Patient-Derived Xenografts in Oncology

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1 Introduction

Cancer is among the leading causes of death worldwide. In 2012, approximately 14 million new cases and about eight million cancer-related deaths were reported. In the United States, it is estimated that approximately 1,685,210 new cases of cancer will be diagnosed and 595,690 people will die from the disease (www.cancer.gov). Scientists for many years have been investigating cancer biology in an attempt to understand its development, mechanisms of progression and identify therapeutic agents to treat malignancies arising from various tissues. Animal models have long been used to investigate cancer biology providing an avenue to explore therapeutic efficacy of anti-cancer drugs and/or contribution of the immune system to tumor immunity. There are many models, ranging from in vitro culture systems utilizing human cell lines, to genetically engineered mouse models harboring specific genetic alterations, to mouse allograft models, to patient-derived xenograft (PDX) models. These existing investigational platforms vary in terms of their potential to recapitulate cancer biology as seen in patients [1, 2].

1.1 Generation of PDX Models (PDX Defined)

Patient-derived xenografts as the name suggests (PDX) encompasses the process of developing and maintaining (tumor) tissue obtained from a cancer patient an which is introduced into a secondary recipient host such as immunodeficient mice or rats typically by direct implantation of the human tumor cells. The source of tumor cells could

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be cell lines derived from human tumors after repeated cultures in vitro, fresh human tumor tissue obtained during re-sectioning surgery or biopsy either from primary site or metastatic lesions, tumor cells collected from malignant ascites or from blood, or tumor cell suspension after disaggregating the whole tumor. In the case of whole intact tumor tissue, this is often cut into small pieces of about 3–8 mm^3 prior to implantation. Upon engraftment of tumors in the first cohort (often termed F_0) of recipient mice, the growing tumors are removed and serially grafted onto another cohort over several passages (from $F_1 \cdots$ to F_n). Use of patient undissociated tumor specimen is considered to be superior in terms of overall tumor engraftment rate or "tumor take," an observation that could be attributed to the preservation of the tumor architecture and clonal population within the tumor [3]. Engraftment rates vary considerably between different models and different cancer types ranging from 25 to 100% [3]. Section 1.1.1 provides a general step-wise protocol for developing a standard PDX in immunodeficient NSG mice using fresh human cancer specimen and Fig. 1 provides a simplistic pictorial explanation of the basic process involved in establishing a PDX model.

Recipient mice commonly used in PDX models are the athymic nude which lack T cells but still have quantifiable adaptive immune system. More stringent than nude mice are the SCID and RAG2 knock out (KO) mice which are generally devoid of lymphocytes that make up the bulk of the adaptive immune system. With the

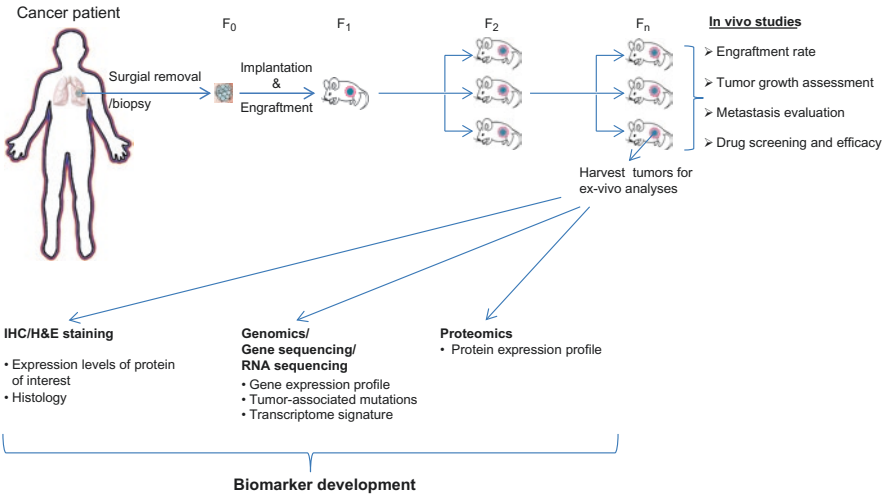

Fig. 1 Patient-derived xenograft establishment, propagation, and utility**.** Tumor specimen obtained from consented cancer patient undergoing surgery or biopsy is implanted unfragmented or dissociated into cell suspension and subsequently implanted either heterotopically or orthotopically into immunodeficient mice. Upon engraftment, tumor is excised surgically from the first cohort of recipient mice (F_1) and serially propagated over a number of passages in vivo (F_2….Fn). Tumor growth properties including engraftment rate and metastasis are evaluated often as part of drug screening and efficacy evaluation studies. Well established tumor grafts can be harvested for ex-vivo studies including immunohistochemistry, H&E staining, genomic studies, DNA and RNA sequencing, and proteomics

demonstrated contribution of NK cells to tumor immunity [4], other models lacking these cells such as the NOD.SCID and NOD.SCID IL-2Rγ KO mice have provided in vivo models considered to be most immunodeficient so far [5–7]. These mice are increasingly being used in both academic and industry-based drug research settings to conduct drug evaluation studies against cancer cells in PDX models. The benefits of these super immunodeficient mice is that they allow for greater tumor engraftment rates ranging from 90 to 100 % and are particularly useful for tumors that are difficult to engraft and tumor-associated leukocyte subsets such as effector memory cells can persist in these hosts for up to 9 weeks after tumor implantation thereby allowing for studying tumor-immune cell cross talks [8].

How PDX implantation is performed differs from cancer types and/or sub-types. Specifically, site of implantation varies and can be heterotopic or orthotopic. Orthotopic simply means implantation at the tissue corresponding to that from which the tumor developed in a patient thereby allowing for studying the behavior of the tumor in its "natural" environment. Heterotopic on the other hand, is any site other than the orthotopic location such as sub-cutaneous (s.c.) or under the renal capsule (sub-renal). The sub-renal implantation method is generally considered better than s.c. transplant model which is typically performed on the dorsal side of the mouse for a number of reasons: (a) engraftment rate is better and tumor architecture is better preserved, (b) most tumor-associated stroma is present at least initially, and most importantly, (c) the tumor genotype and phenotype is closer to the original tumor than tumor of s.c. PDX tumor. The choice of implantation site differs based on cancer types and/or sub-types and varies by investigator. Thus, the tissue of interest could be implanted either sub-cutaneously, sub-renally, or orthotopically. While the former is easier to perform from a technical standpoint, the growth behavior of the implanted tumor may mirror that seen in the patient less well than the orthotopic approach. This would especially be critical for cancer types which derive additional support from the native surrounding tissue.

The number of passages that implanted tumors undergo is also critical to the architecture and overall biology of the cancer cells. It is generally recommended not exceed 5–6 passages as changes in gene expression pattern, gene copy numbers, and chromosomal stability may be altered considerably enough to change the morphology and genetic landscape of the grafts making these parameters divergent from the parental tumor [9–15]. As demonstrated in a number of reports, at low passages, gene expression profiles, histology, and chromosomal stability do not change considerably between the tumor graft and the parent tumor [16–19]. However, the possibility exists that divergent changes may occur with increasing passages and this may be dependent on cancer type/sub-type and genotype. For instance, unlike colorectal PDX tumors with wild type p53 gene, those harboring mutant p53 gene underwent considerable chromosomal changes after several passages [17]. In contrast, genomic alterations were infrequent in a breast cancer PDX model upon passages spanning 2 ½ years [19]. While pancreatic PDX models passaged for up to 39 times revealed only modest genetic alterations [20]. In general, early passage tumors are used for drug treatment studies and there is no cut-off point per say as to which passage is most ideal. For the most part, the general consensus is that the less divergent the PDX tumor is in terms of histology and genetic landscape, the better for ascertaining its clinical relevance.

There are a number of other manipulations that can be performed to the input specimen prior to implantation depending on the aim of the study and endpoints. For example, small tumor pieces or single-cell suspensions can be mixed or coated with matrigel before performing the tissue graft. Other variations of standard PDX include mixing the tumor preparation with cells such as human fibroblasts, mesenchymal stem cells, etc. in an attempt to provide accompanying stromal components of human origin. Regardless of the PDX variation employed, the factors discussed above coupled with tumor type and precision of implantation are considerations that could impact the success of a PDX system and the overall behavior of engrafted tumor.

1.1.1 Materials

- Freshly excised tumor specimens, removed from a patient and stored in RPMI1640/1 % penicillin-streptomycin (Invitrogen 11875-093/15070-063) in a 50-ml conical tube at 4 °C for less than 24 h
- Optional; matrigel basement membrane matrix (BD Biosciences, 356237)
- NSG female mice at 4–6 weeks old (The Jackson Laboratory, 005557)
- Biosafety hood
- Ketamine/xylazine/saline mixture (20:2.5:77.5, v/v/v)
- Buprenorphine (Buprenex, 0.3 mg/ml)
- 0.9 % sodium chloride (BD Biosciences, 306500)
- Hair remover lotion (Pharmacy store)
- Individually wrapped alcohol wipes/sterile gauze pads/cotton swab/10 % povidone-iodine
- *Sterile surgical instruments*, including micro dissecting scissors 4.5-in. straight sharp (Roboz, RS-5916)/castroviejo micro dissecting spring scissors (Roboz, RS-5658SC)/μ dissecting forceps, serrated, full curve (Roboz, RS-5138)/μ dissecting forceps, straight, fine sharp tips (Roboz, RS-5090)/wound clip applier, 9 mm (Roboz, RS-9260)/wound clips, 9 mm (Roboz, RS-9265)/wound clip remover (Roboz, RS-9263)
- *Sterile tissue culture instruments*, including 100-mm Petri dishes/50-ml conical tubes/2-ml microcentrifuge tubes
- Instruments for mouse identification
- *Freezing instruments*, including freezing medium (90 % FBS/10 % DMSO, chilled)/2 ml cryovials (VWR 89094-806)/slower freezing chamber (Nalgene, 5100-0001)

1.1.2 Surgical Implantation Process

1. Wash the tumor sample with ~30 ml of ice-cold RPMI1640/1 % penicillin-streptomycin.
2. Transfer the tumor specimen into a 100-mm Petri dish, cut off cystic or necrotic part of the tumor, and wash the tumor specimen with ice-cold RPMI1640/1 % penicillin-streptomycin.

3. Cut the tumor specimen into about 2×2×2 mm fragments, and then wash the tumor pieces gently with ice-cold RPMI1640/1 % penicillin-streptomycin.
4. Optional; place a 2-ml microcentrifuge tube in ice, add 1-ml matrigel, and then further add the cut-small tumor pieces into the tube (make sure all tumor pieces are covered by matrigel) for minimum of 10 min.
5. Weigh a NSG mouse and anesthetize it with ketamine/xylazine/saline mixture (20/2.5/77.5, v/v/v) at 10 μl/g of body weight by intraperitoneal (i.p.) injection.
6. Dilute buprenorphine 1:10 in sterile saline and inject subcutaneously in the loose skin around the neck and shoulder area at 100 μl/mouse.
7. Apply hair remover lotion to the lower back of mice, wait for 15 min, and then wipe the hair off with gauze pads.
8. Wipe the nude skin area with 10 % povidone-iodine followed with sterilization using alcohol wipes.
9. Using surgical scissors, make a 1–1.5 cm vertical incision on the right flank skin, insert straight forceps into the incision up to ~2 cm, and then spread the skin to create a pocket between the skin and the overlying muscle tissue.
10. Using straight forceps, extract a piece of tumor fragment from microcentrifuge tube in which tumor pieces were collected, insert the tumor piece into the incision, and then gently push the tumor further in the pocket. The tumor pieces in this step may be retrieved already coated with matrigel if this was added into the microcentrifuge tube.
11. Close the skin with metallic staples, wipe the incision site with sterile gauze,
12. Repeat steps 7–11 on the left flank skin.
13. Number the mice and then put them back in their cages.
14. Repeat steps 5–13 for more NSG mice. This gives the first cohort F_0.
15. Add the surgery date to your original cage card
16. Check the mice daily and remove wound staples 7-days after surgery.
17. Monitor tumor engraftment (tumor take) and growth weekly, and prepare to passage it into new mice when the tumor reaches ~10 mm in diameter. It may take 1–12 months for primary tissue to engraft and grow in recipient mice.
18. Euthanize tumor-bearing mice, wipe mouse skin over the tumor with alcohol wipes, surgically remove the skin and surrounding interstitial tissue from the tumor, and then remove the tumor mass from the mouse.
19. See step 1–14 to set up the second cohort F_1. Usually 1–3 generations of transplantation may be required to establish a stable mouse line.
20. Flash-freeze several tumor pieces for biological analyses.
21. If there are excess specimens of original tumor or tumor grafts, transfer them into chilled cryovials containing 1.5 ml freezing medium, cap the vials and invert several times, and then place the vials into a slow-freezing chamber such as Mr. Frosty. Immediately place the chamber in a −80 °C freezer for 24 h and then transfer to liquid nitrogen for long-term storage.

1.1.3 Commercial Resources

As the establishment of PDX studies is cumbersome and requires experience in this approach, commercial companies and other contract research organizations which develop and maintain PDX models world-wide are available. Some of these are listed below:

Aveo Oncology http://www.aveooncology.com *(Cambridge, Massachusetts, USA),*
Charles River Laboratories http://www.criver.com *(Wilmington, Massachusetts, USA),*
The Jackson Laboratory https://www.jax.org (Bar Harbor, Maine, USA)
Champions Oncology https://championsoncology.com *(Hackensack, New Jersey, USA),*
GenScript http://www.genscript.com *(Piscataway, New Jersey, USA),*
AJES LifeSciences, LLC http://www.ajeslifesciences.com/ (Stony Brook, NY, USA)
Taconic http://www.taconic.com *(Hudson, New York, USA)*
SAGE Research Labs https://www.horizondiscovery.com (St. Louis, MO, USA)
Crownbio http://www.crownbio.com *(Santa Clara, California, USA),*
Molecular Response Therapeutics http://molecularresponse.com (San Diego, *California, USA)*
Living Tumor Laboratory http://www.livingtumorcentre.com (Vancouver, British Columbia, Canada)
Pharmaron http://www.pharmaron.com (Beijing, China)
WuXi AppTec http://www.wuxiapptec.com (Shanghai, China)
Oncodesign http://www.oncodesign.com (Dijon Cedex, France)
Urolead http://www.urolead.com (Strasbourg, France)
Urosphere http://www.urosphere.com (Toulouse, France)
XenTech http://www.xentech.eu (Paris, France)
Experimental Pharmacology and Oncology http://www.epo-berlin.com (Berlin-Buch, Germany)
Oncotest http://www.oncotest.com (Freiburg, Germany)
Deshpande Laboratories http://www.deshpandelab.com (Bhopal, Madhya Pradesh, India)

1.2 Other Considerations in PDX Models

1.2.1 Cell Lines Versus Intact Tumor Specimen

In vitro-generated human tumor cell lines are often derived from advanced tumors or poorly differentiated neoplasms. Due to the ease of manipulation, cell lines are commonly used for PDX testing platforms in biopharmaceutical companies. However, selective pressure from cell culture often generates tumor cell population likely arising from least differentiated cells. This may result in a loss

of important biological properties relative to the parent tumor. Secondly, cell lines have the disadvantage that this selective pressure often results in loss of some clones under the in vitro growth condition. Thus, many cell lines may lose the intra-tumor heterogeneity present in the primary tumor and do not represent the inter-tumor heterogeneity found among different patients. PDX models utilizing whole tumor cell preparation either intact or as cell suspension, however, do maintain the architecture, morphology, and histology of the original tumor, hence are closer to providing a more accurate picture of the primary tumor. This allows in principle for investigating different cancers hence capturing the diversity that exists among different sub-type. Furthermore, the cells have adapted to growth in vitro that is different from the natural tumor environment from which they were derived. Adding to this is the observation that genetic mutations do arise as a result of selective pressure in vitro that are distinct from those seen in the patient [21]. For these reasons, mouse xenograft models of human cell lines often have poor predictive value for translating therapeutics of interest to the clinic. While information garnered from their use can say something about drug efficacy in the immunodeficient mice, in many cases, these models only partially predict efficacy in the clinic and in some cases, the PDX data was largely discordant from the clinical trial built upon them [22]. In this regard, PDX models of intact tumor specimen or tumor cell suspension is superior in terms of recapitulating the growth and histopathological features/characteristics of the original patient tumor.

Because of the homogeneity often associated with the tumor cell population in cell lines, they lack primary host-derived stroma. Primary tumor PDX models, on the other hand, have intact stroma, hence, they are an ideal platform to study tumor–stroma interactions. Furthermore, PDX of primacy tumor sample at least in the initial in vivo passage phase generally bear close resemblance in terms of genetic/genomic landscape and overall physiology when compared with the tumors of patients from which the PDX were derived [3, 19]. In the same vein, histology/morphology [21, 23], transcriptome [22] copy number variations [24], and clonal evolution [25, 26] are often not divergent.

While cell lines, intact tumor tissue or dissociated into cell suspension are the more common forms used for implantation, patient cancer biopsy or specimen can be manipulated in other forms prior to implantation. For example, spheroids have been used in some studies. This is essentially a piece of the tumor tissue that is enzymatically digested and grown in specialized chambers with growth medium prior to implantation. This reduces the vigorous manipulation that is typically associated with single cell suspension preparations [27]. Spheroids retain the histology and genetic pattern of parent tumor and likely preserve the tumor-initiating cells. Another option is to sort (tumor cells) from the disaggregated malignant tissue by FACS based on phenotypic expression of relevant cell surface markers. This approach is particularly useful when studies are focused on understanding contributions of certain sub-populations to tumorigenesis such as cancer stem cells, or metastasis such as tumor-propagating cells, etc.

1.2.2 Heterotopic Versus Orthotopic PDX Models

Orthotopic models are generally considered more accurate in terms of histology and gene expression profile between primary tumor and implanted tumor possibly due to effects of the microenvironment. Heterotopic implantation such as sub-cutaneous route, on the other hand, has been shown to produce tumors with growth properties different from orthotopic models, hence may be divergent from what might be seen in a patient [28, 29]. In this regard, they are thought to be better predictors of patient response to drugs compared to heterotopic implantation models. Most s.c. tumors do not metastasize and may be poor models to study highly aggressive tumors with metastatic potential in humans. As demonstrated by others, most s.c. tumor implantations exhibit a rather benign behavior with growth being confined mostly to the local area of implantation [30]. While metastasis is more permissive in orthotopic models, the form in which the tissue is grafted also impacts level of metastasis. For instance, use of tumors disaggregated into cell suspensions or cell lines often yields low frequencies of metastasis [31]. The downside to orthotopic models is that they are time consuming, and may require imaging techniques to confirm successful implantation as well as tumor growth.

1.3 Humanized PDX Models

Given the lack of an intact immune system in the immuno-deficient recipients mice often used in PDX models, they are not suitable for evaluating stroma–tumor interactions and contribution of immune cells which are now being appreciated as key to shaping the course of tumor growth and progression. To circumvent this challenge, immunodeficient mice such as NOD.SCID mice are now being reconstituted with bone marrow or peripheral blood cells along with the patient tumor implant [32]. This approach allows for generation of what is now termed “humanized” xenograft models. These humanized mice provide a valuable platform for studying how the xenogeneic immune cells recruited to the human tumor contribute to the overall anti-tumor immunity. In addition, immunotherapy drugs aimed at mobilizing the effector arm of the immune system can be studied using these models. A key issue that remains unresolved is to what extent the phenotype and function of the human immune cells transferred into the murine environment recapitulate the response of equivalent cells in their primary human host. One might speculate potential reactivity that are driven by human cells recognizing mouse tissue as foreign (not driven by the tumor milieu) may be erroneously interpreted as anti-tumor response especially with ex-vivo assays that attempt to test effector function of T lymphocytes derived from these models using T cell receptor-independent stimulators such as PMA and Ionomycin. There is also the potential graft versus host disease which may develop over time but this can be largely mitigated by keeping the experimental

set up and analysis to a reasonable time frame of 6–10 weeks. As more and more investigators are utilizing this platform as "wholistic" approach to studying tumor biology in concert with associated stroma, efforts are underway to improve these models and apply them to various human cancer types. Currently, there are a number of humanized models mostly for hematologic malignancies such as lymphoblastic leukemia and acute myeloid leukemia [33].

1.4 History of PDX Models

The first PDX was performed by Rygaard and Povlsen when they implanted subcutaneously, a colon cancer specimen from a 71-year-old patient into nude mice which lack T cells. The tumor was reported to have grown as several nodules in the implanted area and exhibited a well differentiated adenocarcinoma histology, similar to that of the patient [34]. PDX performed today are not substantially different from that performed by Rygaard and colleagues [34]. PDX models have been under investigation for several decades with improvements with each succeeding decade. Some of these improvements come from the recipient mice used for the human tissue grafting. Although the use of athymic nude mice has been widespread, newer and more stringent immuno-deficient mice such as NOD.SCID and NOG/NSG mice are gaining traction in their utility especially in experimental settings involving co-implantation of tumor and other cell types.

In a seminal study, Wang and Sordat injected colon cancer cell suspensions into the descending region of the large intestine of nude mice. In this study, local tumor growth as well as metastasis was observed [29]. This was among the first cases of orthotopic implantation as opposed to heterotopic. Hoffman and colleagues were (also) among the pioneers of the orthotopic implantation of intact tumor tissue [35]. Their colon cancer orthotopic PDX model demonstrated robust tumor growth and metastasis. Since then, myriads of various PDX have been conducted in colon, pancreatic, breast, ovarian, lung, head and neck, and stomach cancers [28, 35–40]. The metastatic behavior of these orthotopic tumor grafts seems to be highly concordant with metastatic lesions that developed in patients [28]. In the case of stomach cancer, the primary tumor spread to the peritoneal region and the liver in some patients, an observation that was reproduced when the tumor tissue was placed in mice in PDX studies [28]. Similarly, PDX model of HER2+ cervical cancer was described by Hoffman and colleagues with multi-tissue invasion including lung, liver, peritoneal, and lymph node metastasis in nude mice which reflected the pattern associated with the patient's tumor [32]. Lastly, the efforts of Fidler and co. is thought to be among several key ones that brought some spotlight and interest in the orthotopic tumor models [31].

1.5 Importance of PDX Models

Animal models are invaluable tools for evaluating efficacy, potential toxicity, and side effects of drugs before considering applicability to clinic. PDX models are well suited for this purpose providing an investigational platform to study tumor biology with pre-clinical and translational relevance. Because of their close recapitulation of primary tumors, they allow for investigating tumor biology including cell differentiation, cell death, morphology, architecture, genotype, phenotype, cellular and molecular features associated with tumor growth. As for drug screening, PDX models also offers opportunities to identify biomarkers of drug response/sensitivity providing opportunities for identification of new targets. To a limited extent, they are also of value in pharmacokinetic/pharmacodynamic studies, toxicity studies, and establishing or predicting tolerable dose range at which tumor cells might become sensitive to potential anti-cancer drugs. While PDX models are a great tool for oncology research particularly for pre-clinical efficacy studies, they are often cost and labor intensive. (The formation of) consortia such as the Center of Resource for Experimental Models of Cancer (CreMec), the Translational Proof-of-Concept consortia TransPoc) and the Euro PDX consortium have mechanisms in place to facilitate collaborative research built on PDX models and should be instrumental in making these models of wider application in oncology at reasonable costs.

2 PDX in Various Cancer Types

2.1 Colorectal Cancer

Several investigators have utilized PDX models as platforms to study molecular and genotypic features of colorectal cancer (CRC) [41–44]. Propagation of the tumors in vivo has been conducted with as many as 14 passages as tumor engraftment rate is considered good (at least 70 %) in these models [43]. PDX models are also gaining usefulness in uncovering pathways that may contribute to tumor resistance to drugs. In a CRC study, PDX were treated with cetuximab, an EGFR inhibitor. Assessment of mutation status and gene expression profiling was used to predict sensitivity and response to cetuximab. Mutations in KRAS, NRAS, BRAF and activated MET or low EGFR were assocciated with decreased response. Furthermore, MET activation was considered key mechanism contributing to resistance to cetuximab [45]. In a retrospective study, resistance to cetuximab was predicted based on presence of KRAS, NRAS, BRAF mutations while HER2 amplification appeared to confer resistance in tumors without these mutations demonstrating the utility of PDX models in deciphering molecular mechanisms that may be engaged by tumor cells to circumvent drug efficacy. These studies highlight how data generated from PDX analyses could inform design of treatment regimen to complement or replace existing ones for effective management of cancer sub-types in which such

mechanisms are dominant. As data emerge demonstrating that refractoriness to certain drugs may be in part due to tumor-initiating cancer stem cells, evaluating their distribution within tumors could shed some light on how well as particular sub-type might respond to therapy. Indeed, PDX using patient colorectal cancer tissue was utilized in this context to describe a subset of cancer stem cells [46].

2.2 Pancreatic Cancer

In pancreatic cancer, both cell lines and patient tumor tissue have been used as xenografts. Heavy desmoplasia is a common feature in prostate cancer and one that has implications for drug pharmacokinetics. As cell lines PDX may have less associated stroma compared to whole tissue grafts, results of drug efficacy studies need to be interpreted with caution as they may not mirror drug penetrance in whole tumor xenograft which is often associated with heavy stroma arising from desmoplasia. A number of early studies were conducted in an attempt to identify molecular signatures that could serve as predictive biomarkers [47]. In one study, high basal expression of p70 S6 Kinase was identified as a biomarker predictive of patient's response to mTOR inhibitor treatment [48]. The results from patients, however, were not in alignment with this prediction. By using tumor biopsies and ex-vivo assays to screen for drug efficacies, another pancreatic ductal adenocarcinoma PDX study was able to identify cyclin B1 as a biomarker of response associated with tumor cell growth inhibition by a polo-like kinase inhibitor especially in gemcitabine-refractory pancreatic cancer cells [49]. The use of PDX models as surrogates or xenopatients is also another avenue that has been explored in a number of tumor models including pancreatic cancer. Patient tumor sample is propagated in vivo and tested against a panel of promising agents with the aim of finding regimen that will most likely yield objective response in the patient as informed by the xenograft studies. This approach was explored in a patient whose tumor had PALB2 mutation. Information from genomic studies revealed potential agents with demonstrable anti-tumor activity such as to mitomycin C, a cytostatic agent and cisplatin a chemotherapy drug [50, 51].

The rich stroma often associated with in prostate cancer may in lend itself to immunotherapy. Gemcitabine together with nabplaclitaxel synergized to reduce stroma components and increased the intratumoral levels of gemcitabine, leading to tumor growth inhibition [52]. It is tempting to speculate that the anti-tumor effect seen in this study is in part due to gemcitabine's depleting effect of tumor-associated myeloid cells which are often abundantly represented within the tumor microenvironment [53]. Thus, with a reduction in this immune cell population, which incidentally likely includes immune-suppressive cells (MDSCs, [53], adaptive anti-tumor response becomes less obstructed, potentiating the observed anti-tumor response. Although a phase I/II clinical trial based on these studies showed improved median survival in patients with advanced disease, more studies are warranted to better understand how therapeutic agents with potential to disrupt the tumor cellular composition particularly in pancreatic cancer may shape the outcome of a patient's tumor.

2.3 Lung Cancer

Lung cancer remains among the most prevalent types of cancer. While targeted therapies with small molecule inhibitors have yielded some promising results in patients [54], their effect are often short-lived and there is still a need for agents with potential to effect durable response. In this regard, the use of PDX models have been employed in lung cancer settings to identify mechanisms of drug resistance to existing agents such as docetaxel, and cisplatin or identify biomarkers for predicting therapeutic response such as DNA repair pathways [55, 56].

PDX studies are also well documented for lung cancer [56–58]. In one report, patient tumor specimen were implanted under the sub-renal capsule of NOD.SCID mice which yielded ˃95 % engraftment rate. This study demonstrated the closeness of the PDX tumor to parent tumor in terms of genetic abnormalities but acquired mutation that can occur in persistent tumors [57]. One potential application of PDX models in lung cancer is their use to predict disease course. In a study in which surgically resected tumors from NSCLC patients were used to generate PDX, those samples with successful engraftment were associated with squamous histology with poor differentiation and large tumors in patients who had shorter disease-free survival [59]. This study highlights the fact that post engraftment growth features of xenografts could be considered as predictive of growth pattern in patients and potential for relapse in cases where surgery successfully removed most or all tumor nodules.

Current existing methods of gene sequencing allow for identification of mutations in putative oncogenes or tumor suppressor genes that have the potential to activate or inhibit oncogenic pathways, respectively. What is needed is placing some of these genomic alterations in context with immune activation pathways to gain a more comprehensive understanding of how the regulation of these genes impinges on contribution of the immune system to cancer pathogenesis.

2.4 Melanoma

Murine studies of melanoma have historically used cell lines with varying metastatic potential. While PDX models have been explored, interest in their use in melanoma setting has not been on the same level as other cancers such as breast, lung, ovary, and colon cancer. Reasons for this have been obscure. One early study investigated the effect of anti-tumor agents on cell lines derived from a patient's primary melanoma tissue as well as PDX-derived tumor tissue [59]. There were some concordance in the results seen between the two tumor cell sources but differential sensitivities were also noted for some other agents. These studies were conducted in pre-genomics era, otherwise, they would have benefited from this methodology in terms of understanding molecular networks that may be involved in the discordant findings. More recently, melanoma PDX models have been conducted by multiple

groups including those demonstrating the presence of melanoma-initiating cells that appear to be crucial for tumor propagation [60] and a uveal melanoma PDX model which while demonstrating sensitivity to temozolomide, a chemotherapy drug, had poor engraftment rate [61].

With the availability of gene arrays, one group reported a gene signature (as identified by DNA array analyses) that could be predictive of response to 11 cytotoxic anti-cancer agents in a melanoma PDX study [62]. Given that melanoma harbors genetic mutational load that is atop of the spectrum when considering several cancer types side by side, and neo antigens encoded by these mutated genes have been shown to be immunogenic [63], perhaps, PDX models are no longer as relevant for drug discovery for this particular indication in which the natural immune system may be potentiated to eliminate the disease as opposed to often toxic chemotherapy drugs. Based on this line of reasoning, genetically engineered mouse models or models of spontaneous autochthonous development of tumors may be more clinically relevant.

With the advent of antibodies against CTLA4 which was approved for late stage metastatic melanoma by the FDA in 2011 and anti-PD1, approved in 2014, which show clinical efficacy in a subset of patients with advanced melanoma, we are seeing a shift in the management of melanoma towards immunotherapy. In this regard, PDX models might be of less appeal to research scientists and PDX models which largely focus on biology of tumor cells often in isolation from immune cells may be relegated to the backstage.

2.5 Head and Neck Cancer

Unlike melanoma, mutational load in head and neck cancer is relatively low with reports demonstrating mutations in a few tumor suppressor genes such as TP53 [64, 65]. With limited treatment options including chemotherapy and antibodies such as cetuximab, there is a need for investigational platforms such as PDX models to identify new drugs for this challenging cancer type. A number of PDX models focusing on head and neck cancer have been carried out over the years [66–70] but their predictive value for clinical outcome have been somewhat poor. This may be in part due to sub-optimal tumor take reported in some of these studies or unknown factors intrinsic to the design of the study. Good engraftment of patient tumor in PDX models can be predictive of "aggressiveness" of a tumor type but such correlation has not been well established in head and neck cancers. With respect to efforts to use PDX models for drug testing and validation, a phase II clinical study was conducted testing cisplatin and diaziquone in addition to other experimental agents [71]. This study demonstrated that cisplatin had little efficacy in inhibiting tumor growth in the PDX model while diaziquone showed robust cytostatic effect. However, these results were discordant to clinical observations. Mutations that have been reported to contribute to resistance to cisplatin include *TP53* and *CCND1* amplification, a gene involved in cell cycle regulation [72]. Consistent with this

report, patients with *TP53* mutation had poor prognosis [73–75]. Despite the overall limited efficacy seen with some of these agents, cetuximab remains one of the drugs of choice among targeted therapy drugs. It will be interesting to see whether combining its strong response in a study of head and neck squamous-cell carcinoma (HNSCC) PDX in which 63 % response was seen [45] could be further improved with co-treatment involving an immuno-modulatory agent. In any case, a number of clinical trials are ongoing to bring the use of some of the experimental agents that have been tested in PDX models to the clinic.

2.6 Breast Cancer

Breast cancer PDX models present with unique issues of consideration such as multiple locations for transplantation sites and responsiveness of sub-types to hormones [76]. The murine environment into which they are implanted may affect their growth and behavior. In this regard, building PDX models that span the different sub-types of breast cancer particularly these hormone-driven ones have been somewhat challenging. However, advances in understanding this dependency through molecular pathway methodologies have led to many great PDX studies some of which incorporate hormone supplementation into their PDX platform in an attempt to mimic as closely as possible, the exposure of the tumor cells to these hormones [21, 76–78]. While tumor takes have not been impressively robust in some of these studies, it has allowed for generation of PDX models to study sub-types based on estrogen, progesterone, and HER2-receptor status. These studies have led to data supporting the observation that triple negative (ER-, PR-, and HER2−) breast cancer presents with aggressive growth pattern and metastatic potential in patients. Armed with these observations, PDX models are now continued to be utilized to evaluate potent anticancer drugs to target various breast cancer sub-types. While not applicable to every breast cancer sub-type, it is interesting to note that the engraftment rate and growth of triple negative sub-type in PDX models may be useful as prognostics for patients from which they are derived. In this context, knowledge gained from PDX studies evaluating potential therapeutic drugs of interest could inform of aggressive nature of the disease as well as rational treatment options. While not every PDX model has focused on stromal components, existing reports highlight the contributory role of murine stroma/leukocytes to the tumor microenvironment [21] raising the issue of the extent to which the mouse stromal components alter the human tumor mass. As advances in gene expression profiling methodologies continue, we are likely to see a surge in PDX models aimed at identifying mutations contributing to tumorigenesis. One such mutation, the BRCA2 mutation has been described in which gene expression pattern, the basal-like histology, and stroma showed similarity between the patient tumor and the PDX derivative [78]. With the current era where several "omics" approaches are being explored in an attempt to get a global picture of the overall biology of cancer cells, metabolic pathways through metabolomics is also an area of interest that is being pursued by some investigators [79].

2.7 Prostate Cancer

Limited PDX models exist for prostate cancer in part due to the difficulty in establishing prostate cancer cell lines in vitro. A number of studies have produced successful PDX models with diverse focus [80–84]. The site of implantation is particularly important in prostate cancer PDX as demonstrated by studies in which orthotopic implantation was compared to other sites such as sub-renal and sub-cutaneous spaces [83]. Not surprising, the orthotopic route yielded PDX with best engraftment rate, with tumors with well differentiated histology and which maintained expression of prostate antigen and androgen receptor [83]. Other PDX studies conducted have focused on key biological aspects of prostate cancer pathogenesis including but not limited to angiogenesis [80], genetic mutations [82], tumor stem cells [81], and therapeutic intervention via inhibition of hormones [84]. Given the dearth of PDX studies in prostate cancer, more efforts are warranted for this cancer indication.

2.8 Renal Cell Carcinoma

Both primary patient tumor and cell lines have been utilized for PDX studies in renal cell carcinoma (RCC) [81, 83–88]. The latter has been reported to deviate from the parental tumor as acquired mutations are known to develop with the in vitro culture of RCC cells [89]. A number of treatment options exist for RCC including cytokine therapy and chemo/radiotherapy. However, small molecule inhibitors like Sunitinib and sorafenib, both of which are tyrosine kinase inhibitors [90, 91] are being explored. Using a PDX model, sunitinib was evaluated for anti-cancer effect [90]. This study demonstrated some efficacy but was short lived leading the investigators to implicate epithelial-to-mesenchymal transition as mechanism of resistance [90]. The potential utility of anti-angiogenic drugs as therapeutic agents have also been tested in RCC as studies have demonstrated the human vasculature persists with tumors for a period of time post tumor implantation [80, 92]. Interestingly, sorafenib showed efficacy partially attributed to disrupting inhibition of cell cycle and anti-apoptotic proteins including cyclin B1/D1 and survivin [91]. One of the emerging concepts is combinatorial approaches to target multiple biological processes contributing to RCC. In this vein, an RCC PDX study explored the use anti-angiogenic agent with an inhibitor of mTOR and showed pronounced reduction in blood supply to the xenogeneic tumor [93].

2.9 Glioblastoma Multiforme

Mechanisms driving tumor initiation and progression in Glioblatoma multiforme (GM) are beginning to be unraveled through genomic studies [94]. Due to the complexity of the disease, and the technicality of performing grafts in mice, a number

of studies have used athymic nude rats for PDX studies of GM. In one study, patient tumor biopsy was first cultured in vitro to form spheroids which were then subsequently implanted into the cranium of the recipient mice [94]. Results from this study demonstrated the suitability of this orthotopic-recipient host combination as a PDX platform for the diseases and showed the clinical relevance as the tumor behavior such as vascularization was similar to that seen in patients [94]. In another study, anti-VEGF treatment, which has anti-angiogenic activity led to reduction of blood supply to the tumor. Although some shrinkage in tumor was noted, the hypoxic environment within the tumor promoted tumor cell invasion that appeared to rely on the PI3K/AKT signaling pathway undermining durable and long-lasting inhibition of tumor growth. Nevertheless, the similarity between the primary patient biopsy and the PDX-derived tumor sample based on genomic analyses and vasculature undoubtedly makes PDX models of GM ideal investigational platforms to evaluate targeted therapies either as monotherapy or in combination with agents that inhibit angiogenesis.

3 Clinical Utility of PDX

3.1 PDX Models as Tools for Understanding Genomic and Protein Expression Profile of Cancers

Some investigators have also focused their attention on the regulation of genes and gene products involved in cancer pathogenesis such as those involved in migration and angiogenesis [95]. In a study involving several PDX tumor models, tissue microarray focusing on a number of genes including VEGF-A, proteinase-activated receptor 1, cathepsin B, integrin β1, and MMP1 among others was conducted. This analysis provided some picture of molecular features employed in metastasis and angiogenesis. In this regard, gene set enrichment analyses of multiple canonical pathways involved in angiogenesis could also be informative in terms of evaluating metastatic ability of the tumor cells. Existing reports from genome-wide studies have demonstrated that PDX models derived from primary patient tumor are more preserved with respect to global gene expression pattern and pathways, and closely mirror that seen in patients [13, 55]. In an NSCLC PDX study of 17 PDX-tumor sample pairs, 10 were found to have correlation co-efficient of $^{>}0.9$ as determined from hierarchical clustering analyses [55]. Similarly, a pancreatic cancer tumor-PDX pairing study revealed that 10 out of 12 retain the expression levels of mutant KRAS as well as SMAD4 expression [47]. In a comprehensive study comparing primary small cell lung tumor PDX with cell lines derived from them, substantial changes were reported in gene expression pattern such that cell lines that were derived from an initial PDX and later cultured for several months before implantation had as many as 395 genes that were differentially expressed when compared with the founding PDX tumor [13]. Taken together, these reports highlight the

notion that gene expression pattern can change considerably in patient tumors when subjected to extensive in vitro manipulations such as generation of cell lines [13] and some of these changes may be irreversible, thus permanently altering the behavior of in vitro-generated PDX tumor models.

3.2 PDX Models as Tools for Predicting Clinical Response

A great percentage of oncology compounds fail to enter the clinic in part due to the low predictability of the pre-clinical pharmacological model used to text their efficacy. Several lines of evidence show that PDX models have better predictive value for clinical outcome compared to cell line-derived xenograft models. Models in which the heterogeneity and hierarchical complexity of tumors, i.e. tumor-initiating cells, tumor-propagating cells/tumor stem cells are preserved are likely to generate information that is relevant to patient tumors. In this context, PDX models particularly those utilizing patient's tumor tissue which have this feature are of value.

Many clinical trials are designed based on findings from pre-clinical PDX studies [16, 96, 97]. A number of these studies demonstrate high potential of PDX models in predicting objective response in patients in the clinic. In a study of 34 cancer patients, the predicted response or resistance rate for chemotherapy drugs including cetuximab using PDX models was remarkably high, and correlated with patient response [97]. In another study in which cetuximab was tested in metastatic colorectal cancer PDX models at clinically relevant doses (CRD), results mirrored what was seen in the clinic [16, 96]. Similarly, PDX models of small cell lung cancer evaluating efficacy of topotecan as monotherapy or in combination with other agents yielded outcomes that were similar to those in phase II clinical trials [98]. In an effort to identify potential treatment regimen for patients with refractory tumors, patient tumor graft studies were conducted with several agents with anti-cancer properties. This study led to identification of treatment plan for 11 of 14 patients which resulted in nearly 90 % clinical objective response [99].

While the studies above provide supporting evidence for the utilization of PDX models as predictive platforms for clinical studies, there exists a number of reports in which discordant results were observed [100, 101]. In a phase II clinical trials testing therapeutic index of an experimental drug brequinar sodium, marginal clinical response of about 5 % was only seen in lung cancer subjects despite a more robust (63–80 %) response rate as predicted by PDX models [100]. This could be attributed to the relatively small number of the PDX tumors used for the study which likely makes predictive power less accurate. The experimental drug Sagopilone was also tested in NSCLC PDX models and while demonstrating a robust 50 % tumor regression in mice [102], its effect in clinical studies was dismal [101]. Another issue is that disease stage may factor into the course of tumor development and progression in PDX models. Early stage tumors may yield discordant results compared with late stage tumors due to differences in growth properties and metastatic potentials. Furthermore, in cases where resected tumors are used for

PDX studies, they may offer little in terms of forecasting how patients might perform under drugs of investigation especially where surgical re-sectioning in itself was therapeutic.

3.3 Using PDX Models Towards Personalized Cancer Care

One of the utilities of PDX models is the potential for testing novel agents with the hope of translating them to patient application especially in settings where patient's tumor is refractory to existing standard of care or therapeutic options and is advanced. To do this, a piece of patient's tumor needs to be obtained. This could be achieved by biopsy or when surgical re-sectioning is being performed. Then follow the basic series of steps involved in setting up a PDX: implant patient tissue in immunodeficient recipient mice, test a number of drugs, identify those with promise, and subsequently test those in the patient. An example of this is a clinical pilot study in pancreatic cancer [103].

PDX models can also be particularly useful in the drug discovery process including target identification, validation, and drug screening. Tumors refractory to treatment can be propagated in mice to better understand mechanisms of drug resistance by profiling such tumors pre-implantation and identifying pathways that have been amplified or altered relative to treatment-naive tumor sample.

With the re-invigoration of interest in the utilization of PDX model came the concept of "avatars". Although largely used to describe patient-derived xenografts that are implanted sub-cutaneously, the consensus is that they offer the potential to test various drugs on a patient's currently un-resolved cancer. In this regard, modifications to improve PDX avatar models include better and more stringent immunodeficient mice models such as NOD.SCID, NOG/NSG mice.

3.4 PDX as Platforms for Biomarker Discovery

PDX models can also allow for identification of novel biomarkers that can be useful in predicting drug sensitivity or resistance. By exploring genetic and molecular feature of the tumor, PDX platforms can reveal patterns associated with objective response or those associated with little to no efficacy. This information is often useful in guiding patient stratification to choose those who are more likely to benefit from a particular regimen or those in which other treatment course may be warranted. In a study that evaluated the activity of cetuximab against a panel of PDX tumors that included colon cancer, it was found that mutations in the KRAS, BRAF, NRAS genes when present was associated with resistance to cetuximab in colon cancer [45]. Other molecular patterns were also identified which led to evaluation of small molecule inhibitor to target some of the dysregulated pathways identified as biomarkers. In a large study of colorectal PDX tumors, KRAS mutations or *HER2*

amplification accounted for resistance to cetuximab and these findings were in accordance with response rates noted in the clinic [104, 105]. Similarly, Olaparib, a PARP inhibitor and vemurafenib, a B-Raf inhibitor were tested in clinical trials and showed demonstrable activity in BRCA1/BRCA2 mutant ovarian cancer, and melanoma, respectively [105–107]. These clinical studies resonated with PDX studies [108, 109] demonstrating that PDX models with the employment of genetic screening tools can be effective platform for identification of biomarkers of therapeutic response.

Besides mutational load, expression pattern of genes in primary tumor or PDX-derived tissues as assessed by genomic tools can reveal underlying gene regulatory mechanisms that correlate with sensitivity or resistance to drugs. In one study utilizing PDX of various solid cancers, DNA microanalysis revealed several genes whose differential expression were associated with sensitivity to a number of chemotherapy drugs [110]. The PCR-based method described by Tentler and colleagues [111] is yet another useful tool that could be instrumental in biomarker identification. They have employed this methodology to assess sensitivity or resistance to a number of small molecule inhibitors [55, 112, 113] and biomarkers identified have been employed in some clinical trials. Thus, DNA microarray analysis and deep sequencing of chromosomal segments can aid our efforts in identifying global gene networks or mutations associated with cancer type and its progression. Furthermore, proteomics offers an avenue to dissect expression pattern of aberrantly expressed protein molecules in patient's cancer. By employing these "omics" approaches, multiple pathways enlisted by various cancers which favor growth and survival can be decoded. With the development of biomarkers such as gene mutations, amplifications, post-translational dysregulation, or over expression of protein molecules, drugs could be designed to target those mechanisms employed by the tumor cells to favor growth, survival, and progression.

With the availability of humanized immunodeficient mice, biomarker discovery extending to immunological parameters would be beneficial in terms of understanding the contributory role of tumor expressed markers favoring tumor evasion. Evaluating the expression of inhibitory pathways at the chromosomal level by RT-PCR or at the protein level by FACS or IHC should provide additional opportunities for biomarker discovery. For instance, the expression level of PD-L1 in many solid cancers has been associated with response to immunotherapeutic drugs such as anti-PD-1 antibody [114]. This is one example where comprehensive assessment of suppressive pathways operative on both tumor cells and immune cells could be informative and of substantial predictive value.

4 Challenges in PDX Models

The many challenges that pose barriers to PDX models as "perfect" pre-clinical investigative platform are numerous, some of which have been mentioned briefly in the preceding sections but will be elaborated here.

1. Cell line-derived PDX generally lack heterogeneity resulting from the in vitro selection process of certain clones over others. It is quite common for cultures passaged over many generations to eventually comprise of a near monoclonal tumor cell population. While patient tumor-derived PDX often exhibit clonal diversity similar to the parental tumor, they are also subject to chromosomal changes with extended in vivo passages. Genetic alterations tend arise in grafted tumors probably due to the fact that less differentiated tumors which tend to be the case for early stage cancers are more unstable [115, 116] and the likelihood of development of acquired secondary mutations increases with prolonged passages [47]. Thus, reliable data are best generated at early passage times when architecture, morphology, and histology of the original tumor are still only minimally perturbed.
2. Engraftment rates vary considerably between different models and different cancer types. For example, breast cancer PDX models are generally more challenging compared to other cancers with basal-like cancer models being easier to generate compared to luminal tumors such as the estrogen receptor-positive cancers [18, 76, 77, 103]. The lack of engraftment due to a number of factors including technical failure can also be an impediment to successful PDX model generation. Latency phase is another issue of concern in PDX models. A prolonged latency period [41, 115] before tumor engraftment and growth is confirmed can pose barriers to therapeutic studies in "xenopatient" settings where results are anticipated to guide selection of rational treatment options for aggressive tumor types.
3. Of importance is the stroma or lack thereof surrounding a PDX tumor [117]. Due to the changes in the stroma environment to which the patient tumor is now exposed when present, stromal-associated genes can be dysregulated [19, 116]. Furthermore, the cellular composition of the PDX stroma which may be of human origin initially, eventually becomes replaced with murine equivalent, making inferences about tumor–stroma interactions not so straightforward. Similarly, as with a described renal cell carcinoma model [80, 92], the vasculature of human origin associated with the PDX tumor gradually disappears, paving way for murine vasculature. This is particularly important when considering the impact of soluble factors and angiogenic properties that are important in the biology of a cancer indication. If of mouse origin, they may affect tumor behavior differently than the human equivalents, hence generate outcomes that are divergent from what is seen in a patient. On a related note, PDX models may not faithfully recapitulate their parental counterpart due to differences in hormones present in primary versus secondary host which is relevant for hormone-driven tumors such as sub-types of breast cancer [76, 118–121].
4. While implantation is not particularly difficult, the skills required are notwithstanding beyond that needed for simple maintaining cell cultures in vitro, necessitating an expert in this approach to be a part of the research team. While PDX models are a great tool for oncology research particularly for pre-clinical efficacy studies, they are often cost and labor intensive. Consortia such as the Center of Resource for Experimental Models of Cancer (CreMec), the Translational

Proof-of-Concept consortia TransPoc), and the Euro PDX consortium have mechanisms in place to facilitate collaborative research built on PDX models and should be instrumental in making these models of wider application in oncology at reasonable costs, hence alleviating this potential cost-associated challenge.

5. PDX models might not be very effective in early stage disease as there is about 40–60 % tumor development rate in grafted mice [122] and even then, only malignant, potentially aggressive tumor may effectively engraft and propagate in the recipient mice making PDX model not ideal for early tumors. Additionally, many PDX especially s.c. implants are not metastatic which is a critical determinant in the clinical outcome of disease [30, 31]. This limits the extension of data generated from s.c. PDX systems focused on drug efficacy to human cases especially where such parental tumor has confirmed metastatic potential.
6. Animal use ethical concerns are not to be taken lightly when designing and establishing PDX programs. Many institutions have regulatory committees such as Institutional Review Board (IRB) and Institutional Animal Care and Use Committee (IACUC) that oversee protocols utilized for conducting animal studies and criteria for endpoint assessments including limits of tumor burden allowed in a recipient animal. These regulations ensure smooth operation of animal studies and are not barriers to effectively conducting PDX studies as long as they are abided by.

4.1 Tumor–Host Interactions; Tumor Stroma and Cancer-Associated Cells

The host stromal components and blood vessels contribute to the tumor microenvironment as revealed by studies utilizing secondary recipient transgenic mice expressing fluorescent proteins such as RFP, and GFP [123]. Such studies highlight the contribution of the tumor stroma to tumor progression and behavior in PDX models. The question is: with each passage, does the contribution of the host stroma change enough to alter the behavior of the PDX in a manner that is divergent from the parent tumor? In early stages of PDX implantation, human stroma associated with the tumor is present and has been studied. Both fibroblasts and tumor-infiltrated T cells were readily identified in the implanted PDX tumor of a non-small cell lung cancer model several weeks after implantation [6]. With increasing passaging, stromal components of human origin became replaced with the host (mouse) stroma [76, 78, 123]. Of consideration is the possibility that stroma composition may also impact the pharmacology (pharmacokinetics) of therapeutic agents. In a study evaluating the efficacy of gemcitabine and nab-paclitaxcel in pancreatic PDX tumors, the combination of the drug interestingly was associated with near 3-fold increase in the levels of gemcitabine present in the tumor [124]. Given the potential for gemcitabine to cause a depletion in myeloid cell subset in tumor-bearing mice [125],

such increased penetrance of drug may be attributed to re-shaping of the tumor cellular dynamics. Whether the mouse stroma that eventually envelopes the human PDX makes for easier penetration of therapeutic drugs of interest compared to human stroma remains to be seen.

In some PDX models, lack of intact tumor stroma similar to the patient's may essentially preclude studying interactions between tumor cells and both innate and adaptive immune system which are critical to tumor progression and metastasis. Even when present, studying the cross talk between these immune cells and cancer cells may be challenging as the mouse environment may cause some tumor-resident immune cells not to persist, hence become lost due to absence of key growth/survival factors in the secondary host. With this realization in mind, evaluation of immunotherapeutic compounds which rely on the host's immune cells for anti-tumor therapy may be difficult to achieve in PDX models as recipients (immunodeficient mice) are generally devoid of an intact immune environment.

5 Pushing PDX Beyond the Status Quo

Genomics and sequencing are often tools employed to identify genetic changes favoring tumor growth. These changes provide the recipe for designing targeted therapies for cancers harboring such genomic alterations. In similar vein, epigenetic modifications deserve our attention even in PDX models as information continue to emerge demonstrating how methylation and acetylation patterns impact gene expression. Lastly, proteomics approach can be relied upon for evaluating expression levels of protein of interest as relevant to specific cancer indications such as receptor tyrosine kinases, hormone receptors, growth factor receptors, inhibitory ligands, etc. PDX models have come a long way but could still benefit from refinements. Incorporating many of these analytical tools in PDX tumors will likely provide researchers a more comprehensive picture of regulatory networks at play in cancer tumorigenesis and pathogenesis as well as clues to how components of these networks might be connected and employed by cancer cells to evade drugs or immune recognition.

6 Conclusion

Summarily, PDX models are a useful investigational platform to study the many facets of cancer biology. Importantly, they offer an approach to generate an expansive array of various human tumors which is essential in order to capture the heterogeneity that exists among human cancers both within the same cancer type or between different cancers. They are also a great tool for drug discovery, as well as uncovering mechanisms of resistance and sensitivity to known anti-cancer drugs. PDX models are thus a great pre-clinical model given the information they provide us such as histology, genomic landscape, architecture, growth behavior, genetic and epigenetic features as they relate to the primary patient tumor.

PDX models are also a great alternative to GEMMs as they appear to mimic more closely the overall biological parameters associated with the patient's cancer. Lastly, toxicity and tolerability issues need to be weighed carefully when utilizing PDX models given the difference in the body mass between mice and men to avoid over or understating effects when extending drug efficacy studies from PDX mice to human trials. It would be advantageous if there exists certain ground rules on when the results of a PDX study should be deemed robust enough to extrapolate from it and use findings to guide design of clinical trials. As PDX models continue to be employed in oncology, modifications including adoptive transfer of human cells should provide a more rounded approach to understanding tumor biology. After all, cancer cells are never in isolation and their surrounding stroma which includes immune cells are key to course of disease progression.

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