Review

# Patient-derived xenograft models for oncology drug discovery

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## ABSTRACT

The success of targeted therapies for cancer patients rests on three major components: the right target(s), the right drug and drug combination, and the right patient population. Although much progress has been made in understanding the mechanism of disease and in refining pharmaceutical properties of therapeutic agents, the attrition rates between target discovery and drug marketing approval have been high, especially in oncology. One of the main reasons underlying this undesirable statistics is believed to be the lack of predictive power of the model systems used in the preclinical setting. Several strategies have been employed with the aim of improving the predictive value of the preclinical studies, such as incorporating genomic profiling and molecular segmentation into model selection, and enhancing the development and application of patient-derived xenograft models even during early stage of drug discovery. This brief review will summarize some of the recent concept and practice in incorporating patient-derived models into all stages of drug discovery process, from target to clinical development.

**Key words:** Animal models, drug discovery, oncology, patient-derived xenograft, translational research

## Introduction

The past decades have witnessed an explosive growth of scientific understanding of human diseases especially those of highly unmet medical needs. In the field of oncology, the significant progress in basic research coupled with technology advancement in drug discovery has resulted in a significant number of breakthrough therapies with improved efficacy and manageable toxicity. However, the overall track record of oncology drug research and development remains one of the worst in all therapeutic areas, with high attrition rate and prohibitive cost.[1,2] Recent survey indicated that in oncology drug development, close to 95% of drugs tested in Phase I trials failed to reach marketing authorization stage.[3] Significant efforts have been invested in scrutinizing every aspect of the drug discovery and development process and looking for ways to improve the success rate and efficiency. Among all, three pivotal areas have received much attention. First, it is commonly accepted that more refined, clinically relevant preclinical models are critical for accurately predicting patient response in clinical trials. Second, as we have fully embraced the concept and practice of personalized medicine and targeted therapy, tumor profiling and patient segmentation based on predictive biomarkers need to be an integral part of preclinical and clinical research and drug development. Finally, there is a need for bi-directional flow of information between preclinical and clinical investigators, and for increased collaboration between industry, academia and regulatory agencies to ensure optimal alignment of interests and resources. This short review will only focus on patient-derived models as a promising approach for improving the successful rate of oncology programs.

Access this article online

Quick Response Code:

Website: www.jcmtjournal.com

DOI: 10.4103/2394-4722.152769

## Patient-derived Xenograft Models for Target Identification and Validation

In the past 4 decades, significant progress has been made in the understanding of cancer biology and emergency of new classes of targeted therapies that have significantly changed the landscape of cancer treatment and management. The key to these successes has been the identification and validation of cancer targets that distinguish cancer cells and tissues from normal ones, as elegantly summarized in the landmark articles by Hanahan and Weinberg.[4,5] Although a dauntingly complex disease, cancer can be viewed as evolved around a number of rational commonalities, or hallmarks, necessary for tumor initiation, progression, metastasis, evasion of immune surveillance and resistance to therapeutic intervention. These processes involve not only genetic and epigenetic changes in the cancer cells themselves, but also recruitment and alterations in the tumor-associated stroma and micro-environmental factors. Therefore, it is conceivable that therapeutic approaches involving targeting multiple hallmark functions will continue to be the cornerstone for targeted cancer therapy and management.[6]

Cancer target identification traditionally involves the search for differential expression and function between cancer

and normal cells and tissues at the DNA, RNA, protein and microRNA levels. Multiple approaches of various through-put have been developed to identify differentially expressed genes and proteins.[7,8] Recent advances in transcriptomics, proteomics, genomics, functional genomics, epigenomics and metabolomics have significantly expanded the scope and depth of novel targets as well as utility of existing targets.[6,9-11] Although cell lines have been traditionally used due to their availability and accessibility, most recent efforts have been focused on patient samples, tumor biopsies and resections, for example, for their clinical relevance and heterogeneity. Once a potential candidate target is identified, the next key step is to functionally validate the target in the context of relevant patient population. The routinely employed approaches include tool compound, blocking antibody, dominant negative and RNA interference/short hairpin RNA. In addition, it is imperative to investigate whether the target identified in a small set of cells and tissues are reflected in a larger population ideally identifiable with selective biomarkers. To this end, a collection of large number of clinically collected tumor samples and patient-derived tumor models are critical to ensure translatability from target to drug and from laboratory to clinic.

Although cancer cell lines are the most widely used starting material as they are readily available and propagated to provide sufficient material for *in vitro* manipulation and *in vivo* tumor growth, most of them have been established long time ago and have been selected and cultured under nonphysiological conditions. In contrast, the least manipulated samples are those directly obtained from patients through surgical procedures or needle biopsies. However, one of the major challenges of using primary patient tumors is their limited "shelf-life" and very low quantity in most cases. Compared with cell line models and patient tissues, patient-derived xenografts (PDXs) provide a practical solution by both preserving the fidelity of clinical characteristics and providing tumor supply sufficient for most target identification and validation strategies.[12,13] Another significant benefit of using PDX for target identification and validation is that the process from target identification to validation and then to efficacy screening can be streamlined around the same models, therefore, offering a complete circle from patient to mouse and then back to patient.

## Patient-derived Xenograft Model Characterization

Typically, when patient samples are obtained for establishing PDX models, basic patient information (such as age, sex, ethnicity, clinical diagnosis) with the exception of patient identity will be provided. Once the tumors are established in immune-compromised mice, comprehensive characterization at DNA, RNA and protein levels will be carried out to gain detailed understanding of the histological, biochemical, molecular and genomic characteristics of the models.[14-16] As many of the technologies have become more efficient and affordable, whole-genome or transcriptome sequencing is increasingly being used to replace traditional microarray-based gene expression profiling and copy number variation studies. Next generation sequencing (NGS) approaches such as exome sequencing or whole genome sequencing also provide information on mutations and chromosomal aberrations such as duplication, deletion and translocation, many of which identify tumor suppressors or oncogenic drivers[17] and potentially predict drugs likely to be efficacious in particular patient subgroups.[18]

A number of studies were carried out to study the impact of successive passages on the gene expression, chromosomal stability and copy number variation. Although not definitive and most likely model-dependent, the general consensus in the field is that PDX models should be used at early passages.[19] At relatively low passage, the histological features, gene expression profile, copy numbers and chromosomal stability remains very similar to the matching tumor directly harvested from patient.[20-23] On the other hand, with each passage to a new mouse host, subsequent genetic changes may occur at different tendencies intrinsic to individual tumors, although the extent and impact of these alterations remain unclear.[24]

In reality, each cancer patient's tumor is heterogeneous and unique. And within each of the tumor indications mainly defined by anatomic locations of tumor incident (e.g. lung cancer, breast cancer), many subtypes can be identified by histopathology and immunohistochemistry (IHC) of an abbreviated panel of markers. Although these approaches have been widely used to describe and categorize tumors, they have largely failed to capture the variation of disease within indications. Recently, gene expression profiling and NGS have helped further refine the models via molecular subtyping within individual cancer indications.[25-29] Such molecular subtyping can be particularly helpful in delineating subtypes that can be challenging to distinguish with routine histopathology or IHC. For example, traditionally, breast cancer subtyping is mainly based on histology findings of IHC staining of selected markers. Recent molecular profiling has identified six distinct subtypes (luminal A, luminal B, human epidermal growth factor receptor 2, basal-like, claudin-low, and a normal-like) with clinically significant differences in risk factors, incidence, prognosis, and treatment response.[30-33] A similar approach has also been used in lung cancer to define clinically relevant subtypes to which targeted therapy can be applied to achieve optimal efficacy. In lung cancer, especially in non-small cell lung cancer (NSCLC), recurrent oncogenic drivers such as epidermal growth factor receptor, KRAS, anaplastic lymphoma kinase, as well as their related pathways can

be successfully employed to select responsive patients and predict response and resistance.[34-36]

## Patient-derived Xenograft Models More Accurately Reflect Human Cancer

Accumulating evidence has indicated PDX models are superior to traditional cell line xenograft models because they maintain more similarities to the tumors found in actual patients.[14] For example, a detailed cytogenetic analysis of PDX models revealed strong preservation of the chromosomal architecture observed in patients.[23] Furthermore, other studies have shown strong fidelity in histology,[37,38] transcriptome,[39] polymorphism[40] and copy number variations.[41] In some cases, certain oncogenic gene amplification can be found in cell lines at levels that are several-multitude higher than in patient rumors, a cell culture-derived artifact that may lead to over-predict drug response in the clinic (unpublished data). On the other hand, emerging data started to show that PDX models may be more accurately reflect clinical response when treated with therapeutic agents at clinically relevant doses (CRDs).[21]

## Modeling Drug Resistance

Despite the continuously growing arsenal of new and improved anti-cancer drugs, for most cancer patients with advanced diseases, treatment failure remains an inevitable outcome. To a given treatment, only a fraction of the patients would respond the regimen favorably (responders), which stresses the importance of selecting patients with the appropriate molecular and pathological characteristics for maximal therapeutic benefit. On the other hand, even when a particular treatment is initially efficacious in selected patients, drug resistance will develop overtime. Therefore, drug resistance is a fundamental cause of therapeutic failure in cancer therapy. Numerous studies have attempted to unravel the mechanisms of drug resistance to traditional chemotherapeutic agents and to recently developed targeted, small molecule and antibody based drugs. Briefly, the mechanisms of resistance can be roughly mapped to four categories: (1) Multi-drug resistance (MDR). MDR is caused by expression and/or induction of efflux proteins, which are members of the ABC transporter superfamily involved in the transport of both hydrophobic and hydrophilic compounds.[42] This mechanism is relatively more common for cytotoxic drugs and payload of antibody-drug conjugates[42] than targeted agents; (2) Tumor initiating cells/cancer stem cells (TICs/CSCs). As discussed earlier, these cells have the capability of self-renewal and differentiation, remain relatively quiescent, and can tolerate higher level of DNA damaging agents and oxidative stress. These characteristics are important for TICs to survive chemotherapy and radiation and ignite tumor re-growth when the condition permits;[43-46] (3) Tumor genetic and epigenetic alterations. These alterations can take place at multiple points during tumor initiation, progression and treatment, and they can be preexisting mutations, acquired mutations, or changes in downstream genes and pathways. For example, resistance to EGFR tyrosine kinase inhibitors can be attributed to multiple mechanisms, such as gatekeeper mutation (T790M),[47-49] c-Met amplification,[50] activation of alternative pathways such as insulin-like growth factor receptor and AXL,[48,51] trans-differentiation to mesenchymal cells[52] or small cell features;[53] and (4) Tumor microenvironment. Emerging data has indicated tumor microenvironment as a key mediator of drug resistance.[54] For example, several potential mechanisms of resistance to anti-angiogenic drugs are microenvironment-derived, including up regulation of alternative pro-angiogenic signals,[55,56] recruitment of bone marrow progenitors,[57] and increased pericyte coverage.[58] Another example can be found in pancreatic ductal adenocarcinoma, in which gemcitabine resistance has been attributed to inefficient drug delivery due to poorly perfused tumors.[59]

There are obvious advantages of using PDX models to study drug resistance mechanism and to characterize therapeutic agents for efficacy. As discussed earlier, PDX models are heterogeneous in nature, and more closely reflective of tumors in actual patients,[60] and a more appropriate system for understanding acquired and de novo drug resistance through enrichment of preexisting changes in subsets of cells.[61,62] A large collection of PDX models can best represent a broad patient population with various preexisting mutations and susceptibility to generate additional mutations, which cannot be achieved by other models including cell line xenografts. In addition, PDX models contain TICs/CSCs, and proper tumor stroma (albeit controversial) that can potentially contribute to resistance as well. Furthermore, it has become possible to establish PDX models with tumors that had already been treated and later became refractory. This is an important point because in clinic, most patients entering clinical trials have been treated with standard of cares previously and have relapsed with refractory disease. Compared to cell line xenografts, PDX models should better recapitulate patients with refractory and metastatic cancer.[63]

A number of studies have taken the advantages of PDX models to study drug resistance. Krumbach *et al*.[60] investigated response to cetuximab in 79 PDX models generated from colon, gastric, head and neck, lung and mammary cancer. After an in-depth analysis of different molecular characteristics of the tumors, they identified c-MET activation as a key mechanism for drug resistance, especially in NSCLC adenocarcinomas. In another study: using PDX models of NSCLC, Dong *et al*.[64] identified foci of resistance cells after cisplatin treatment as a single agent or in combination with vinorelbine, docetaxel, or gemcitabine. The authors

suggested that these drug-resistant cells were TICs-like and could be responsible for tumor recurrence.

## Patient-derived Xenograft Models for Pharmacology and Biomarker Studies

Traditionally, pharmacology, biomarker and pharmacokinetics/pharmacodynamics studies for oncology programs almost exclusively relied on tumor xenograft and to a much lesser degree, syngeneic models. With the significant increase in the availability and affordability of PDX models offered by both academic institutions and contract research organizations, PDX models have seen increasingly their utility in routine research activities. A quick survey of oncology discovery programs published in the past 3 years shows that increasing number of programs use PDX models at some point during the preclinical discovery and translational research stages.[14,65-67] In addition, there is an industry-wide trend to include PDX model readout as a key component of the required data package for both internal use as well as regulatory submission. The history of using incorporating PDX models in drug discovery can be traced back to several decades ago. For example, one of the earliest reports involving cancer drugs and PDX models by Fiebig *et al*.[68] studied a number of chemotherapy drugs at their respective maximal tolerated doses (MTDs) in PDX models derived from 34 patients, and demonstrated 92% accuracy in predicting efficacy and 97% in predicting no-response. Similar predictive value was seen in a later study by the same group.[69] However, additional studies suggest that the predictive value can fluctuate due to factors such as tumor histology and location, stage of disease from which the models are derived, the quality of PDX models, sample size and dosing regimen.[64,70,71] In addition to selecting models that are histologically, molecularly and genetically relevant to the patients in clinical, another important factor for improving translatability of preclinical findings is the drug exposure. Not surprisingly, preclinical model species, in most cases immunocompromised mice, can exhibit different tolerability and adsorption, distribution, metabolism and excretion property than those in human. It is commonly seen that drug exposure levels at MTD dose in mice are higher than clinically achievable levels in human.[72] Therefore, a compound given at mouse MTD to xenograft, allograft or syngeneic models may generate exaggerated efficacy that over-predicts human response in the clinic. This phenomenon has been seen for both chemotherapy agents[12,73,74] as well as targeted agents such as vascular endothelial growth factor receptor inhibitors and PI3K inhibitors.[75] A key concept and practice to avoid the pitfalls of using mouse MTD dose and exposure as the sole basis for efficacy prediction is to use CRD or clinically relevant exposure (CRE) whenever a CRD or CRE can be determined.

## Patient-derived Xenograft Models for Mouse Clinical Trial

An evolving concept and practice, PDX mouse clinical trial, has started to yield positive results that had real-life impact on selected patients.[76] In this setting, PDX models established from the very same patients on trial are being treated ahead of patient therapy or concurrently, and results from the mouse trial is provided in real-time to help guide clinical management of the patient's tumor. Further powered by the molecular characterization of the tumors, this highly personalized approach has the potential to revolutionize the drug development and patient care.[77] For example, a recent study by Stebbing *et al*.[78] reported 22 sarcoma PDX models were successfully established from 29 patients (76% take rate) and screened for drug sensitivity to a panel of therapeutic agents. The entire process typically took 3-6 months depending on individual tumor growth characteristics and treatment regimen. Of the 22 patients, 6 died before data became available. Of the 16 remaining patients, 13 (81%) demonstrated a correlation between the results from their PDX mouse trial and clinical outcome. Similar approach has also been reported in advanced adenoid cystic carcinoma,[79] ovarian,[80] and other cancer types.[81] The current data, although limited, appears to support the use of PDX models to prioritize therapeutic agents against individual tumors. However, some key challenges remain before this strategy can be broadly implemented in clinical practice. For example, establishment of PDX models is still a technically challenging and time-consuming process, even after much progress has been made to improve the take rate and optimize the expansion scheme. In addition, the algorithm for the selection of agents to be tested needs to be further developed and refined. Lastly, to effectively demonstrate the feasibility and clinical benefit of the PDX-guided treatment prioritization in the patient care setting, properly controlled clinical trials are needed.

## Limitations of Patient-derived Xenograft Models

Although PDX models present an exciting opportunity for improving predictive value of preclinical and translational studies, and offer a number of advantages over conventional cell line xenograft models, just like any other preclinical model platforms, there are several limitations that one needs to be aware of. First, the utilization of severely immune-compromised host mouse strains, particularly the nonobese diabetic severe combined immune deficiency gamma mice, while allowing higher take rate and more consistent growth of xenografted human tumors, is inherently inadequate in modeling immune responses. Although human stroma components including immune cells originally present in the tumor biopsy can be grafted together with the tumor tissue,[82] they normally cannot survive beyond the first passage, and will be completely lost in the subsequent expansion.[83] The other stroma components including fibroblasts and vasculature are quickly replaced by murine counterparts.[83] The lack of functional immune system limits the utility of these models in studies where immune responses are required. For example, immunotherapy cannot be readily studied in the PDX

models established in immune-compromised mice. It is well documented and accepted that immune system is an important part of tumor stroma and significantly contributes to tumor initiation, progression, metastasis and therapeutic response.[84,85] The introduction of mice with partially or completely humanized immune systems can potentially ameliorate this issue, but significant technical challenges still exist.[86,87]

Second, although technical advances have gradually improved the tumor take, different tumor types, and different subtypes within the same tumor type, have varying rates of success. This has led to imbalanced representation of tumor types/subtypes that is more determined by take rate rather than clinical incidence rate. Although PDX models can avoid artificial selection in extended culture on plastic, the *in vivo* selection process exists as soon as the tumors are implanted. For example, high-grade, fast proliferating tumors tend to be easier to establish as PDX models than low-grade, slowly growing but progressive tumors.[88,89]

Additionally, compared to cell lines, PDX models are difficult to manipulate genetically. Most PDX models are established from and passaged as tumor fragments, and conventional transfection or transduction are not efficient to genetically modify the tumors or introduce detection markers (such as luciferase or fluorescent proteins). Therefore, PDX tumors are rarely established as orthotopic models, unless there is a surrogate biomarker that be readily used to measure tumor burden noninvasively.[90]

## Conclusion

Although hardly a new concept, PDX models have gained much attention and premium status in the past few years as they are becoming increasingly available and affordable, and are believed to offer a superior predictive value over conventional cell line xenograft models. Ample data indicated that PDX models maintain heterogeneity and tumor initiation ability, as well as molecular and genetic characteristics reflective of human tumors. Emerging data indicated an improved predictive value of the PDX models; however, it is still early to conclude whether the advantage in translatability is applicable to large sample size and to various therapeutic mechanisms and modalities. The mouse clinical trial has the potential to accelerate and de-risk human clinical trials and hopefully reduce clinical attrition rates for novel compounds, and to prioritize therapies by allowing parallel testing of multiple treatment schemes for an individual patient. However, there are still much to be done to address technical challenges to make this approach feasible and affordable and to convince the medical and insurance community of the value this approach can offer. At the same time, one cannot overlook the limitations of PDX models and should take into consideration of their shortcomings when design and interpret studies. Collectively, these new developments emphasize the importance of employing PDX models in key areas of oncology drug discovery and development.

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**How to cite this article:** Li G. Patient-derived xenograft models for oncology drug discovery. J Cancer Metastasis Treat 2015;1:8-15.

**Received:** 05-11-2014; **Accepted:** 27-01-2015.
**Source of Support:** Nil, **Conflict of Interest:** None declared.