Commentary

Advances in patient-derived tumor xenografts: From target identification to predicting clinical response rates in oncology

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ABSTRACT

Most oncology compounds entering clinical development have passed stringent preclinical pharmacology evaluation criteria. However, only a small fraction of experimental agents induce meaningful antitumor activities in the clinic. Low predictability of conventional preclinical pharmacology models is frequently cited as a main reason for the unusually high clinical attrition rates of therapeutic compounds in oncology. Therefore, improvement in the predictive values of preclinical efficacy models for clinical outcome holds great promise to reduce the clinical attrition rates of experimental compounds.

Recent reports suggest that pharmacology studies conducted with patient derived xenograft (PDX) tumors are more predictive for clinical outcome compared to conventional, cell line derived xenograft (CDX) models, in particular when therapeutic compounds were tested at clinically relevant doses (CRDs). Moreover, the study of the most malignant cell types within tumors, the tumor initiating cells (TICs), relies on the availability of preclinical models that mimic the lineage hierarchy of cells within tumors. PDX models were shown to more closely recapitulate the heterogeneity of patient tumors and maintain the molecular, genetic, and histological complexity of human tumors during early stages of sequential passaging in mice, rendering them ideal tools to study the responses of TICs, tumor- and stromal cells to therapeutic intervention.

In this commentary, we review the progress made in the development of PDX models in key areas of oncology research, including target identification and validation, tumor indication search and the development of a biomarker hypothesis that can be tested in the clinic to identify patients that will benefit most from therapeutic intervention.

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1. Introduction

The drug discovery and development cycle in oncology has been associated with high clinical attrition rates. Retrospective analysis of the success rates of cancer drugs from first-in-man studies to registration, range from 5% to 20% [1,2]. Importantly, 75% of new cancer drugs tested in Phase I clinical safety studies advance to Phase II efficacy testing. Unfortunately, the highest attrition of experimental drugs in the clinic occurs during the resource intensive Phase II and III evaluations, which explore the robustness of pharmacological responses. Such unusually high clinical attrition rates in proof-of-concept clinical studies renewed the interest in developing preclinical efficacy models that are more predictive for clinical outcome. Tumor models in oncology are employed at all stages of the drug development cycle, starting with the identification of therapeutic targets, selection of lead compounds and identification of the most promising cancer indications and patient enrichment strategies. The types of preclinical pharmacology models currently employed by cancer researchers can be divided into four categories: the most widely used models are conventional, human CDX implanted either subcutaneously or orthotopically in immune-compromised mice, followed by genetically engineered mouse models, mouse tumor allografts and PDX models (reviewed in [3,4]).

PDX tumors were first described more than 40 years ago ([5–8]). Since then, the variety of immune-deficient host strains have increased significantly, enabling improved tumor engraftment rates and more widespread use of PDX models in academia and industry.
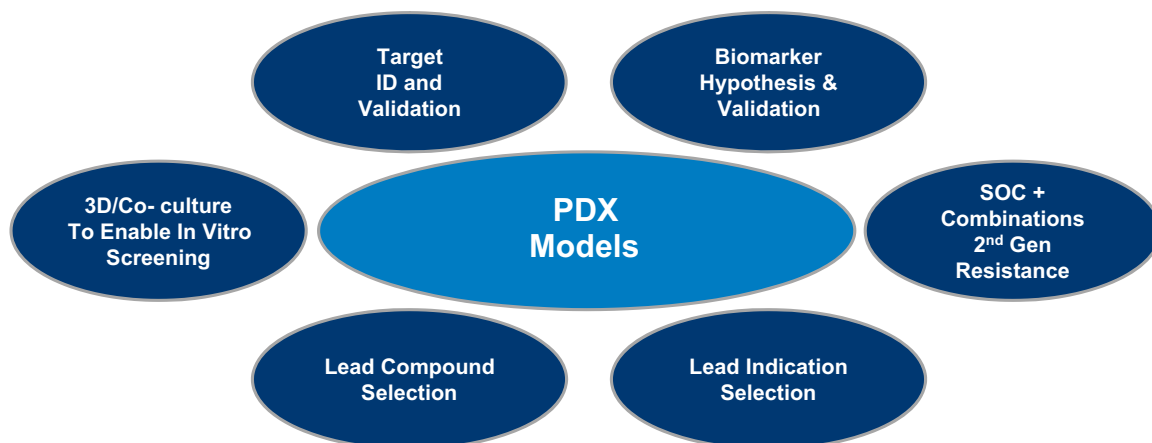
Fig. 1. The impact of employing PDX models in key area of oncology research and development. PDX models enable better decision making at all steps of the drug development cycles and the improvements may be cumulative. Employing PDX tumor for Target ID and Validation ensures that that all relevant experiments are conducted with almost identical tumor cell materials. PDX tumors can be grown *in vitro* as 3D co-cultures to support assay development. Furthermore, tumors that were used for target ID can be used during all subsequent steps of drug development, including Lead Compound Selection, Lead Indication Selection, identification of a Biomarker Strategy and combination studies with SOC. To support clinical decision making, novel therapeutic compounds can be screened in panels of PDX tumors to identify tumor indication(s) that display the most promising response rates. Alternatively, testing of compounds in larger panels of PDX tumors of the same indication may help in the identification of biomarkers to select for patients populations which are most likely to respond to treatment. Finally, by developing PDX models from cancer patients that became refractory to SOC treatment, potential activities in refractory or late-stage cancer patients, compared to naïve or early stage cancer subtypes, can be studied.

Some early reports investigating the histopathology of PDX tumors emphasized their close resemblance with the histopathology found in patient tumors. Another key observation was that PDX tumors grow in the presence of an integrated stroma and tumor vasculature, indicating that PDX tumors have utility in the evaluation of therapies targeting tumor vasculature and/or stromal compartments, in addition to targeting cancer cells. There is a substantial body of experimental evidence in support of the notion that PDX models resemble the pathophysiology of human tumors more closely than traditional CDX models [9]. For example, a detailed cytogenetic analysis of PDX tumors revealed strong preservation of the chromosomal architecture found in patients [10]. Other studies showed strong fidelity in histology [11,12], transcriptome [13], polymorphism [14] and copy number variations [15]. These early studies demonstrated clonal evolution of PDX tumors during serial passaging at similar rates as reported in patient tumors [16,17].

Interestingly, some of the key challenges and questions around the utility of PDX tumors in the evaluation of cancer therapeutics were already anticipated and discussed over 30 years ago [18]. At that time, it remained unclear whether the resistance or sensitivity of patient tumors toward cancer chemotherapeutics is retained in PDX models. It was anticipated that a major challenge for pharmacology experiments in PDX tumors was the administration of agents at clinically relevant dose levels and treatments schedules, which match the measured or anticipated human exposure profiles. The answers to both questions remain the central focus of the current debate over the utility of PDX models. Furthermore, PDX models have their own sets of limitations, including the imperfect cross-talk between murine and human cells and the disappearance of certain immune components following early passages in severely immune-compromised mice. At this point, PDX models may have limited utility to study therapeutic compounds whose pharmacological activities depend mostly on the presence of an intact host immune system, including immunotherapeutic compounds. Additional model refinement will be required to make PDX feasible for the evaluation of immunomodulatory compounds. In conclusion, the development of more predictive preclinical models may potentially impact several critical steps in key areas of oncology drug development, and the benefits may be cumulative (Fig. 1).

2. PDX model development and characterization

PDX tumors are generated by direct transfer of human tumor fragments or cell isolates from patient tumors to immune-deficient mice. Serial passages of tumors in rodents permits the investigation of tumor biology and pharmacology without subjecting tumor cells to artificial *in vitro* cell culture conditions. It is hypothesized that serial passage in mice retains the genetic and morphological characteristics of the original human tumor. A number of studies were carried out to better understand the impact of serial passaging of PDX tumors on gene expression, chromosomal stability and copy number variations. At low passage numbers, histological features, gene expression profiles, copy numbers and chromosomal stability of PDX tumors are comparable with the corresponding patient tumors [10,19–21]. With each passage to a new mouse host, genetic changes occur at rates that are intrinsic to the tumor types tested [22]. Importantly, the degree of clonal evolution within PDX tumors is dependent on the cancer indication studied. For example in colorectal PDX tumors with mutant APC or p53 genes, significant levels of chromosomal changes over a span of eight passages were reported [20]. In contrast, PDX tumors lacking mutations in either APC or p53 developed only few chromosomal changes over a similar time span. Analysis of a luminal breast cancer PDX identified very few chromosomal changes over a span of six passages, a time period of approximately 30 months [10]. Lastly, an evaluation of eight pancreatic PDX propagated up to 39 passages identified only a few genetic changes [23].

To optimize the value of the PDX models, patient information including age, sex, ethnicity, clinical diagnosis and prior treatment regimens can be included in correlative studies. A comprehensive characterization of DNA, RNA and protein levels is frequently carried out to gain a detailed understanding of the histological, biochemical, molecular and genomic characteristics of PDX models [9,24,25]. The combined data using historic patient information, chromosomal aberrations including duplication, deletion and translocation [26] were shown to identify compounds likely to be efficacious in certain patient subgroups, including the hormone receptor status, EGFR, Her2 status and other molecular markers [27].

Despite the recent progress made in translational oncology research, enabling the transfer of patient tumors to oncology research laboratories for preclinical research, many obstacles in sustaining the PDX support system remain to be addressed. The formation of several PDX consortia such as the Center of Resource for Experimental Models of Cancer (CReMEC) (described in [20,22,28]) the Translational Proof-of-Concept consortia (Trans-PoC) [29] and the Euro PDX Consortium [30] will leverage the full potential of PDX models. These consortia will help to further refine ethical considerations, minimize the cost of running PDX experiments and enhance the collaborations between surgeons and researchers.

3. Molecular and cellular heterogeneity of PDX tumors

Cancer is a heterogeneous disease that manifests as intratumoral heterogeneity as well as heterogeneity comparing between tumors from different cancer patients within the same indication. However, such heterogeneity is often lost once a tumor is removed from a patient and then cultured *in vitro* [31]. For example in prostate cancer, only about two dozen cell lines have been described and among them, only a very small set have been repeatedly used for *in vitro* experiments, and even fewer were tested *in vivo*. These circumstances suggest that the majority of preclinical data in prostate cancer was generated based on a small number of cancer models with a narrow representation of intra-patient variability [32,33]. In contrast, PDX tumors maintain the original tumor heterogeneity, which allows for modeling of a wide spectrum of cancer types and capturing of the patient heterogeneity [20]. For example, colorectal tumors with a mutated PI3KCA and wild-type KRAS/BRAF were successfully established as PDX models, while this subtype is not readily found in conventional colorectal cancer cell lines [34].

One of the main challenges encountered when building comprehensive panels of PDX tumors is that their engraftment frequencies or "take rates" are highly variable, depending on the tumor indication. For example, breast cancer PDX models have been more difficult to establish compared to lung, melanoma and colorectal cancer [35,36]. Within breast cancer, basal-like cancer models were more readily developed compared to luminal tumors, including estrogen receptor (ER) positive tumors, which inherently display lower pathological grades and slower growth rates [21, 37–39]. By combining a broader panel of immune-compromised mouse strains such as NOD scid gamma (NSG) [40] with optimized tumor implantation procedures, engraftment efficiency was improved and a better representation of patient heterogeneity can be obtained. Additional measures may be taken to ensure a PDX collection reflects the human cancer subtypes by employing gene expression profiling and other tumor characterization methods to match the distribution of a panel of PDX tumors with the distribution found in human cancer patients.

4. Tumor stroma

While histological and genetic heterogeneity are now widely accepted as key features of PDX models, the functional contribution of tumor stroma to the growth of PDX models is still controversial. It has been demonstrated that certain components of human stroma, including mesenchymal cells and infiltrating lymphocytes, are present during early PDX passages. However, during subsequent passages, the human stroma is progressively replaced by stroma of murine origin [20,39,41]. The exact timing and sequence of these events remain unclear [42,43]. Nonetheless, the presence of human stroma in early passages of PDX models and of murine stroma during later stage passages permitted investigation of the interactions between tumor cells and their microenvironment. One example reported by Simpson-Abelson and collaborators included PDX models of non-small cell lung cancer (NSCLC) at early passages [44]. In this study, freshly harvested pieces of primary human lung tumor were implanted subcutaneously in NSG mice to obtain early passage PDX models with well-preserved human stromal structures, including tumor-associated leukocytes and stromal fibroblasts. The authors described that the immune cells in the tumor remained functional for up to 9 weeks after implantation based on the presence of circulatory human immunoglobulin levels during this time period. Moreover, tumor-associated human T-cells were found to migrate from the tumor location to the lung, liver, and spleen at 8 weeks post implantation [44].

For pancreatic cancers, desmoplastic stroma has been shown to influence cancer therapy diffusion and pharmacology [45]. When testing a panel of 11 pancreatic PDX tumors to evaluate the combination of gemcitabine and nab-paclitaxel on tumor growth [46], gemcitabine treatment led to tumor regressions in 2 of the 11 PDX and nab-paclitaxel resulted in regression in 4 of 11 PDX tumors. The combination of gemcitabine and nab-paclitaxel resulted in tumor regressions in 7 of the 11 pancreatic PDX and a reduction in desmoplastic stroma in pancreatic tumors, associated with a 2.8-fold increase in the intratumoral concentration of gemcitabine. Subsequent Phase III evaluation of gemcitabine and nab-paclitaxel demonstrated a survival benefit in pancreatic cancer patients [47]. These finding suggest high predictive value of pharmacology experiments in PDX tumor models with significant stromal contributions, including pancreatic tumors.

5. Tumor initiating cells/cancer stem cells

Accumulating experimental evidence suggests that both hematological [48] and solid tumors [49–54] contain a distinct subpopulation of TICs (tumor-initiating cells) or CSCs (cancer stem cells) [55–57]. By definition, TICs are capable of self-renewal and differentiation and remain largely quiescent in cancer tissues. Although their roles in cancer initiation has not been conclusively defined, preclinical studies suggested that TICs are intrinsically more resistant to radiation, chemotherapy and targeted therapies, and that their enrichment is critical for the study of drug resistance and tumor recurrence. In many cases, TICs can be isolated from the bulk of the tumor mass by using specific markers [58]. For example, clinical studies have shown that TICs were enriched in breast cancer patients undergoing neoadjuvant chemotherapy [57]. Therefore, therapeutic targeting of TICs may represent a new treatment paradigm to improve the effectiveness of cancer treatments [59–62].

TICs may only make up a small fraction of the total cancer cell population of the tumor and their ability to differentiate has made it challenging to consistently isolate sufficient amounts of TICs from primary human tumor biopsies. Expansion of PDX tumors in mice can yield sufficient quantities of TICs from patient tumor tissues without compromising the heterogeneity of the original tumor. Using the PDX approach, a highly tumorigenic CD133+ subpopulation with TIC features was found to be involved in mediating cisplatin resistance in NSCLC [63]. In another study with melanoma PDX models, ABCB5+ cells were identified as TIC-capable cells that, upon re-implantation, were able to regenerate tumor heterogeneity [64]. Moreover, selective targeting of the TIC subpopulations resulted in tumor growth inhibition [65], consistent with the notion that targeting the most malignant tumor cells may improve the therapeutic benefit. In conclusion, PDX models capture key aspects of TIC biology and therefore are essential to study the pharmacology of therapeutic compounds interfering with TICs [66].

6. Target identification and validation in oncology using PDX models

Clinical samples obtained directly from cancer patients are undoubtedly the most relevant biological source to support identification and validation of cancer targets for drug development. Unfortunately, acquisition of primary human tumor samples for target identification has been challenging, due to lack of continuous supply of sufficient quantity and quality of tumor materials and the prohibitive costs to secure fresh tumor samples. In contrast, conventional cancer cell lines are readily available and can be easily propagated to generate sufficient materials for biochemical studies. However, conventional tumor cell lines expanded *in vitro* do not capture many the key contributions of the tumor microenvironment, oxygen tension and other physico-chemical parameters controlling transformation and growth of tumors. PDXs models can fill this gap because serial passaging in mice generates sufficient quantities of tissue to support target identification and validation experiments [67,68]. In conclusion, the use of PDX tumors for target identification and validation purposes ensures that all relevant stages for target identification and validation are conducted with almost identical tumor materials.

7. Drug resistance screening

PDX tumors display cellular and molecular heterogeneity [69], a feature which is increasingly recognized as a key component of the processes leading to drug resistance through selection and enrichment of pre-existing genetic or epigenetic mutations in subsets of cells during prolonged treatment periods [70,71]. More recently, it has become possible to establish PDX models from cancer patients that became refractory to standard of care treatment. This has largely been accomplished by obtaining metastatic samples during an autopsy performed soon after the patient's death. Such rapid tumor biopsy methods were successfully performed with pancreatic cancers [72] and prostate cancers [73].

8. Pharmacology studies with PDX tumors that correlate with clinical outcome

There exists only a small number of oncology therapeutics that were tested in PDX models and which subsequently completed clinical evaluation, rendering a definitive assessment of the predictive value of PDX experiments in prospective settings a rather difficult task. However, there is a growing body of experimental evidence in support of superior predictability of PDX models for clinical outcome, largely based on retrospective analysis of drugs that were previously approved in the clinic, as summarized in Tables 1 and 2. For example, a retrospective analysis of the pharmacological effects of approved cancer drugs, including bevacizumab and cetuximab was performed in PDX tumors from 34 cancer patients with solid tumors dosed at CRD [6,74]. Overall, 50 treatment comparisons were conducted with standard of care (SOC) administered at the maximum tolerated dose (MTD) levels in mice. The response in PDX models predicted the response in the patients in 12 out of 13 times (92%) and a lack of response was predicted in 36 out of 37 times (97%). In subsequent studies, PDX models correctly predicted response in 19 of 21 cases (90%) and correctly predicted resistance in 57 out of 59 cases (97%) [75]. Overall, the responses in PDX tumors correlated with the patient response in 125 of 138 cases (90.6%).

In a more recent study, a cohort of 85 metastatic colorectal cancer (mCRC) PDX models were treated with cetuximab (anti-EGFR antibody) at a dose approximating the CRD and reported

Table 1
Summary of PDX studies at CRD and MTD and predictive value for clinical outcome.

Drug	Preclinical dose levels	Tumor type	Response rate to clinical	Number of PDX	References
Cetuximab	CRD	CRC	Similar	22	[69,76]
Cetuximab	CRD	mCRC	Similar	66	[19,76]
Bevacizumab	CRD	CRC, NSCLC, Breast, RCC	Similar	72	[74]
Bevacizumab nab-paclitaxel, Cetuximab, Irinotecan	CRD	Refractory advanced cancers	88% predictive	14	[79]
PARP inhibitor	CRD	Ovarian	Similar	2	
Gemcitabine nab-paclitaxel	CRD	Pancreatic	Similar	11	
5-Fluorouracil, oxaliplatin, irinotecan cetuximab	CRD (70% of MTD)	CRC	Similar	52	[20,76]
Many SOCs	MTD	Colorectal, head and neck, non-small cell lung, small cell lung, melanoma, ovarian cancer	Concordance for doxorubicin (active) amsacrine (inactive)	35	[67]
SOC	MTD	34 different tumor types	97% predictive	34	[6]
SOC	MTD	NSCLC	Discordance	32	[85]
Topotecan Etoposide Ifosfamide Cisplatin	MTD	SCLC	Similar	6	[77,78]
Irofulven	MTD	Pediatric solid tumors neuroblastomas, rhabdomyosarcomas	Discordance, drug levels in mice not achieved in man.	20	[80,81]
Doxorubicin Amsacrine Brequinar sodium	MTD	Ovarian, SCLC, head and neck, NSCLC	Discordance with Brequinar sodium	35	
Sagopilone Carboplatin Paclitaxel Gemcitabine	MTD for all	NSCLC	Discordance with Sagopilone	22	[86,87,104]

Summary of published pharmacology studies conducted with various therapeutic compounds in PDX models and correlation with clinical responses. SOC, standard of care; CRC, colorectal cancer; NSCLC, non-small cell lung cancer; Br, breast cancer.

Table 2
Comparison of the pharmacological activities reported for the anti-VEGF antibody bevacizumab in standard tumor cell lines models and PDX models.

	Standard tumor cell lines	PDX
No. of studies conducted	31	2
No. of tumors tested	25	72
No. of tumor indications	10	5
Reports with significant tumor inhibition (>80% TGI)	>50%	0%
Reports with tumor stasis (50% to 80% TGI)	>20%	0%
Reports with progressive growth (11–41% TGI)		15–20%
ORR in single agent clinical trials	0–10% (RCC)	
References	[102]	[74]

Comparison of published response rates of the anti-VEGF neutralizing mAb bevacizumab in preclinical models using standard tumor cell lines *versus* PDX models.

response rates that were nearly identical to the clinical findings [19,76]. Further stratification identified enrichment of tumors with HER2 amplification in cetuximab-resistant, KRAS/NRAS/BRAF/PIK3CA wild-type tumors. The authors concluded that information regarding the HER2 amplification status may be useful not only for resistance to EGFR inhibition but also as a positive predictor of response to HER2-targeting agents. This type of analysis can only be performed in the context of a larger panel of heterogeneous PDX models [19].

In another study, a panel of six small cell lung cancer (SCLC) PDX tumors was treated with topotecan and combinations of topotecan with etoposide, ifosfamide, or cisplatin at MTD, to evaluate the activity of these regimens with SOC combinations [77]. Three of the six PDX models displayed >90% growth inhibition when dosed with topotecan alone. This was similar to the therapeutic response observed in Phase II clinical trials with topotecan [78]. The response was improved when topotecan was combined with ifosfamide or etoposide. The authors concluded that the evaluation in PDX tumors is a useful and informative preclinical assessment for new treatment regimens.

Lastly, an extensive prospective analysis was conducted with refractory tumors from 14 patients with the goal to identify the most promising compounds and to apply these therapeutic regimens to the respective cancer patients [79]. In this study, 63 different cancer agents were tested in 232 experimental arms at MTD. Effective therapies were identified for 11 of the 14 patients with an overall objective response rate of 88%.

An evaluation of the efficacy of irofulven (a DNA alkylating agent) against a panel of 20 pediatric PDX tumors determined that partial and complete tumor remissions could be obtained at the MTD in 14 of the 20 models. At the minimum efficacious dose, only one rhabdomyosarcoma model had an objective response [80]. Comparison of the pharmacokinetic data from Phase I clinical trials of irofulven [81] to the circulating drug levels of irofulven in the PDX studies identified much higher drug exposure levels in mice compared to humans [82]. At the minimum efficacious dose, the circulating drug levels of irofulven were approximately sixfold higher than the MTD in humans [81,82]. Irofulven has subsequently been evaluated in several clinical trials and has resulted in a partial response and stable disease in 37 out of 60 patients with ovarian cancer [83]. At present, clinical studies have not identified single agent efficacy for irofulven.

9. Pharmacology studies conducted with PDX models that failed to correlate with clinical outcome

A series of PDX studies with solid tumors was conducted in a collaboration between European laboratories to determine whether PDX models can improve the predictive value of preclinical evaluations of cancer drugs for clinical outcome [67]. The compounds tested included two approved agents, doxorubicin, amsacrine and the experimental drug brequinar sodium, administered at the MTD in mice. In the evaluation of PDX models, doxorubicin was effective against ovarian and SCLC in addition to head and neck cancers and NSCLC. In contrast, amsacrine, which was primarily used for the treatment of hematological malignancies, was not active when tested in solid tumors. The preclinical evaluation of brequinar sodium determined that it was effective in 5 of 8 (63%) NSCLC, and 4 of 5 (80%) SCLC, and in 4 of 5 (80%) head and neck cancer. These preclinical response rates were markedly different from the results reported in phase II clinical trial conducted with brequinar sodium in NSCLC and SCLC patients [84]. The clinical study identified a response rate of 5% in NSCLC and 6% in SCLC. In light of these discrepancies it was speculated that constant high exposure of brequinar sodium was necessary for effectiveness, which was achieved in the PDX study, but not in the clinical trial [67]. Another potential explanation is that the number of PDX tumors tested was too small, as only four to eight PDX tumors were tested in each indication. Recent studies summarized below have generally evaluated larger numbers of PDX tumors, which has allowed for more accurate evaluations of sensitivity and resistance and better correlation with human efficacy studies.

Another limitation of the PDX approach was identified when testing a panel of NSCLC [85] from early, stage 1B patients. In this study, the fraction of PDX tumors failing to respond to therapy indicated high levels of disease recurrence (6 out of 7; 86%). With regard to efficacy prediction, 2 out of 4 xenograft tumors did not respond to treatment. However, none of these four original patients had disease recurrence after 2 years of follow up. The authors concluded that the lack of efficacy prediction of the four tumors without disease recurrence represented stage 1B cancers, which had not yet metastasized. Likely, these cancers were entirely removed during the initial resection which could account for the discordance in the efficacy prediction. In conclusion, PDX models derived from early stage resections of tumors in patients with a possibility of surgical cures may have led to lower predictive values.

Another example for a discordance between preclinical efficacy and clinical response rates caused by a lack of clinically relevant dose levels in mice are experiments conducted with the epothilone sagopilone [86]. This study evaluated carboplatin, paclitaxel, gemcitabine and the preclinical agent sagopilone in a panel of 22 NSCLC PDX models. Sagopilone treatment at the MTD induced tumor regression in 11 out of 22 (50%) PDX tumors, and resulted in stable disease in 3 of 22 (14%) PDX tumors. This was a better response compared to carboplatin, paclitaxel, and gemcitabine in PDX tumors. However, in Phase II clinical trials, sagopilone induced partial responses in 8 patients out of 128 (6%) [87]. Sagopilone is also being evaluated in melanoma, prostate, and ovarian cancer.

In conclusion, for several experimental therapeutic compounds, including sagopilone, brequinar sodium, and irofulven, superior activities were observed in PDX tumors, compared to their activity observed in the clinic. The importance of adjusting the dose and schedule of oncology compound tested in preclinical efficacy models to the CRD was confirmed independently in a study conducted with CDX lines [88].

10. The utility of PDX models to develop a clinical biomarker hypothesis

PDX tumors enabled the discovery of novel biomarkers predicting drug sensitivity or helped to understand the molecular and cellular mechanism underlying drug resistance [89]. In addition to their ability to recapitulate the disease of an individual patient, the power of PDX models raises the possibility to enroll a collection of PDX tumors for preclinical testing, mimicking in size a Phase II cancer patient population. Three larger size PDX studies have been conducted with the goal to evaluate the efficacy of cetuximab in PDX models [19,20,69].

The activity of cetuximab was evaluated in a panel of 79 PDX tumors including colon cancer, gastric cancer, head and neck cancer; lung cancer and breast cancer to identify biomarkers that would predict drug resistance. Importantly, in this study cetuximab was dosed at 30 mg/kg which is equivalent to the maintenance dose in humans [69]. Analysis of the data to identify features associated with sensitivity or resistance to cetuximab identified that for colon cancer, 16 out of 19 resistant tumors had mutations in KRAS, BRAF, or NRAS. The observation that colorectal PDX tumors with mutations in the RAS pathway are resistant to cetuximab confirmed the main conclusions from studies with cancer patients [90–92]. In total, data from the PDX tumors identified a collection of ten biomarkers including RAS pathway mutation, expression of EGFR, phosphorylation of EGFR, expression of the EGFR ligands amphiregulini and epiregulin, expression of Erb3, activation of Akt, and phosphorylation of MET that could predict sensitivity or resistance to cetuximab [69]. Evaluation of these biomarkers identified a highly correlative signature that could predict resistance or sensitivity to cetuximab. Based on these findings, siRNA and a small molecule inhibitor to MET were tested in clonogenic growth assay in the context of cetuximab resistant lung cancer PDX. The authors concluded that MET activity should be considered along with *ras* mutation status as an additional biomarker for cetuximab resistance.

The identification of biomarkers for predicting resistance or sensitivity to cetuximab in colorectal cancer was examined in a large collection of 85 PDX models derived from mCRC patients [19]. The authors evaluated the responses of 47 tumors to cetuximab (20 mg/kg) and observed regressions in 10.6% of the cases and stable disease in 29.8% of cases [19]. These findings were very similar to the response rates observed in the clinic [76], with 10% of colon cancer patients responding to monoclonal antibodies to EGFR, including cetuximab or panitumumab (reviewed in [93]). The authors further confirmed that mCRC PDXs harboring a KRAS mutation at codon 12 or 13 were resistant to cetuximab. Additional pharmacology experiments were conducted in a larger set of 66 PDX tumors which did not have KRAS mutations at codon 12 or 13. In this cohort, 11 of the 66 PDX (16.7%) had regressions and additional 27 of 66 (40.9%) had stable disease. These preclinical findings were similar to the response rates observed in the clinic, with up to 17% tumor regressions and 34% stable disease in colon cancer patients lacking KRAS mutations at codon 12 or 13 [90,94]. The authors also analyzed a subset of PDX tumors that were cetuximab resistant but did not have mutations in KRAS, BRAF, or PI3K. They discovered that 4 of 11 resistant cancers which did not have a mutation in KRAS, BRAF or PI3K had amplifications in *Her2*. These results suggested that a collection of PDX tumors can be instrumental to identify new biomarkers predicting sensitivity and resistance of cancer patients.

Lastly, a panel of colorectal PDX tumors was analyzed for mutations in genes associated with colon cancer development and progression [20]. The panel of PDX tumors was then tested for their responses to 5-fluorouracil, oxaliplatin, irinotecan, and cetuximab. Importantly, the selected doses of oxaliplatin,

irinotecan, and 5-FU were 70% of the highest non-toxic dose in mice, which may be closer the to the CRD levels in humans compared to MTD. The best response to chemotherapy was observed for irinotecan, where tumor regressions were found in 19 of 49 (39%) of the colorectal PDX models. This response rate is similar to the response rate of 19–32% observed in newly diagnosed cancer patients when irinotecan was used as a single agent therapy (reviewed in [95]). In agreement with the previously described PDX studies, the responses to cetuximab (40 mg/kg) observed in colorectal PDX models depended partially on whether the colon cancers expressed mutant or wild-type KRAS. Overall, mice implanted with colorectal PDX tumors harboring wild-type KRAS displayed longer median survival on cetuximab compared to tumors with mutant KRAS. In this study, 18 of 52 (35%) colorectal PDX demonstrated either stable disease or tumor regression. These are superior response rates when compared to the clinic, where only 10% of colon cancer patients responded to monoclonal antibodies to EGFR cetuximab or panitumumab. When the response in PDX was examined further, 42% of PDX expressing wild type KRAS did not respond to cetuximab. Noteworthy, some of the PDX that did not respond to cetuximab had mutations in BRAF, PI3K, or in other parts of the EGFR response pathway.

In conclusion, in all 3 studies discussed above [19,20,69], the dose of cetuximab administered was close to the human maintenance dose (30 mg/kg). The experimental endpoints reported in these studies, tumor regression, stable disease, and tumor resistance were similar to the responses observed in patients. All 3 studies resulted in the identification of K-Ras mutations as a predictor for lack of activity of cetuximab. Importantly, all 3 studies were retrospective, as they were published after the data from the clinical trials with cetuximab were available, and the correlation between cetuximab activity and wild-type RAS pathway status was established.

11. Conclusions and future directions

Both prospective and retrospective studies conducted with PDX models demonstrated improved predictability for clinical outcome, in particular when cancer therapeutics were administered at CRD levels (Tables 1 and 2). A key role in the development of tumor refractoriness toward conventional cytotoxic therapies has been attributed to TICs, infiltrating stromal cells such as cancer associated fibroblasts or inflammatory cells, tumor associated macrophages, MDSCs. In addition, the high variability in the clonality of tumors between patients [96] contributes to the development of resistance. The failure of conventional tumor cell line models to reproduce many of these hallmarks of tumor growth may account for the improved predictive value of PDX models, as many of these biological drivers of tumor growth were shown to be present in PDX tumors, more closely modeling the human tumor pathobiology.

12. PDX models to develop a clinical biomarker hypothesis

One of the current frontiers in cancer research is focused on the development of a biomarker hypothesis in preclinical models, identifying patients that are most likely to respond to treatment. For example, the exquisite sensitivity of BRCA2 mutant tumors to PARP inhibitors was identified in preclinical PDX models [97]; reviewed in [98]. For example, the pronounced activity of the PARP inhibitor olaparib in BRCA1 or BRCA2 mutant ovarian tumors [99,100] and the robust pharmacological responses of melanoma tumors treated with the B-Raf inhibitor vemurafenib were mirrored in PDX models [89]. In conclusion, these studies have validated PDX models as tools to generate a clinical

Table 3
Comparison of frequency of tumor regressions in PDX dosed with standard of care therapies to the clinical objective responses reported in human clinical trials.

Standard of care	Cancer indication	Tumor regressions Preclinical PDX	Ref.	Clinical objective response	References
Irinotecan	CRC	19/49 (39%)	[20]	19–32%	[95]
Cetuximab	CRC	14/49 (29%)	[20]	11%	[76]
5-FU	CRC	0/49 (0%)	[20]	5–18%	[105]
5-FU	CRC	1/6 (17%)	[82,106]	5–18%	[105]
Methyl CCNU	CRC	1/6 (17%)	[82,106]	2/21 (9.5%)	[107]
Etoposide	NSCLC	1/25 (4%)	[104]	2/49 (4%)	[108,109]
Carboplatin	NSCLC	3/25 (12%)	[104]	20–26.7%	[110]
Gemcitabine	NSCLC	3/25 (12%)	[104]	21%	[111]
Paclitaxel	NSCLC	4/25 (16%)	[104]	21–24%	[112]
Cisplatin plus Vinorelbine	NSCLC	9/32 (28%)	[85]	24.5%	[113]
Cisplatin plus Docetaxel	NSCLC	8/19 (42%)	[85]	31.6%	[113]

Tumor regression frequencies observed in PDX panels with standard of care treatments. Clinical objective response rates observed in clinical trials conducted in patients. CRC, colorectal cancer; NSCLC, non-small cell lung cancer.

biomarker hypothesis that can be implemented in the clinic for patient enrichment purposes.

13. The importance of using clinically relevant doses (CRDs) and pharmacological endpoints in PDX studies

Mostly driven by practicality, the dose and schedule of SOC compounds selected for most preclinical pharmacology studies are based on their MTDs. In general, the MTD in mice is defined as the dose level, where 20% body weight loss is observed. Because of the different sensitivities between mouse strains toward cytotoxic compounds, differences in the route of drug administration, a wide variety of MTDs have been reported in the literature. Given the critical role of the exposure levels on the pharmacology of cancer therapeutics, additional focus on modeling of the CRD levels of standard anti-tumor agents in mice has great potential to further improve the predictive value of preclinical PDX studies in mice.

A similar variability exists in the experimental endpoints used to report the result of pharmacology studies conducted in PDX models. Over 16 different experimental endpoints are used to report *in vivo* pharmacology data, including % TGI (tumor growth inhibition), TTE (time to endpoint), Kaplan Meyer survival curves (summarized in [101]). In addition, the time between tumor implantation and treatment initiation can have a major impact on the magnitude of anti-tumor responses. In general, treatment initiation at timepoints before tumor implantation are called prevention settings, 1–7 days post-tumor implantation are called intervention setting [102] and at tumor volumes >200 mm^3 are called regression setting [103]. In the clinic, most experimental compound will initially be tested in patients with established tumors, representing the regression settings. There is a strong correlation of compounds that induced regression of established PDX tumors >80% and the objective response rates in the clinic, with approvable outcome, as shown in Table 3). Therefore, to select compounds with the highest probability of success in the clinic, complete regressions of established PDX tumors may represent important selection criteria. Finally, for most cancer types and treatment modalities, the onset to therapy resistance is frequently limiting the durability of clinical responses. The mechanism leading to such adaptive resistance toward treatment is the focus of intense preclinical- and clinical investigations. The development of PDX models from refractory patient tumors will be invaluable as they may allow for the identification of the mechanism causing resistance and treatment options to overcome such resistance.

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