived organoids (PDO) from various healthy [12] and cancerous [13,15] human tissues. In general, following the procurement of a patient sample, (pathologically assessed) tissue pieces are dissected and subjected to enzymatic digestion. Dissociated cells are then incorporated into a basement membrane matrix and covered with tissue-specific medium (Figure 1).

Initial efforts in PC research, using normal prostate tissue from radical prostatectomy (RP) specimens, resulted in human prostate organoids that were cultured for >12 months, without obvious phenotypical or genetic changes over time [16]. Prostate epithelial organoids formed cystic structures composed of an outer basal layer and an inner luminal layer, thus displaying an architecture that resembles prostate glands *in vivo* [16]. Applying this approach to generate renewable primary cancer organoids, however, failed due to overgrowth by normal prostate epithelial cells present within each tumor sample [16]. In a parallel study, Gao et al.

Figure 1
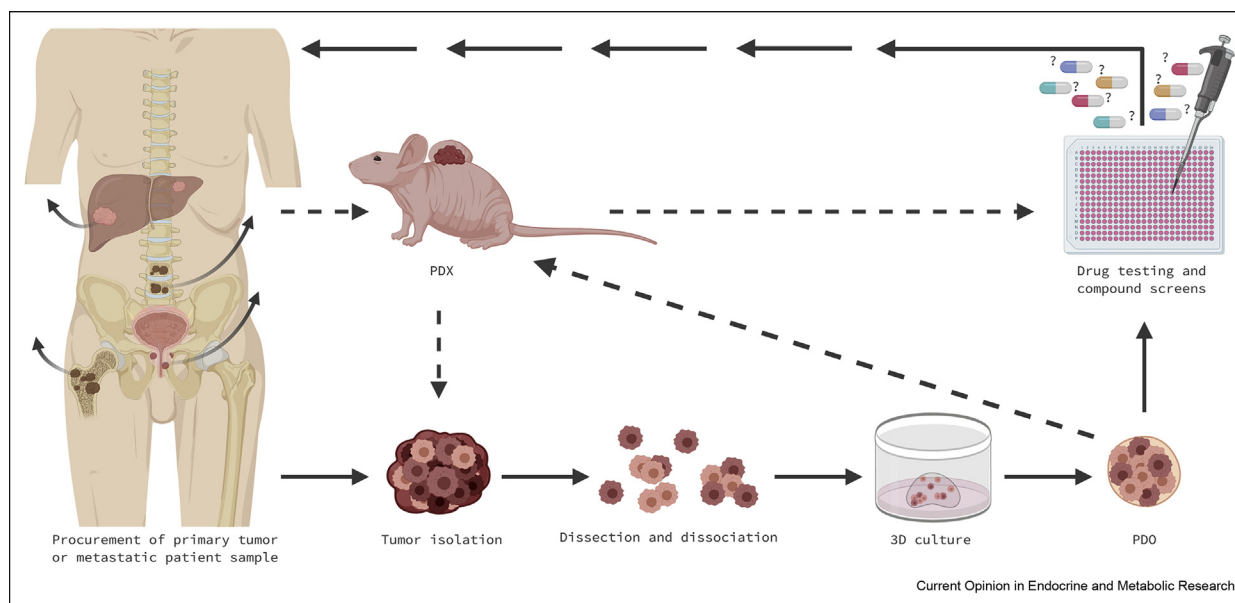
Schematic workflow for generating prostate cancer patient-derived organoids and downstream applications. Dotted lines represent alternative approaches (Created with BioRender.com).

Table 1

Primary patient-derived prostate cancer organoids.

Author	Sample origin	Success Rate (%)	Number of Organoid Lines Reported	Tissue origin of organoid line	*In vitro* maintenance	Organoid Classification
Gao et al., 2014 [17]	Distant metastases (32 attempts: 18 bone, 9 lymph node, 2 liver, 1 brain, 1 bladder, 1 pleural effusion)	18,75%(6/32 attempts)	6	Bone (2), lymph node (2), pleural effusion (1), bladder (1)	>6 months	- Intraductal carcinoma - Adenocarcinoma (high-grade; mucinous; Gleason 4 with cribriform growth; adenocarcinoma with extensive squamous differentiation) - NE tumor
		~ 30%	NR	Bone	1–2 months	NR
		~ 70%	NR	Soft tissue	1–2 months	NR
	Blood (17 attempts)	5% (1/17 attempts)	1	CTCs	>6 months	NR
Puca et al., 2018 [18]	Distant metastases (34 attempts)	11,75%(4/34 attempts)	4	Liver (1), lymph node (1), soft tissue (1), sternum (1)	median 12 months Passage ≥10	NE tumors (pure small cell carcinoma and high-grade carcinoma with extensive neuroendocrine differentiation)
		61%(21/34 attempts)	21	NR	Passage <10	NR
Welti et al., 2018 [19]	Distant metastases (36 attempts)	61%(22/36 attempts)	9 lines available for growth experiments of 7 days	Lymph node (5), bone marrow (4)	NR	NR
Beshiri et al., 2018 [24]	Distant metastases	NR	4	Spine (1), neck (2), liver (1)	Passage>6	- AR + adenocarcinoma - AR + carcinoma with NE features - AR- carcinoma without NE features
Shenoy et al., 2017 [25]	Distant metastases (bone, lymph node, liver)	NR	5	NR	NR	NR
Lambros et al., 2018 [26]	Blood	NR	2	CTCs	NR	NR

NR = not reported; NE = neuroendocrine; CTCs = circulating tumor cells; AR = androgen receptor; CRPC = castration resistant prostate cancer.

successfully generated a panel of seven human prostate cancer organoid lines, using metastatic biopsies and circulating tumor cells, therefore avoiding the presence of normal prostate epithelium. These organoids were continuously propagated for >6 months and demonstrated to harbor known genetic alterations associated with PC [17]. More recently, Puca and colleagues reported on the establishment of four neuroendocrine PC organoid lines derived from needle biopsies of metastatic lesions [18]. Organoids were propagated for a median of 12 months. All four lines lacked AR expression, but expressed classical neuroendocrine markers, thus representing rather rare late-stage PC phenotypes [18]. In both reports, PC organoids displayed (immuno) histological characteristics present in the original patient samples [17,18].

Overall, success rates for generating long-term primary patient-derived PC organoid cultures are low and seem to be dependent on starting material, ranging from 10 to 20% in metastatic biopsies [17,18], over 5% in circulating tumor cells [17] to 0% in RP samples [16]. Nevertheless, it is worth mentioning that success rates for establishing short-term primary PC PDOs are markedly higher, ranging from 60 to 70% [17−19] (Table 1).

Alternatively, human tumor organoids can be generated from PDX tumor tissue [20−22]. Beshiri et al. reported on a PDX-derived organoid biobank to model advanced PC subtypes, by processing 20 LuCaP CRPC PDX models, including adenocarcinoma and neuroendocrine tumors [23,24]. Proliferative organoids were established from all 20 PDXs, and 11 of them were amenable for long-term propagation (>10 organoid passages). Short-term PDX-derived organoids preserved the characteristic genomic features of the original PDX [24].

Drug testing and screening in organoid models of prostate cancer

Prostate cancer is primarily an androgen driven disease, with androgen deprivation therapy (ADT) founding the treatment of advanced and metastatic disease [4,27]. Inevitably, tumors acquire resistance to castration, but even in this castration-resistant state, AR remains the key driver of PC progression as the majority of tumors remains dependent on AR signaling for their growth and survival, making the AR pathway a major target for subsequent treatments [1,28,29]. A prerequisite for preclinical models to represent the clinical disease, therefore, involves the presence and activity of this pathway. Organoids derived from AR-positive PC patients, or PDXs, have shown to preserve an active AR signaling pathway (Figure 2) and/or castration driven AR alterations, making them suitable for AR targeting drug screens and studying resistance mechanisms [17,24]. Welti et al. were the first to use CRPC patient and PDX-derived organoids exhibiting AR mutations, AR amplification, and AR-V7 protein expression to investigate the effect of bromodomain and extra-terminal (BET) protein inhibitors on aberrant AR signaling in CRPC. They reported a growth-inhibitory effect upon the treatment of these organoids, supporting the ongoing clinical studies of BET inhibitors in CRPC patients [19]. Additional to the dominant role of the AR pathway, whole-exome and genome sequencing of metastatic CRPC sample sets revealed that a large subset of these patients harbors additional (potentially actionable) aberrations in other cancer-related genes [29,30]. Reports on PDOs of various cancer types, including PC, show genomic consistency with the original patient or PDX tumor from which the organoids were derived [13,17,18,24,31−33]. This strongly supports the application of PDOs for the *in vitro* testing of relevant, targeted therapies. Recently, Marzi et al. reported on

Figure 2
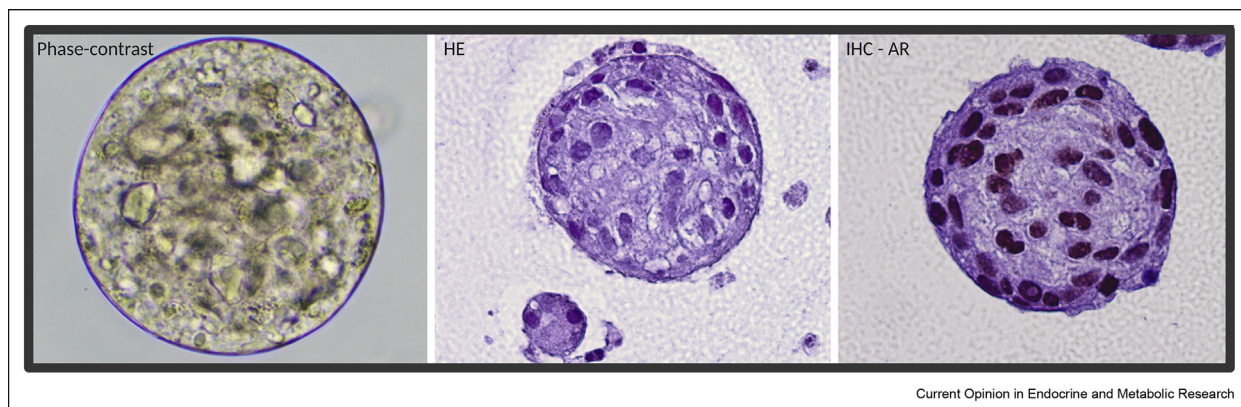
Phase-contrast image (left), hematoxylin, and eosin-stained histological section (middle), and positive immunohistochemical staining of nuclear androgen receptor (right) of a PC PDX-derived organoid (unpublished data).

selective cytotoxicity of TOP1 inhibitors in BRCA2-deficient PC PDX-derived organoids [34]. Shenoy and colleagues generated PDOs from CRPC patients with and without CHD1 loss, a common genomic alteration in PC, and showed its relationship to sensitivity to DNA damaging therapy [25].

In spite of the limited number of compounds and PC organoids included in these first drug testing studies, a major advantage of PDOs is their potential suitability for high-throughput drug screening. This approach is currently under intense exploration in various cancer types, including PC. Some early successes of tumor organoid-based (semi-) high-throughput screens [31–33,35] suggest that this platform could similarly be highly promising in PC research. Jansson et al. reported on the first attempt in PC, by performing a screen on 15 PDX-derived PC organoids using 110 drugs/compounds [36].

Personalized medicine and coclinical applications

With treatment strategies for advanced PC becoming increasingly diverse, the need quickly arises for personalized medicine [1]. This need, however, is not exclusive to PC and has been a major focus of cancer researchers over recent years. Tumor genomics alone is insufficient to identify optimal therapeutic options for many advanced cancer types, suggesting the need for parallel preclinical drug testing to personalize medicine [37]. Vlachogiannis and colleagues reported on a living biobank of colorectal and gastroesophageal cancer organoids and compared organoid responses to targeted agents or chemotherapy with the responses of matched patients. They observed 100% sensitivity, 93% specificity, 88% positive predictive value, and 100% negative predictive value for organoids to forecast responses in patients [38]. In parallel studies, Tiriac and colleagues generated a pancreatic cancer PDO library and established a drug-testing pipeline to generate drug–sensitivity profiles for each organoid. By retrospectively analyzing a small subset of patients from whom the organoids were established, they showed that organoid chemotherapy sensitivity profiles reflect matched patient responses [39]. Recently, in a multicenter prospective study, colorectal cancer organoids were able to accurately predict response in >80% of patients treated with the chemotherapeutic irinotecan. However, organoids failed to predict the outcome for other included chemotherapeutics, with the lack of tumor microenvironment suggested by the authors as one possible reason for this failure [40]. In another study, Ganesh et al. generated 65 patient-derived rectal cancer organoids and demonstrated that organoid responses to clinically relevant chemotherapy and radiation treatment correlated with clinical responses in individual patients [41]. Very interestingly, a proof of concept in

using PC organoids to predict drug responses in matched patients was recently published by Beltran and colleagues [42]. Organoids from two patients with neuroendocrine PC, one exceptional responder and one nonresponder to alisertib, demonstrated a similar trend in response compared to the corresponding patients [42]. Overall, this data suggests that organoids, including PC organoids, can recapitulate patient responses, and therefore, might be suited to guide clinical decision-making.

Challenges and discussion

CRPC remains an incurable disease, and optimal treatment strategies to maximize survival benefit remain unclear [1]. PDOs that preserve patient-specific tumor characteristics and allow high-throughput screening approaches might pave the way to an early bench-to-bedside translation of new drug discoveries and personalized medicine.

Although the establishment of primary patient-derived organoids from various cancer types is well described, limited successes have been achieved in generating long-term PDOs from PC [16–18]. There are several reasons for this limited success. First, as experienced by other groups and us, prostate tumor cells exhibit low proliferation rates, and initial cultures easily become overtaken by noncancerous cells [16–18]. After dissection and digestion of a tumor sample, all cells present in the starting material are embedded in basement membrane matrix, including tumor-associated spindle cells and normal epithelial cells (e.g., normal prostate cells in RP samples or normal epithelial cells present in metastatic biopsies, e.g., lung or liver cells), which easily overgrow PC cells *in vitro* [16,17]. It is unfortunate that detailed information on the persistent presence of these noncancerous cells in long-term cultures is lacking in the original papers [16–18]. Secondly, the availability of PC samples with high tumor cell load is particularly limited as (skeletal bone) metastatic sampling is invasive and is not standard of care. Samples from RP or transurethral resection of the prostate (TURP) are more commonly available, but present difficulties in selecting for tumor organoids during *in vitro* culturing, as a robust PC selectable marker is lacking and normal prostate organoids tend to dominate the cultures (16, unpublished data). Finding the missing link that would enable substantial improvements towards successful *in vitro* PC culture remains a vital concern. Extensive efforts by us and others are directed to fulfill this quest through testing of various manipulable factors, including physical factors, such as oxygenation and pressure, medium components, cocultures with cancer-associated fibroblasts, and conditional reprogramming [24,43–47].

As an alternative, human PC organoids can be cultured with higher efficiency from PC PDX tissue [24], which

is composed of human tumor cells without the presence of human normal epithelial cells. The extensive number of publically available PC PDXs [8] holds the promise that generating a PC organoid panel that reflects a wide diversity of clinical tumors is feasible and this constitutes one of our current aims. By representing several PC patient subtypes, these PDX-derived organoids can be exploited to study mechanisms of cross-resistance, to determine optimal sequential and/or combinational use of existing drugs and to test relevant, targeted therapies within these subtypes. Moreover, they can serve as a base to pioneer organoid-based high-throughput drug discovery. Although the organoid culture technology itself is gradually becoming custom, its downstream drug testing and screening applications are still under full development. Preliminary results of large-scale drug screens on organoids are so far encouraging but are exclusively founded on ATP-based cell viability assays (CellTiter-Glo, Promega) [32,33,35,36]. Besides these measurements, phenotypic profiling using additional alternative screening approaches, such as high-content imaging (Figure 3), will contribute to the assessment of more complex biological responses that may better correlate with clinical endpoints. If we can further improve existing success rates for short-term primary patient-derived PC organoids and in parallel develop pipelines to perform large-scale drug testing on these early cultures, we believe this approach could eventually lead the way to personalized medicine.

First coclinical trials to test the feasibility of using organoids for predicting drug responses in matched patients yielded promising results [38−42]. However, for PC, there are still some challenges. As PC is typically a slow-growing tumor *in vitro*, the feasibility to generate,

expand, and screen primary patient-derived organoids within a clinically relevant time frame needs consideration. Also, the heterogeneity of the disease brings the risk of sample bias and/or *in vitro* selection of subclones [48]. Both may result in the incomplete coverage of the full tumor heterogeneity within a single patient, with the consequence of inaccurate prediction of drug response [49]. Although genomic profiles of PDOs showed high concordance to those of the original tumors, several studies have reported differences, confirming selective representation of the patients' disease [33,49−51]. Also, evaluation of genomic changes during long-term PDO propagation is crucial, as genomic drift in cancer models is inevitable [51,52]. Clearly, the way for coclinical trials in PC is to focus on short-term primary PDOs and their feasibility to predict patient responses to conventional treatments (i.e., anti-androgens and chemotherapeutics), eventually allowing for more efficient clinical decision making. In parallel, efforts for long-term cultures should aim to generate a biobank representing relevant subtypes of PC that will further aid fundamental and early translational research.

Finally, current organoid cultures are reported to exclusively contain epithelial constituents [13]. Although these epithelial organoids preserve cell−cell interactions, 3D tumor morphology, and cell-matrix interactions, they still lack critical cell type and supportive stroma components that are assumed to impact tumor behavior and drug response [53,54]. New culture techniques are rapidly advancing to coculture epithelial tumor organoids with various cell types (e.g., stroma [43,55], immune cells [56], bone [57], lung, and endothelial cells [58]), to fully recapitulate the complex *in vivo* tumor micro-environment.

Figure 3
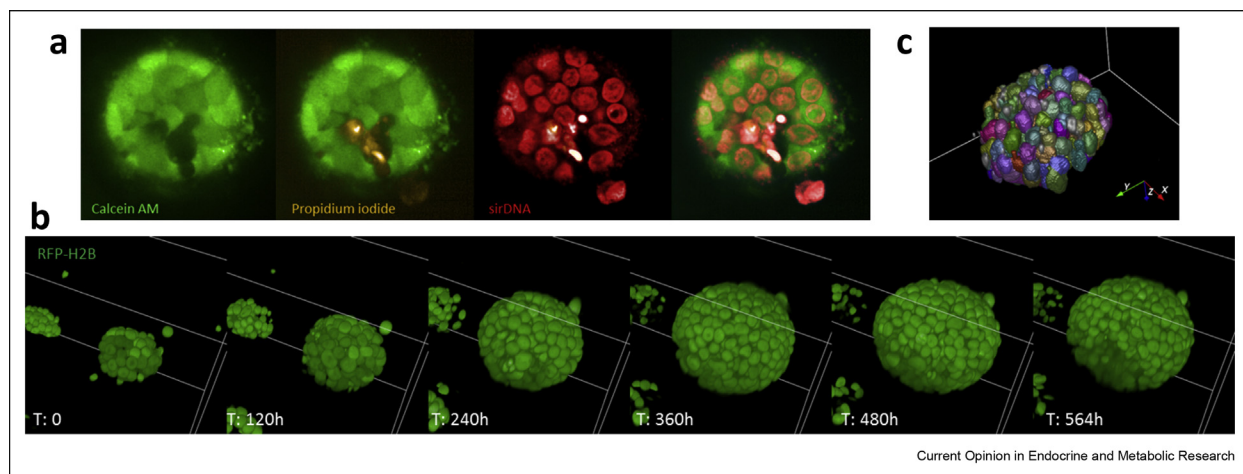
High-content live-cell imaging as an additional approach for organoid-based drug screening. (**a**): Single plane confocal image displaying live/dead cell staining using Calcein AM, Propidium Iodide and SiR-DNA nuclear staining of untreated PC cell line-derived organoids; (**b**): Long-term time-lapse imaging of an untreated PC organoid (3D projection); (**c**): Selection of individual cell nuclei within a PC organoid as a basis for downstream 3D analysis (unpublished data).

Overall, PC PDOs are not without limitations and joint efforts are vital to tackle current challenges. Organoid cultures are likely to stay and may hold great promises in the PC research field. We envision future guidance of patient-tailored disease management by preclinical drug testing in PDOs combined with genomic tumor profiling, that will ultimately meet the clinical need for more personalized medicine.

Conflict of interest statement

Acknowledgements

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Papers of particular interest, published within the period of review, have been highlighted as:

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