Patient-derived xenografts: A platform for accelerating translational research in prostate cancer

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ABSTRACT

Recently, there has been renewed interest in the development and characterization of patient-derived tumour xenograft (PDX) models. Numerous PDX models have been established for prostate cancer and, importantly, retain the principal molecular, genetic, and histological characteristics of the donor tumour. As such, these models provide significant improvements over standard cell line xenograft models for biological studies, preclinical drug development, and personalized medicine strategies. This review summarizes the current state of the art in this field, illustrating the opportunities and limitations of PDX models in translational prostate cancer research.

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1. Introduction

The use of preclinical model systems is central to each step of translational cancer research, ranging from the fundamental biological understanding of the disease to the development of new treatment paradigms. With regard to drug development, the advent of cancer cell line culture techniques in the 1970s fuelled the rapid acceleration and expansion of preclinical testing of anticancer agents both *in vitro* and *in vivo* (Venditti et al., 1984). Currently, xenografts developed by growing cell lines subcutaneously in immunodeficient mice is the ubiquitous platform for preclinical drug development and screening. However, the harsh reality is that about 85% of anticancer therapies fail in early clinical trials, despite significant efficacy in *in vivo* models (Arrowsmith, 2011; Ledford, 2011).

The randomized, phase 3 SYNERGY trial in patients with metastatic, hormone-refractory castration-resistant prostate cancer (CRPC) treated with a standard chemotherapy regimen of docetaxel and prednisone with or without custirsen, an antisense oligonucleotide designed to inhibit production of the cytoprotective protein clusterin, led to unexpected disappointing results (Chi et al., 2015). The addition of custirsen to standard chemotherapy failed to significantly improve survival. Preclinical studies, however, suggested that inhibition of clusterin could be beneficial as treatment with custirsen slowed tumour growth and resensitized treatment-resistant cell lines and tumours to chemotherapy (Sowery et al., 2008; Zellweger et al., 2001; Gleave and Miyake, 2005). Also, a small randomized phase II trial testing the combination of custirsen with docetaxel/prednisone showed an increase of sensitivity of tumours to combination therapy, leading to a 50% reduction in the rate of death in patients receiving custirsen (Chi et al., 2010). Why, then, was there not a benefit in the phase III setting? The observations described above confirm, once again, a failure in the translational process and an urgent need to develop more relevant preclinical models of prostate cancer.

Preclinical models, unfortunately, seldom mirror drug efficacy and outcomes in clinical trials (Johnson et al., 2001). Although the underlying cause of this poor predictive value is not fully understood, emerging evidence suggests that the process of generating cell lines yields major alterations in biological properties, including gain and loss of genetic information as well as modifications in invasive capabilities and the reliance on specific growth and survival pathways (Gillet et al., 2011; Hausser and Brenner, 2005;

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Daniel et al., 2009). In addition, cell line models are not representative of the complex heterogeneity evident in the clinic, partly due to increased homogeneity after long-term *in vitro* culturing. Finally, these model do not possess the tissue architecture of the original tumour and, consequently, do not accurately recapitulate the complex interactions between the tumour cells and various components of their microenvironment (reviewed in (Choi et al., 2014)).

In an attempt to circumvent these issues, there has been increasing interest in the application of more advanced preclinical models, including PDX as well as genetically engineered mouse (GEM) models and short-term primary cultures or organoids (Fig. 1). PDX models are not new; studies conducted in the 1980s demonstrated a high degree of correlation between clinical response in lung cancer patients treated with cytotoxic therapy and PDX models generated from these patients (Fiebig et al., 1985). In recent years, there has been a renewed interest in developing and utilizing PDX models to improve the drug discovery process. Indeed, a recent phase II clinical trial integrated PDX models to better assess the efficacy of cabozantinib in CRPC patients (Varkaris et al., 2016). Utilizing more relevant preclinical models to test anticancer agents before the implementation of clinical trials can possibly reverse the failures of phase III trials and open a new era of translational research.

2. Methodological aspects of prostate cancer PDX models

The process of generating PDX models in mice from fresh primary or metastatic human prostate tissue has been extensively described in the literature (Wang et al., 2005a; Priolo et al., 2010; van Weerden et al., 1996; Zhao et al., 2010). Briefly, tumours maintained as tissue structures are procured by surgery or biopsy. These tumours are subsequently implanted as small pieces or single-cell suspensions, either alone or in some studies coated with Matrigel or mixed with mouse seminal vesicle mesenchyme (SVM), into immunodeficient mice. Tumour take *in vivo* can be measured by serum level of prostate-specific antigen (PSA), which is not produced by mice and thus must be synthesized and secreted into the blood by the grafted human tumour tissue (Priolo et al., 2010). Table 1 provides a summary of approaches used to generate prostate cancer PDX models.

Defining the most appreciate host mouse strains to develop PDX models is an important consideration. It is generally assumed that more severely immunocompromised models are better suited for PDX generation due to higher engraftment rates. Indeed, NOD/SCID mice and NOD/SCID/IL2γ-receptor null (NSG) mice are routinely employed for developing prostate cancer PDX models. However, one study found no significant difference in engraftment rate between nude (nu/nu) mice (which lack T cells) and NOD/SCID mice (which lack both T and B cells), suggesting the type and extent of immunodefiniency in the murine host does not affect tumour take (Priolo et al., 2010).

A more substantial difference between methods is the site of implantation. The most common site of implantation is on the dorsal region of mice (subcutaneous implantation), although several approaches have implanted primary tumours in the sub-renal capsule (SRC; subcapsular implantation) and anterior prostate (orthotopic implantation) in an effort to increase engraftment success rates. The various sites (subcutaneous, subcapsular, and
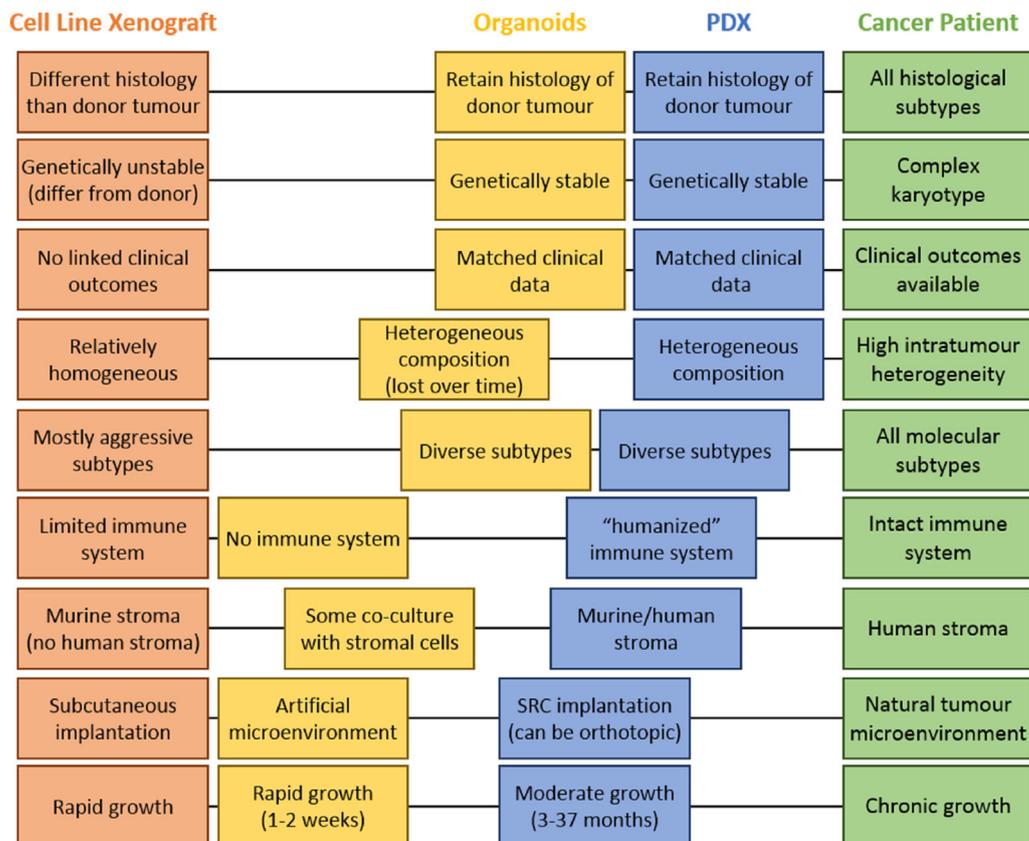
Fig. 1. Comparison of preclinical models utilized in prostate cancer translational research. A "tumour model abacus" illustrates the strengths and weaknesses of patient-derived xenografts in modeling human prostate cancer, as well as a comparison to cell line xenografts and three-dimensional organoid cultures.

Table 1
Summary of Prostate Cancer PDX Models. BHP, benign prostatic hyperplasia; CTC, circulating tumour cell; NR, not reported; ortho., orthotopic; s.c., subcutaneous; SRC, subrenal capsule; TURP, transurethral resection of the prostate.

Tissue origin and number	Source	Processing	Mice strain	Implant site	Take rate	Reference
BHP (15) Primary (26)	Surgery/ biopsy	Fresh tumour pieces	SCID	SRC, s.c, ortho.	93% SRC 58% s.c. 72% ortho.	(Wang et al., 2005a)
Primary (30)	Surgery	Fresh tumour pieces	nu/nu and NOD/SCID	SRC, s.c.	100% SRC 17% s.c.	(Priolo et al., 2010)
Primary (9) Metastasis (3)	Surgery/ biopsy	Fresh tumour pieces	NOD/SCID	SRC	94%	(Lin et al., 2014)
Primary (12)	Surgery	Fresh tumour pieces	NOD/SCID and NSG	SRC	64%	(Toivanen et al., 2013)
Primary (1)	Surgery	Fresh tumour pieces	Rag2$^{-/-}$γc$^{-/-}$	SRC	NR	(Zhao et al., 2010)
Primary (1)	Surgery	Transplanted SRC graft	NOD/SCID	ortho.	95%	(Wang et al., 2005b)
Primary (2) TURP (1) Metastasis (5)	Surgery	Fresh tumour pieces in Matrigel	SCID	s.c.	75%	(Klein et al., 1997)
Primary (2) TURP (3) Metastasis (2)	Surgery	Fresh tumour pieces	BALB/c and NMRI	s.c.	35%	(van Weerden et al., 1996)
Primary (6)	Surgery	Fresh tumour pieces in Matrigel	nu/nu	s.c.	60%	(Pretlow et al., 1991)
Primary (4)	Surgery	Fresh tumour pieces in Matrigel	nu/nu	s.c.	20%	(Pretlow et al., 1993)
Primary (1)	Surgery	Single cell suspension in Matrigel	nu/nu	s.c.	NR	(Wainstein et al., 1994)
Metastasis (1)	Surgery	Fresh tumour pieces	BALB/c	s.c.	NR	(Liu et al., 1996)
CTCs (2)	Peripheral blood	Mononuclear CD45-negative cells	NSG	s.c.	NR	(Vidal et al., 2015)

orthotopic) have theoretical advantages with regard to higher and faster engraftment rates and generation of models that better recapitulate the heterogeneity of human tumours. In earlier studies, tumour tissues were largely grafted subcutaneously; however, the development of these models was hampered by low take rates (20—75%), with engraftment only successful when applied to advanced, metastatic tumours (Wang et al., 2005a; van Weerden et al., 1996). In contrast, subcapsular grafting consistently yields impressive take rates (64—100%), likely because the kidney is highly vascularized (Wang et al., 2005a; Priolo et al., 2010; Toivanen et al., 2013). These grafts were also found to preserve the heterogeneity of the parent tumour with respect to histopathology, genetic profile, and metastatic ability (Priolo et al., 2010; Lin et al., 2014). Orthotopic grafting into the mouse prostate has also been used, which had a 72% take rate (Wang et al., 2005a). While technically challenging to perform, orthotopic grafting may confer a translational advantage as the tumour develops in the same anatomic microenvironment; however, this is partially addressed by using SVM in subcapsular grafts (Lawrence et al., 2013).

Engraftment success can also be affected by hormonal stimulation. As androgens are required for growth and survival of prostate cells, it has been suggested that supplementing mice with exogenous testosterone may enhance the take rate and growth of xenografted human prostate cancer (Wang et al., 2005a; Lin et al., 2014). Indeed, in one study comparing the effects of testosterone on prostate PDX growth, plasma testosterone levels were markedly increased from 1.3 ± 1.1 nmol/L to 30.6 ± 11.4 nmol/L when testosterone implants were used. A higher tumour take rate was observed in mice implanted with testosterone tablets than in the absence of testosterone and, moreover, tumours failed to grow when implanted in female nude mice (Russell et al., 2015).

Most recently, Williams and colleagues developed a standardized protocol for subcutaneous grafting of circulating tumour cells (CTCs) isolated by liquid biopsy from peripheral blood (Williams et al., 2015). They demonstrated that CTCs from CRPC patients with chemorefractory disease are tumourigenic in immunocompromised mice, and the resultant xenografts mirror the castration- and taxane-resistant properties of the donor patient's tumour (Vidal et al., 2015). These unique CTC-based PDX models (colloquially referred to as CTC-derived explants or CDXs), generated from sequentially available, minimally invasive clinical samples could be advantageous for studies seeking to implement real-time PDX data for personalized medicine strategies.

Overall, generating PDX models by sub-renal capsule grafting in highly immunocompromised mice, such as NSG, is the most efficient in terms of take rate (94%) and success rate of transplantable tumour line development (44%) (Wang et al., 2005a; Lin et al., 2014). The use of various extracellular matrices, for example Matrigel, along with exogenous testosterone supplementation provides additional support for the implanted prostate cancer tissue, which is necessary to achieve consistently high tumour take rates of >90% (Russell et al., 2015). Importantly, prostate tissue grafted under the sub-renal capsule maintains the histopathology, differentiation status, and proliferation rate of the donor tumour, even after successive passages through new hosts (Wang et al., 2005a).

3. Prostate cancer PDX models preserve the salient features of the human disease

As mentioned, conventional preclinical models have been criticized for their poor predictive power with regard to clinical outcome (Johnson et al., 2001). Cell lines, and by extension cell line xenografts, undergo extensive evolutionary adaptation to grow indefinitely in artificial culture conditions and thus rarely recapitulate the histology of parental tumours when reimplanted (Wenger et al., 2004; Esquenet et al., 1997). The rationale for developing PDX models is based on the expectation that these models will retain the key biological properties of the original malignancy (for example, histopathology, growth rate, metastatic ability, and response to androgen-ablation therapy) and, therefore, better capture the human disease state and be more predictive of patient response to treatment.

Indeed, studies have demonstrated that prostate cancer PDX models retain the principal characteristics of the donor tumour and that these characteristics are maintained through successive mouse-to-mouse passages *in vivo* (Priolo et al., 2010; Lin et al., 2014; Wang et al., 2005b; Beltran et al., 2015; Kohli et al., 2015).

In particular, fine tissue structure and subtle microscopic details, such as gland architecture, grade of differentiation, and relative abundance of tumour and stroma, are preserved in PDX models. Furthermore, analysis of copy-number alterations and exome sequencing data show extraordinary concordance between patient tumours and the PDX models derived from them. For example, PDX models developed from *TMPRSS2-ERG*-positive tumours, which marks 20%—50% of prostate cancer (Tomlins et al., 2005), expressed the *TMPRSS2-ERG* fusion gene and exhibited high levels of ERG protein (Lin et al., 2014). In other cases, a PDX developed from a neuroendocrine prostate cancer (NEPC) tumour retained the histopathology of the original tumour and expressed markers of neuroendocrine differentiation, namely chromogranin A, synaptophysin, and neuron-specific enolase (Lin et al., 2014). Finally, in a panel of PDXs derived from seven prostate cancer patients, unsupervised hierarchical clustering of copy number segmentation profiles confirmed that paired donor tumours and PDXs cluster together (Lin et al., 2014), further confirming conservation of gross genomic structure.

A principal limitation of cancer cell lines is their inability to capture the inter- and intra-tumoural heterogeneity of clinical prostate cancer, skewing toward subtypes with increased affinity to grow in monolayer culture. Profiling tumours using whole-transcriptome sequencing and microarray analysis in the prostate cancer PDX xenograft panel described by Lin and colleagues suggests that a more complete spectrum of molecular subtypes may be present (Lin et al., 2014). For example, NEPC tumours, which lack expression of the AR and PSA, were successfully established — although this subtype is exceedingly rare in the available prostate cancer cell lines. Similarly, with respect to intra-tumoral heterogeneity, multiple tumour tissue lines have been developed from biopsy foci of a single prostate cancer patient's primary tumour (Lin et al., 2014; 2008; 2010). These tumours retained the histopathology of the original heterogeneous primary tumour, but individually differed in growth rate, metastatic ability, and response to androgen-ablation therapy (Lin et al., 2014). Together, these studies suggest that PDX models preserve the molecular diversity and complex heterogeneity of human prostate cancer.

Of even greater relevance is the remarkable one-to-one concordance between an individual patient's response to chemotherapy and clinical outcome with that of their PDX (Varkaris et al., 2016; Zhang et al., 2013; Fichtner et al., 2008; Garrido-Laguna et al., 2011). In particular, prostate cancer patients whose grafted tumours develop into transplantable tumour lines were found to have significantly poorer overall survival compared to those whose grafts remained static (Lin et al., 2014). The latency (time from initial engraftment to tumour line development) was correlated with matched patients' time to PSA recurrence, providing retrospective prognostic information (Lin et al., 2014). Furthermore, treatment with bicalutamide (an AR-pathway inhibitor commonly used as a front-line therapy in the clinic) initially yielded a dramatic drop in tumour volume and PSA levels in all PDX models developed from adenocarcinoma patient tumours; however 20% of tumours relapsed as CRPC after several months, a scenario also observed in the clinic (Lin et al., 2014). Finally, the NEPC lines continued to grow despite anti-androgen therapy (Lin et al., 2014), further supporting the notion that the therapeutic response in PDX models correlates with activity observed in the clinic.

It is import to note, in contrast to cancer cell lines, PDX models exhibit relatively stable genomes. Mouse to mouse propagation does not significantly change the functional and molecular characteristics of the grafted tumour (Zhang et al., 2013; Li et al., 2013; Rubio-Viqueira et al., 2006; DeRose et al., 2011). A recent study reports that prostate cancer PDX models from different passages (up to 16 generations) do not radically diverge from the original tumour with respect to copy number and gene expression profile (Lin et al., 2014). This phenotypic stability further supports the fidelity of PDX models for translational research.

4. Applications of prostate cancer PDX models in translational research

4.1. Drug screening and biomarker discovery

Achieving success in oncology drug development continues to be challenging. Many compounds are advanced into large phase III trials, at a cost of billions of dollars, to end up failing due to efficacy and safety issues (Hay et al., 2014). Part of the reason for this high rate of attrition is the poor predictive value of the preclinical cell line xenograft models used to screen new agents for clinical development. Moreover, new drugs are often tested without appropriate biomarkers for patient selection and response monitoring.

The rationale for implementing PDX models into preclinical drug discovery relies on the fact that these models preserve the histopathological features and molecular circuitry of the original patient tumours. Recently, using a PDX derived from an AR-driven, metastatic tumour (termed LTL313H) it was demonstrated that tumour regression could be achieved using a novel small molecule inhibitor targeting the amino-terminus domain of the AR, EPI-001 (Andersen et al., 2010). Moreover, using the same LTL313H metastatic xenograft model, it was reported that Aneustat, a multivalent botanical drug candidate, synergizes with docetaxel to elicit strong anti-tumour activity (Qu et al., 2014). Finally, in a CRPC PDX model (LuCaP 96CR) the combination of enzalutamide (ENZ) with prostate specific membrane antibody drug conjugate (PSMA ADA) yielded strong antitumour activity and significantly improved survival over ENZ monotherapy (DiPippo et al., 2016). On the basis of these findings, PDX models may play an important role in developing combination clinical trials to improve the efficacy of current first-line therapies.

Likewise, the search for targeted therapies for NEPC has been hampered by the lack of clinically relevant *in vivo* models. In this regard, a PDX model was recently developed from a NEPC patient tumour (termed LTL352). Utilizing this model, it was discovered that cisplatin in combination with irinotecan, a topoisomerase I inhibitor, decreases NEPC tumour volume without major host toxicity (Tung et al., 2011). Furthermore, the NEPC PDX was integrated into proof-of-principal studies aimed at uncovering the efficacy of the Aurora Kinase inhibitor, PHA-739358 (Beltran et al., 2011). It was found that Aurora Kinase inhibition may potentially benefit NEPC patients, a premise currently being investigated in a phase II clinical trial (NCT01799278).

Equally important to drug screening is the discovery of predictive biomarkers to stratify patients into treatment groups and anticipate emergent resistance. Serial Analysis of Gene Expression (SAGE) of paired metastatic (PCa1-met) and non-metastatic (PCa2) PDX sublines derived from a single patient's cancer led to the identification of *ASAP1*, a gene associated with prostate cancer metastasis that may represent a biomarker for metastatic disease (Lin et al., 2008). Similarly, next-generation sequencing was applied to identify differentially expressed microRNAs in matched metastatic and non-metastatic prostate cancer PDX sublines, of which many were found to target key metastasis-associated genes that could serve as potential biomarkers for prostate cancer metastasis (Watahiki et al., 2011). Most recently, modeling the development of NEPC from a conventional prostatic adenocarcinoma using a PDX (LTL331 model) identified PEG10 as the most upregulated gene during the transdifferentiation process (Akamatsu et al., 2015); thus, it may represent a progression-related biomarker to aid in the

early diagnosis of NEPC.

4.2. Avatar mice and co-clinical trials

Personalized PDX models can be integrated into clinical trials to allow for real-time tumour profiling, with the goal of guiding treatment decisions. So called "mouse avatars," a PDX model developed from a patient enrolled in a clinical trial, are treated with the same experimental agents to emulate clinical response (Malaney et al., 2014). This strategy permits the assessment of drug response simultaneously in the patient and mouse model (known as a co-clinical trial). As tumours evolve and resistance develops, novel combination strategies can be evaluated for efficacy and safety first in the avatar, thereby avoiding ineffective and/or highly toxic therapies in patients.

To evaluate the clinical utility of mouse avatars, Gao and colleagues performed in vivo compound screens on over 1000 established PDXs and obtained strikingly similar results when comparing the drug responses in the PDXs with available clinical data (Gao et al., 2015). In another study, investigators generated avatars from 14 patients with advanced refractory solid tumours, which were subjected to various therapeutic regimes. Notably, in a subset of tumours, actionable alterations such as mutations in NF1 and PI3KA failed to provide any benefit when targeted in the avatar and, accordingly, treatment of the patients with these drugs was not effective (Hidalgo et al., 2011). To date, of the 13 patients that received prospectively guided treatments, 6 achieved a durable partial remission (Hidalgo et al., 2011; Garralda et al., 2014). Together, these studies illustrate the immense potential of the avatar platform for monitoring clinical response and guiding empirical treatment.

A recent study in prostate cancer, the Prostate Cancer Medically Optimized Genome-Enhanced Therapy (PROMOTE) study (Mayo Clinic, USA), has integrated mouse avatars to aid with the development of more effective neoadjuvant therapy for CRPC patients. In this study, tumour samples will be biopsied from patients before and after treatment with abiraterone acetate. The tumours will be sequenced and the most promising agents based on genomic analysis will be tested for efficacy in the avatar model to determine patient treatment.

4.3. Personalized medicine

The approach to cancer therapy is rapidly evolving from "one size fits all" to an era in which individual patients' tumours are molecularly profiled to select the most appropriate treatment. Efforts are underway to develop molecular signatures of prostate cancer to improve patient stratification, predict clinical behaviour, and guide rational treatment decisions (Lapointe et al., 2004; Latil et al., 2003). Furthermore, prostate tumours are now routinely profiled using next-generation sequencing technology to identify genomic alterations that can be therapeutically exploited or act as biomarkers of drug efficacy (Frampton et al., 2013; Grasso et al., 2015; Miyamoto et al., 2015; Azad et al., 2015). For example, BRAF alterations are found in 2–4% of prostate tumours (Grasso et al., 2015); therefore, these patients may benefit from therapies targeting the BRAF/MEK signaling axis. Recently, CTCs and cell-free DNA (cfDNA) have begun to be analyzed as they reflect the intra-tumour heterogeneity and can be acquired though a minimally invasive liquid biopsy to identify biomarkers and actionable therapeutic targets, such as AR splice variants and mutations (Miyamoto et al., 2015; Azad et al., 2015).

The use of PDXs to personalize cancer therapy is actively being explored. Interest in using these models stems from studies that have demonstrated a strong correlation between drug efficacy in PDX models and clinical response. In prostate cancer, for example, a PDX was developed from a NEPC tumour that harboured a homozygous deletion on chromosome 9p21 spanning the 5'-deoxy-5'-methylthioadenosine phosphorylase (MTAP), CDKN2A, and ARF genes. Treatment of mice with methylthioadenosine in combination with 6-thioguanine led to a marked regression in tumour transplants without any significant host toxicity, indicating this drug combination may be efficacious in the patient (Collins et al., 2012). Similarly, whole-genome sequencing of a patient's advanced prostate tumour uncovered a hemizygous deletion of the DNA repair gene FANCA, suggesting the patient may benefit from platinum-based chemotherapy. A PDX was generated from the FANCA-deficient patient's tumour and mice were treated with cisplatin, which, as predicted, caused inhibition of tumour growth (Beltran et al., 2015). Together, these studies demonstrate that combining next-generation sequencing of patient's tumours with PDX models can lead to the identification of novel therapeutic paradigms that will contribute to personalized cancer treatment.

5. Current limitations and opportunities for next-generation PDX models

As with all preclinical models, there are limitations with PDXs that need to be addressed to improve their use in translational research. Several of these issues are technical in nature and include: (i) developing models from tissues obtained via minimally invasive procedures such as fine-needle aspirates and even CTCs; (ii) defining extrinsic factors, such as Matrigel and SVM, that contribute to engraftment efficiency to establish protocols to increase engraftment rates; (iii) establishing orthotopic tumours to improve fidelity by virtue to replicating some aspects of the microenvironment of the donor tumour; and (iv) reducing the latency between engraftment in mice and tumour formation from currently, on average, 22 months (Lin et al., 2014). These technical issues preclude the widespread adoption of PDX models into the clinical decision-making process. Thus, some groups have begun creating artificial PDX models by implanting tumours in three-dimensional culture systems (Gao et al., 2014; Drost et al., 2016). While still in their infancy, organoid models may have important advantages for real-time translational applications, including higher throughput, shorter time to achieve results, and lower cost and animal utilization.

To more closely mimic the genesis of human tumours, several aspects of PDX models require optimization. One important issue is the rapid replacement of human stromal components – including cancer-associated fibroblasts, endothelial cells, and inflammatory and immune-mediating cells – with murine stroma. This new murine stroma may result in changes to paracrine regulation of the tumour and its biological properties, such as drug responsiveness (Ostman, 2012). However, in general, prostate PDX models in early passage retain stromal components and microenvironment features of the donor patient tumour (Choi et al., 2014), and can be used reliably to address the effect of the microenvironment on tumour biology and response to therapy.

Another major limitation of PDX models is the use of mouse strains with varying degrees of immune deficiency, ranging from nude mice to NSG mice, to avoid allograft rejection. This precludes the evaluation of immune-based therapies for prostate cancer, such as checkpoint inhibitors (for example, anti-PD1) and immunostimulants (for example, the vaccine sipuleucel-T). Furthermore, how the suppressed immune system affects drug response and predictability for therapeutic efficacy in human tumours is not known. Interestingly, a recent study suggests that immune-tumour interactions are, at least partially, captured by PDX models as immune cell aggregates and natural killer (NK) cell infiltration was observed

in a PC-3 prostate cancer cell line xenograft established in nude mice (Choi et al., 2016). However, to develop more robust preclinical models, efforts are underway to engineer humanized PDX models, in which the patient-derived tumour is co-engrafted with hematopoietic stem cells to reconstitute the full repertoire of immune cells from the individual donor (Rongvaux et al., 2014; Morton et al., 2016).

Finally, on the point of predictive value, a number of factors are not addressed by PDX models. Of particular concern is the fact that many drugs have pharmacokinetics that are considerably different across species (Peterson and Houghton, 2004). Agents therefore need to be administered based on clinically achievable exposure, not just weight-adjusted clinical dose. Likewise, to utilize empiric testing in PDX models to guide clinical decisions the definition of success needs to be more closely aligned with the desired outcome in patients (namely, stable disease or regression), and not simply reduced tumour growth. For example, a 70% reduction in tumour volume may be highly significant in a PDX model, but in the clinic 30% tumour growth is defined as progressive disease and a treatment failure. While PDX models, at least initially, closely resemble the patient tumour, the next step will be to ensure treatment regimens resemble what can be achieved in patients.

Even if the abovementioned technical challenges are overcome, the cost of developing personalized PDX mice stands as the reigning barrier to their widespread integration into translational research. Tumour grafts can only be maintained in mice and, accordingly, require a more specialized skill set compared to the simple maintenance of cultured cell lines. Moreover, multiple xenograft mice must be established from a single patient tumour to test different drugs and drug combinations. The cost of creating and maintaining the mice can put the price tag of a PDX experiment at tens of thousands of dollars.

In an attempt to better characterize and annotate PDX models as well as develop new methodologies to overcome the current limits of PDXs, a number of groups are actively developing PDX collections and establishing collaborative networks. These networks house hundreds of models with well-annotated biological, clinical, and drug-response data available for dissemination to research laboratories conducting translational research. The Living Tumour Laboratory (Vancouver, Canada) has established over 200 transplantable human cancer tissue lines from a range of primary patient tumours, including prostate cancer. Likewise, the EurOPDX consortium is spearheading the development and characterization of PDX models in Europe and, to date, has established over 1500 models from more than 30 different solid tumour types. Similarly, the newly formed International Breast Cancer Patient-derived Xenograft Consortium has 537 individual PDX lines representing 500 individual breast cancer patients in a biobank available to the research community (Dobrolecki et al., 2016). Together, these PDX collaborative initiatives will facilitate the dissemination of PDX models as well as harmonize study designs and procedures to accelerate the development of novel therapeutic strategies.

6. Conclusions and future outlook

The primary impetus for the advancement of new preclinical models is the well-recognized limitations of cell line xenografts for predicting therapeutic efficacy in the clinic. In many ways, PDX models, which maintain fidelity when compared to the originating tumours, represent a major advancement in that direction. Accordingly, these models are becoming an integral part of the oncology drug development process. PDX models can be used as avatars to guide personalized therapeutic recommendations as well as help identify precision medicine strategies for future patients who have similar molecular characteristics to the originating

patient. However, widespread adoption of PDX methodologies has been precluded by inherent limitations, including low take rate and long latency to model generation, in addition to an underappreciation for human stroma and immune-related elements. Clearly, at present, PDX models need to be viewed as complementary to other preclinical models, such as organoid cultures, which are well suited for large-scale drug screens. Although the concept and initial establishment of PDX models has been in existence for decades, their value in translational research is just now becoming realized.

Conflict of interest

Authors do not have any conflict of interest.

Acknowledgements

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