

Minireview

# An Integrative Approach to Precision Cancer Medicine Using Patient-Derived Xenografts

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Cancer is a heterogeneous disease caused by diverse genomic alterations in oncogenes and tumor suppressor genes. Despite recent advances in high-throughput sequencing technologies and development of targeted therapies, novel cancer drug development is limited due to the high attrition rate from clinical studies. Patient-derived xenografts (PDX), which are established by the transfer of patient tumors into immunodeficient mice, serve as a platform for co-clinical trials by enabling the integration of clinical data, genomic profiles, and drug responsiveness data to determine precisely targeted therapies. PDX models retain many of the key characteristics of patients' tumors including histology, genomic signature, cellular heterogeneity, and drug responsiveness. These models can also be applied to the development of biomarkers for drug responsiveness and personalized drug selection. This review summarizes our current knowledge of this field, including methodologic aspects, applications in drug development, challenges and limitations, and utilization for precision cancer medicine.

## INTRODUCTION

Emerging evidence has indicated that cancer is a genomic disease caused by diverse alterations in oncogenes and tumor suppressors associated with oncogenic signaling pathways (Garraway et al., 2013). Recent advances in high-throughput sequencing technologies have elucidated the mutational landscape of several cancers and provided a comprehensive catalogue of cancer genes (Bell et al., 2011; Koboldt et al., 2012; Muzny et al., 2012; Verhaak et al., 2010; Weinstein et al., 2013). These and other genomic analyses of cancers have demonstrated that cancer is often a highly heterogeneous disease, even with respect to genomic mutation levels and tumor histology in the same cancer type. However, traditional cancer clas-

sification based on the cellular origin of tumors and on tumor histology cannot explain these heterogeneous characteristics. This widespread heterogeneity can explain, to some degree, why some patients with a given cancer type are more or less resistant to a particular therapy than other patients. The concept of precision medicine, whereby patients are grouped into subpopulations based on sophisticated genomic profiling, aims to address this problem by enabling development of therapies designed to specifically target the unique cancer of an individual or subgroup of individuals.

For some cancers, extensive genomic profiling has yielded identification of many cancer-related genes, which in turn, has led to identification of genomic alterations that can be targeted for therapy. Examples of drugs that target specific genomic alterations and have been translated to the clinic include imatinib, which targets the BCR-ABL1 fusion protein in chronic myeloid leukemia (Quintas-Cardama and Cortes, 2009), trastuzumab, which targets HER2 amplification in breast and gastric cancer (Slamon et al., 2011), gefitinib and erlotinib targeting the EGFR mutation in lung cancer (Pao et al., 2004), and crizotinib targeting the EML4-ALK fusion protein in lung cancer (Shaw et al., 2011). In addition to enabling the translation of such drugs to the clinic, the accelerated development of targeted therapies has promoted the concept that treatment decisions might eventually be guided by the molecular profiling of individual tumors (Macconail and Garraway, 2010). The integration of genomic profiling and targeted therapy are now broadly investigated in almost all kinds of cancer types.

However, drug development in oncology is usually hampered by high clinical attrition rates (DiMasi et al., 2013). The clinical approval success rates of cancer drugs are lower than 15%, especially lower in solid tumors (DiMasi et al., 2013). Despite the great promise of genomic alteration targeted therapy, it has proven difficult to identify targetable genomic alterations in quite a lot of cancers. Genomic instability of cancer increases the complexity of the cancer phenotype, as genomic instability in a given cancer can result in simultaneous alterations in several oncogenes and tumor suppressors involved in various cancer-associated signaling pathways. In addition, a tumor is a mixture of heterogeneous cells including cancer cells, fibroblasts, vessel cells, and immune cells. Even cancer cells from a single patient exhibit diverse characteristics in the genomic and transcriptomic signature, contributing to the phenomenon of the intra-tumoral heterogeneity of cancer. Therefore, in many cases, drug responses cannot be fully anticipated via simple genetic profiling.

To overcome these limitations, more precise and compre-

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Received 17 December, 2015; accepted 23 December, 2015; published online 2 February, 2016

**Keywords:** cancer drug development, cancer genomics, patient-derived xenografts, precision medicine

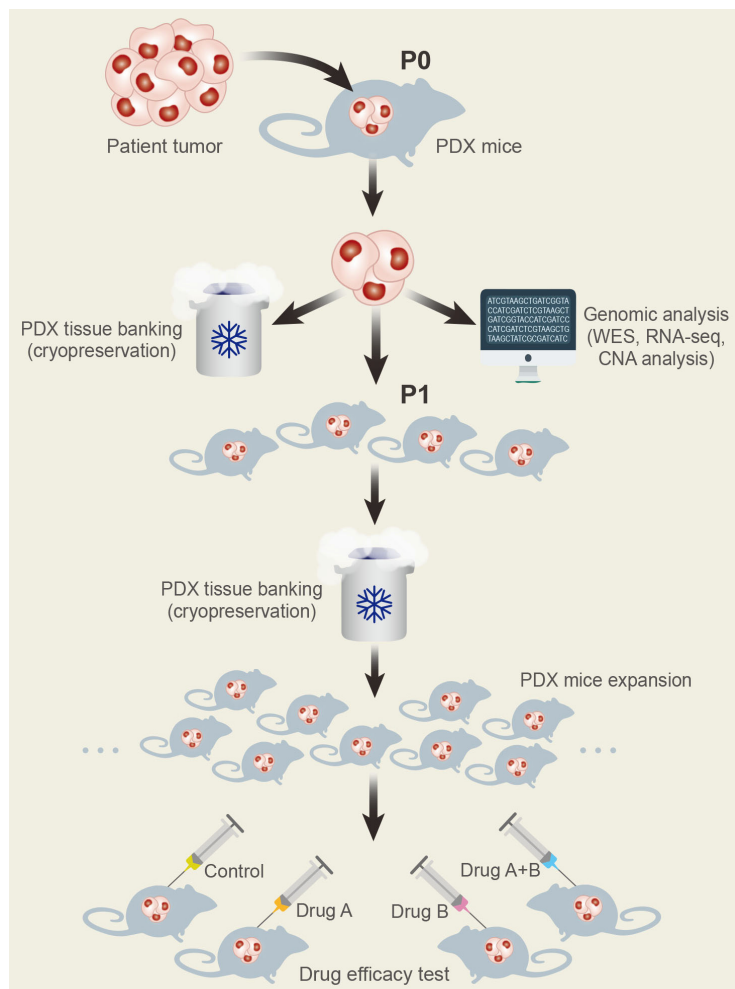
hensive profiling of genomic alterations in tumors, and of drug responsiveness, is needed. Patient-derived xenografts (PDX), a research platform whereby patient tumors are transplanted into immunodeficient mice, are designed to meet this need by providing clinically predictive models of human cancers. This review delineates recent advances of PDX models and their significance for precision cancer medicine.

### PATIENT-DERIVED XENOGRAFT MODELS: OVERVIEW

PDX models are generated by implanting sectioned patient tumor fragments into immunodeficient mice, subcutaneously or orthotopically (into the organ of the cancer origin). Although previously used methods for drug testing such as *in vitro* cell culture and organoid cultures are advantageous in easy genetic manipulation and high-throughput screening, they showed strong selective proliferation, adaptation to culture condition and loss of tumor heterogeneity (Gao and Chen, 2015). An important advantage of PDX models is that they retain key characteristics of patients' tumors. The histologic characteristics and genomic signature of the patient tumor, and the heterogeneity of cancer cells, are highly preserved in PDX tumors (Kopetz et al., 2012). PDX tumors also contain stromal and immune cells originating from the patient (Kopetz et al., 2012). Thus, PDX models are the most clinically relevant cancer models developed

to date, and represent a highly predictive drug response platform that recapitulates the therapeutic outcome in human patients.

The procedures for the generation of PDX are very straightforward, comprising the collection of fresh surgical tumor-containing tissue, division of the tumor into pieces, and then implantation, either subcutaneously or orthotopically, into an immunodeficient mouse (Fig. 1; Tentler et al., 2012). In some cases, investigators put tumors into the renal capsule for effective blood flow. The tumors, which are established in immunodeficient mice, are cut into 2-4 mm fragments and reimplanted into new hosts for next passage. Several different mouse strains are used for generation of PDX models, and they differ with respect to the degree of immunosuppression, research benefits, and experimental considerations (Table 1). Nude, B6 *Rag 1* and BALB *scid* mice have intact innate immunity and NK cells, and can be used for engraftment of human and mouse cancer cell lines. On the contrary, NOD *scid* and NOD/*scid*/IL2 $\gamma$ -receptor null (NSG) mice have defects in both humoral and innate immunity, and support implantation of human hematopoietic cells and solid tumors. Human acute myeloid leukemia and acute lymphoblastic leukemia primary cells showed faster leukemic symptom and more leukemic cells in NSG mice compared to other immunodeficient mice (Agliano et al., 2008). The time needed for engraftment of PDX mice differs according to tumor type, site of implantation, and mouse strain,



**Fig. 1.** Generation of PDX models. Surgical specimens from cancer patients are divided into small pieces and transplanted into immunodeficient mice (P0). When tumors are grown in P0 mice, xenografts are used for genomic analysis including whole exome sequencing (WES), RNA sequencing (RNA-seq), and copy number alteration (CNA) analysis, and then maintained in cryo-banks for preservation. After expanding tumor xenografts in immunodeficient mice (P1 and more), *in vivo* drug responsiveness is screened in these models.

**Table 1.** Comparison of immunodeficient mouse strains [Modified from The Jackson Laboratory's JAX<sup>®</sup> Mice and Services website (<https://www.jax.org/jax-mice-and-services/find-and-order-jax-mice/most-popular-jax-mice-strains/immunodeficient-mouse-and-xenograft-host-comparisons>)].

	<b>NOD <i>scid</i> <math>\gamma</math> (NSG)</b>	<b>NOD <i>scid</i></b>	<b>BALB <i>scid</i></b>	<b>B6 <i>Rag1</i></b>	<b>Outbred nude</b>	<b>Inbred nude</b>
<b>Mature B cells</b>	Absent	Absent	Absent	Absent	Present	Present
<b>Mature T cells</b>	Absent	Absent	Absent	Absent	Absent	Absent
<b>Dendritic cells</b>	Defective	Defective	Present	Present	Present	Present
<b>Macrophages</b>	Defective	Defective	Present	Present	Present	Present
<b>Natural killer cells</b>	Absent	Defective	Present	Present	Present	Present
<b>Complement</b>	Absent	Absent	Present	Present	Present	Present
<b>Leakiness</b>	Very low	Low	Low	Absent	N/A	N/A
<b>Irradiation tolerance</b>	Low	Low	Low	High	High	High
<b>Lymphoma incidence</b>	Low	High (thymic lymphoma)	High (thymic lymphoma)	Low	Low	Low
<b>Median survival</b>	> 89 weeks	36 weeks	Not determined	Not determined	Not determined	Not determined
<b>Benefits</b>	<ul style="list-style-type: none"> <li>- Adoptive transfer recipient for study of autoimmune type 1 diabetes</li> <li>- Supports engraftment of human peripheral blood &amp; bone marrow</li> <li>- Xenotransplantation of human tissues, cells, &amp; tumors</li> </ul>	<ul style="list-style-type: none"> <li>- Adoptive transfer recipient for study of autoimmune type 1 diabetes</li> <li>- Engrafts hematopoietic cancer cell lines</li> <li>- Xenotransplantation of some human tumors</li> </ul>	<ul style="list-style-type: none"> <li>- MHC haplotype (H2d) allows adoptive transfer from BALB/c donors</li> <li>- Common BALB/cBy inbred background simplifies creation of compound immunodeficient mutants</li> <li>- Therapeutic Ab testing</li> <li>- Engrafts hematopoietic cancer cell lines, some primary cells</li> </ul>	<ul style="list-style-type: none"> <li>- MHC haplotype (H2b) allows adoptive transfer from B6 donors</li> <li>- Common B6 inbred background simplifies creation of compound immunodeficient mutants</li> <li>- Therapeutic Ab testing</li> </ul>	<ul style="list-style-type: none"> <li>- Engraftment of human &amp; mouse tumor cell lines</li> <li>- Well published/characterized</li> <li>- Segregating genetic background improves hybrid vigor</li> <li>- Hairless phenotype enhances assessment of tumor growth</li> </ul>	<ul style="list-style-type: none"> <li>- Engraftment of human &amp; mouse tumor cell lines</li> <li>- Well published/characterized</li> <li>- Uniform genetics improve reproducibility</li> <li>- Hairless phenotype enhances assessment of tumor growth</li> </ul>
<b>Considerations</b>	<ul style="list-style-type: none"> <li>- No thymic lymphomas, can be used for long &amp; short-term experiments</li> <li>- Sensitive to irradiation</li> </ul>	<ul style="list-style-type: none"> <li>- Develops thymic lymphomas by 8-9 months, best used in short-term experiments</li> <li>- Sensitive to irradiation</li> </ul>	<ul style="list-style-type: none"> <li>- Innate immunity intact NK cell activity limits engraftment</li> <li>- Sensitive to irradiation</li> </ul>	<ul style="list-style-type: none"> <li>- Innate immunity intact</li> <li>- Poor host for primary cell transplantation</li> </ul>	<ul style="list-style-type: none"> <li>- Innate immunity intact</li> <li>- Little engraftment of hematopoietic cancer cells</li> <li>- Not suitable for primary cell transplantation</li> </ul>	<ul style="list-style-type: none"> <li>- Innate immunity intact</li> <li>- Little engraftment of hematopoietic cancer cells</li> <li>- Not suitable for primary cell transplantation</li> </ul>

but in general it takes between two and four months (Morton and Houghton, 2007). For drug treatment testing, tumor-bearing mice can be subsequently expanded from early passages.

Reported success rates of PDX models estimated by obtaining successful PDX tumors for next passages have ranged between 23% and 75%, depending on the tumor type (Table 2; Siolas and Hannon, 2013). Generally, colorectal (64-89%) and pancreatic (62%) tumors have had high engraftment rates, but breast cancers (13-27%), especially estrogen receptor-positive breast cancers, have shown low success rates, even in the laboratories most successful with PDX engraftment in general (Hidalgo et al., 2014; Williams et al., 2013). Clinically aggressive and metastatic cancers have exhibited high PDX model

engraftment rates compared to less aggressive and non-metastatic cancers (Zhao et al., 2012), and patients with tumors that had a high engraftment rate in PDX mice showed lower overall survival and increased metastatic characteristics compared to patients whose tumors showed a low engraftment rate (Garrido-Laguna et al., 2011), suggesting that the engraftment rate can sometimes be considered a predictive marker of disease prognosis (DeRose et al., 2011). Engraftment success can also be affected by experimental protocols. In breast cancer, supplementation with estradiol pellets enhanced stable xenograft take rate from 2.6% to 21.4% (Zhang et al., 2013), and in prostate cancer, male NOD *scid* mice supplemented with testosterone showed successful engraftment in 38.9% (7/18) of samples (Lin et al., 2014). Among immunodeficient mouse

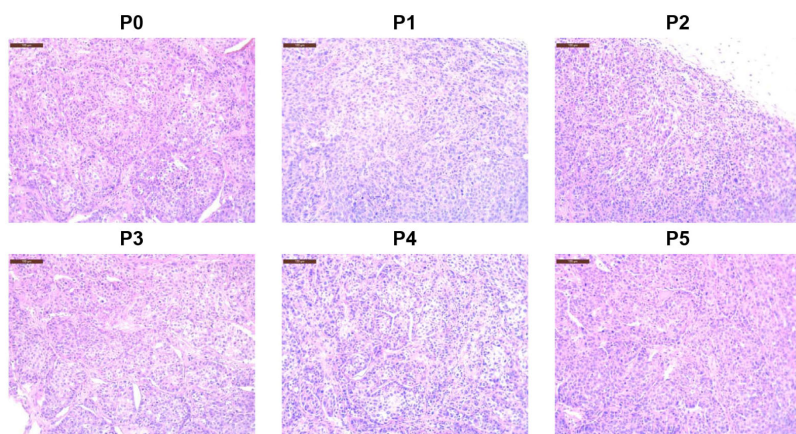
**Table 2.** Summary of engraftment rates of PDX tumors

Tumor type	Mice strain	Implantation site	Engraftment rate	References
Breast cancer	Nude	Subcutaneous	13%	Marangoni et al., 2007
	NOD <i>scid</i>	Mammary fat pad	27%	DeRose et al., 2011
	<i>scid</i> /Beige, NSG	Mammary fat pad	19-21%	Zhang et al., 2013
	NOD <i>scid</i>	Humanized mammary fat pad	13%	Li et al., 2013
Colorectal cancer	NOD <i>scid</i>	Subcutaneous	87%	Bertotti et al., 2011
	Nude	Subcutaneous	64%	Julien et al., 2012
	Nude	Orthotopic	89%	Aytes et al., 2012
Head and neck cancer (squamous cell carcinoma)	NSG	Subcutaneous	85%	Kimble et al., 2013
	Nude	Subcutaneous	54%	Keysar et al., 2013
Medulloblastoma	Rag2 <i>scid</i>	Orthotopic	52%	Zhao et al., 2012
Non-small cell lung cancer	NOD <i>scid</i>	Subcutaneous	25%	Fichtner et al., 2008
	NOD <i>scid</i>	Renal capsule	90%	Dong et al., 2010
Pancreatic cancer (ductal adenocarcinoma)	Nude	Subcutaneous	61%	Garrido-Laguna et al., 2011
	Nude	Orthotopic	62%	Reyes et al., 1996
	ICR <i>scid</i>	Subcutaneous	67%	Mattie et al., 2013
Uveal melanoma	NOD <i>scid</i>	Subcutaneous	28%	Némati et al., 2010

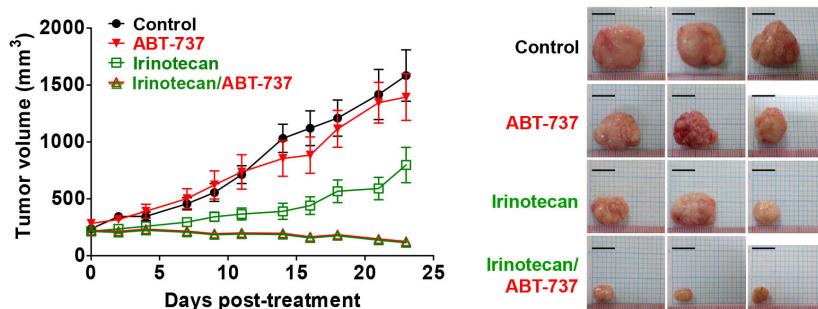
strains, *scid*-based strains, such as BALB *scid*, NOD *scid*, and NOD/*scid*/IL2 $\gamma$ -receptor null (NSG), demonstrated more successful engraftment compared to nude (nu) mutation-based strains due to further immune suppression (Landis et al., 2013). Therefore, tumor type, cancer aggressiveness, hormonal supplements and mouse host strains are important experimental factors to determine the engraftment rates.

The tumor implantation site is one of considering factors for the generation of PDX models. Subcutaneously transplanted PDX models have been widely developed due to their simple procedure and easy measurement of tumor size, and suitable for massive expansion of PDX models. Orthotopic models are anticipated to show more similar behavior of patient tumors, especially in metastasis (Hoffman, 2015), but well-trained surgical skill is required for appropriate PDX models generation. The subrenal capsule site is considered as tumor plantation site due to its hypervascularity (Lin et al., 2014), and exhibited successful PDX generation in several cancers including prostate and ovarian cancer (Lin et al., 2014; Scott et al., 2013). Considerable evidence indicates that PDX tumors retain the histological and genomic characteristics of the original human tumor (Rosfjord et al., 2014). At low passages, the morphological

features and chromosomal stability of PDX tumors are comparable with the corresponding patient tumors (Fig. 2; DeRose et al., 2011; Rosfjord et al., 2014; Zhang et al., 2013). In addition, comprehensive gene-expression data analyses have indicated that PDX tumors cluster with their original tumors in clustering studies, and that the majority of the key genes and signaling pathway activity in original tumors are preserved in PDX models (Reyal et al., 2012; Tentler et al., 2012). In breast cancer PDX models, even spontaneous metastatic patterns appear to be recapitulated to clinically relevant sites including thymus, lung, bone, and peritoneum (DeRose et al., 2011). In serial passage experiments in which the repeated transplantation of the same tumor has been performed, a relatively high degree of genomic aberration has been preserved in colorectal, breast, and pancreatic cancers (Julien et al., 2012; Mattie et al., 2013; Reyal et al., 2012), but the degree of preservation is dependent on the particular cancer indication studied (Rosfjord et al., 2014). In addition, some studies have reported PDX-unique single nucleotide mutations, which are caused either by adaptation of tumor cells to a new microenvironment or by overgrowth of subclones below detectable limits in the original tumor (Ding et al., 2010; Li et al., 2013).



**Fig. 2.** Histologic comparison among PDX tumors from passage 0 (P0) to passage 5 (P5). Breast cancer PDX model #45356922 was generated in NSG mice and PDX tumors were serially implanted from P0 to P5. Tissues were formalin-fixed and stained by H&E staining. Scales bars: 100  $\mu$ m.



**Fig. 3.** *In vivo* drug efficacy testing of combination therapy using PDX models. Combination effects of BCL2L1 inhibitor (ABT-737) and cytotoxic drug (irinotecan) were tested in BCL2L1-amplified (11 copies) gastric cancer PDX models. Average tumor sizes of treated groups are plotted (left panel) and representative tumors after treatment are shown (right panel). Scale bar: 10 mm. (reproduced from Park et al., 2015).

from these studies shows a notable similarity of drug activity between PDX mice and patients, indicating remarkable predictive value of PDX models for clinics (Hidalgo et al., 2014; Rosfjord et al., 2014). For example, PDX models and human patients exhibited comparable responsiveness rates in anti-EGFR antibody cetuximab treatment for colorectal cancers and gemcitabine treatment for pancreatic cancers (Bertotti et al., 2011; Garrido-Laguna et al., 2011; Julien et al., 2012). In addition, selected drugs that were effective in PDX models produced similar responsiveness for patients in terms of resistance and sensitivity (Hidalgo et al., 2011).

In predicting the therapeutic effects in clinic, PDX models are valuable research tools which can overcome well-recognized limitations of conventional cell line-derived xenograft (CDX; Johnson et al., 2001; Kung, 2007). Although the anti-angiogenic agent, bevacizumab prolonged survival in glioblastoma (GBM) CDX study (de Groot et al., 2010), bevacizumab failed to extend overall survival of patient in clinic (Lai et al., 2011). Like these outcomes, the survival length of GBM PDX was not altered by bevacizumab treatment and tumor became more aggressive and invasive (Joo et al., 2013).

In sum, these evidences indicate that PDX models can be a stable preclinical research platform that retains the characteristics of original patients' tumors with regard to histology, genomic and transcriptomic signatures, and drug responsiveness.

## APPLICATIONS OF PDX MODELS

PDX models have proven to be valuable platforms for preclinical drug testing in many types of cancer. The effectiveness of the combination of nab-paclitaxel and gemcitabine in pancreatic cancer was demonstrated in a PDX experiment (Von Hoff et al., 2011), and later this regimen was reported to provide a survival benefit for patients with advanced pancreatic cancer in a randomized phase III study (Von Hoff et al., 2013). PDX models are especially advantageous in preclinical studies of targeted agents, because, after evaluating the genomic characteristics of a PDX model, a subset of PDX models with the same genomic characteristics can be tested for targeted therapeutics. For example, the effect of the pan-RAF and SRC-family kinase dual inhibitor was evaluated by previously known RAF inhibitor-sensitive and resistant melanoma PDX models (Girotti et al., 2015). In addition, combination treatments of targeted drugs and cytotoxic drugs can also be tested in previously characterized PDX models. For example, our group recently validated the efficacy of combination therapies using a BCL2L1 inhibitor and irinotecan in genomically defined gastric cancer PDX models (Fig. 3; Park et al., 2015).

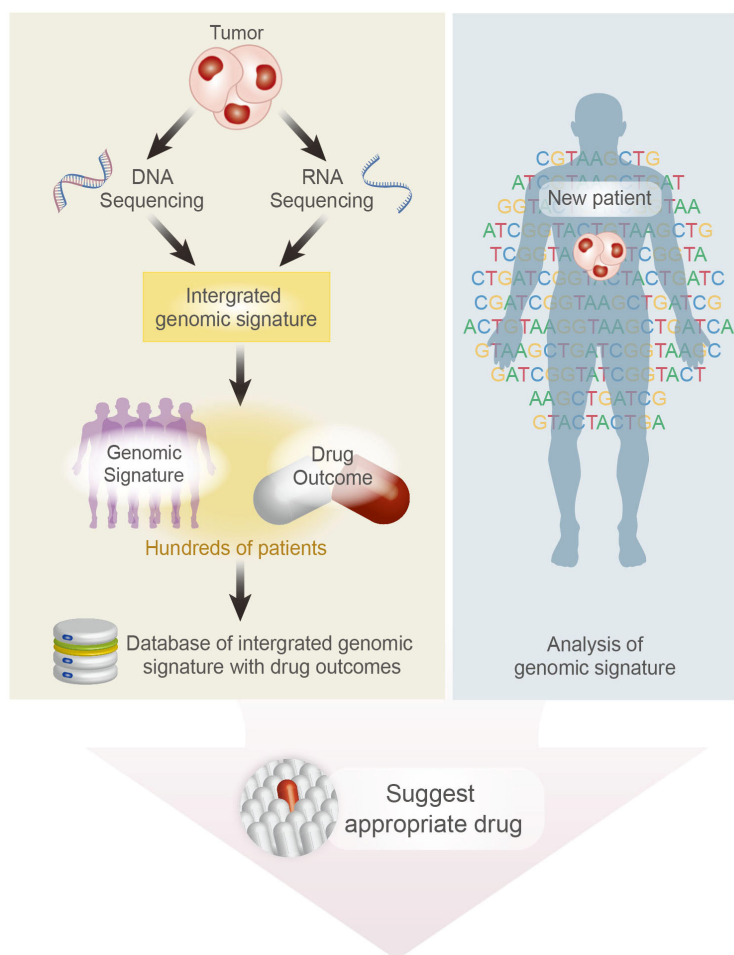
For more systematic application of PDX models to drug development process, biorepositories, or biobank of PDX tumors are required. Especially, genomically well-characterized PDX

repositories are valuable resources for developing improved diagnostics and therapeutics of cancers (Dowst et al., 2015). Recently, several groups, both in academia and industry, developed PDX repositories, and have now tried to develop collaborative networks for PDX biobanking (Hidalgo et al., 2014). For example, EurOPDX is a consortium of centers working on PDX models in Europe, and aims at the development of network of clinically relevant and annotated PDX models (Hidalgo et al., 2014).

With genomically well-defined PDX collections, biomarkers associated with drug sensitivity and resistance can be identified, which facilitates precision cancer medicine (Fig. 4). PDX tumors recapitulated previously known sensitive biomarkers including *KRAS* wild-type status for cetuximab sensitivity in colorectal cancers (Bertotti et al., 2011; Julien et al., 2012) and *KRAS* wild-type status for erlotinib sensitivity in lung cancers (Fichtner et al., 2008). By comparing drug-sensitive and -resistant PDX groups in colorectal cancers, amplification of *HER2* was identified as a resistant biomarker for cetuximab treatment (Bertotti et al., 2011). PDX models are also valuable tools for generating drug-resistant tumor models, which is achieved by repeated administration of a given drug. These resistant tumors are more clinically relevant compared to previous *in vitro* cancer cell culture systems, and are used for investigation of drug resistance mechanisms and identification of drug resistance biomarkers. For example, continuous administration of the BRAF inhibitor vemurafenib resulted in vemurafenib-resistant melanoma models with BRAF mutations, and elevated mutant BRAF protein resulting from this continuous drug treatment was reported as a critical factor in vemurafenib resistance (Das Thakur et al., 2013).

The predictive power of PDX models is now being applied to co-clinical trials for novel cancer therapeutics. Phase I/II clinical trials take more than 5 years to complete, and during these trials, real-time analysis and integration of data for stratification of responders and resistant populations are limited. To overcome this hurdle, co-clinical trials are carried out, whereby drug treatment experiments for mouse tumor models are performed in parallel with clinical trials, and preclinical and clinical data are integrated (Nardella et al., 2011). The concept of co-clinical trials was first developed for genetically engineered mouse models, but this strategy can be applied to genetically well-defined PDX models (Hidalgo et al., 2014). The co-clinical trial approach using PDX models will facilitate rapid stratification of responders, prioritization of attractive drug combinations, identification of resistance mechanisms, and determination of biomarkers (Nardella et al., 2011).

Although genomic profiling of tumors provides significant improvement in patient stratification and targeted drug selection, additional decision-making processes may be required when multiple potential targets are identified or no targetable mutation



**Fig. 4.** An integrated strategy for precision medicine using PDX models. Database of integrated genomic signature with drug outcome can be generated from hundreds of PDX models. By integrating clinical data from patients, genomic profiling data, and drug screening data, PDX models are valuable platforms for precision cancer medicine and personalized drug selection.

is found. To facilitate drug selection in such cases, PDX models are used as “Avatar” models for personalized cancer treatment. The avatar models are mouse substitutes of specific human patients for drug testing. In one prospective study, avatar models were generated from 14 patients, and effective treatment options were explored with 63 drugs in 232 treatment regimens (Hidalgo et al., 2011). Following identification of the most effective treatment regimens in the xenograft model, the 17 regimens were tried in 11 patients, and durable partial remission was detected in 15 treatments.

And in another study, PDX clinical trial (PCT) was demonstrated to be assessed by comparing the targeted drug response in PDX to responses in patients. The 67% melanoma PDX with BRAF mutation showed response to encorafenib, a selective BRAF inhibitor and this was concordant with the reported phase II clinical trial response rates in patients. And also, insensitive BRAF-mutated melanoma PDX to BRAF inhibitor responded to combined therapy with MEK inhibitor. This is consistent with combination phase I/II trial in BRAF-mutant melanoma patients (Gao et al., 2015). Thus, personalized PDX models showed high positive predictive value for drug testing, and can be beneficial for therapeutic decision-making and guiding treatment options.

PDX tumors can recapitulate the intratumoral heterogeneity of original patients’ tumors, and a growing body of evidence shows that tumors contain a distinct subpopulation, usually termed cancer stem cells (CSCs). CSCs have the capacity for

self-renewal and differentiation, and have been reported to be more resistant than other cancer cells to chemotherapy and radiation (Rosen and Jordan, 2009). A large number of CSCs can be obtained through expansion of PDX tumors in mice without compromising the heterogeneity of the original tumor (Rosford et al., 2014). Using specific surface markers, CSCs have been purified and characterized in several types of PDX tumors (Bertolini et al., 2009; Schatton et al., 2008). In addition, the heterogeneity profiles of patients’ tumors evolve in response to environmental changes, and the microenvironment of tumors in a mouse background provides selection pressure for tumors, recapitulating tumor evolution and clonal selection in patients (Whittle et al., 2015). PDX tumor from primary basal-like breast cancer retained the primary tumor mutations and showed additional *de novo* mutations similar with the brain metastatic lesion, suggesting that the PDX models exhibit genomic adjustments compatible with cancer evolution in patients (Ding et al., 2010). The clonal dynamics in PDX propagation was evaluated through deep-genome and single-cell sequencing, and the results showed that the degree of clonal selection varied from extremely selective engraftment of minor clones to moderate, polyclonal engraftment, and similar clonal expansion patterns were reproduced in independent grafts of the same tumors (Eirew et al., 2015). In sum, the above studies demonstrate that PDX models can be applied to the study of tumor heterogeneity, CSCs, and cancer evolution.

PDX models in early passages retain stromal components

and acidic conditions of microenvironment in patient tumors, and can be used to study the effects of microenvironment on tumor biology (Choi et al., 2014). The microenvironment-targeting agent, ibrutinib was effective at inhibiting tumor growth and increasing survival in PDX models of pancreatic ductal adenocarcinoma (Masso-Valles et al., 2015). Tumor architecture can be conserved through multiple passages by recruitment of murine stromal cells instead of human stromal elements (Delitto et al., 2015).

PDX models can also be applied to study the effect of cancer immunotherapy. For example, mice engrafted with patient-derived gastric cancer tissue and peripheral mononuclear cells from same patient exhibited slow tumor growth by combination therapy of anti-hCD137 and anti-hPD1 antibodies (Fernandez de Sanmamed et al., 2015). However, humanized PDX models, which have patient-derived tumor and full repertoire of human immune cells by engrafting human hematopoietic stem cells, are required for proper preclinical models of cancer immunotherapy.

## CHALLENGES IN PDX MODELS

Although PDX models maintain the histology, genomic architecture, and drug responsiveness of the original tumors, during engraftment into immunodeficient mice the clonal profiles of PDX tumors can change, in comparison to the patients' tumors. Analysis of genome-wide variant allele frequencies in serial passages of PDX tumors showed that clonal selection occurs more frequently in initial engraftment steps than in propagation steps, but the detailed clonal dynamics differs depending on the different tumor samples of the same tumor type (Eirew et al., 2015). This clonal dynamics in PDX tumors is probably generated by selection acting on preexisting clones, rather than the generation of new clones (Aparicio et al., 2015). As a result, it is probable that the more aggressive clones become dominant in PDX tumors, and in some cases, PDX models indeed showed the genomic and transcriptomic signature of metastatic and relapsed cancers (Rosfjord et al., 2014). These aggressive clones could be particularly important targets in cancer therapeutics.

For use of PDX models in decision-making processes of cancer therapeutics, engraftment failure and the long periods of time required for PDX establishment are limiting factors. Because success rates of PDX models vary depending on the cancer type and tumor aggressiveness as detailed above, there is a probability that PDX collections are skewed toward more aggressive type of cancers (Hidalgo et al., 2014). The time for establishment and expansion of PDX models for drug efficacy testing—four to eight months, as noted above—is usually not feasible for immediate application in patient decision-making for therapeutics. An alternate strategy would be to perform PDX-based drug testing during the period of initial standard therapy and pursue the second-line treatments based on PDX drug screening.

The depletion of human stromal and immune cells in engrafted tumors of late passages is a major limitation of PDX models for studies of tumor microenvironments and metastasis. The interaction of tumor-associated cells, such as vascular cells, fibroblasts, and inflammatory cells, with malignant cells plays an important role in tumor biology, and recent studies have shown that tumor microenvironments are also associated with drug responsiveness (Ostman, 2012). In addition, the application of immune-modulating agents such as anti-PD1 antibody into PDX models is limited, as immunocompromised mice used for PDX generation lack the essential components of an immune system (Hidalgo et al., 2014). To overcome this hurdle, immunocompromised mice that are transplanted with human hemato-

poietic stem and progenitor cells, i.e., humanized mice, are promising options. Some studies have demonstrated that diverse lineages of functional human immune cells have developed via the infusion of human hematopoietic stem cells, and of human immune and stromal cells, in tumor xenografts (Morton et al., 2015; Rongvaux et al., 2014).

Another pitfall of PDX models is lymphomagenesis of human tumors in mice. This phenomenon has been reported in PDX models of several cancer types including non-small cell lung cancers, hepatocellular carcinomas, prostate cancers, and gastric cancers (Chen et al., 2012; John et al., 2012; Wetterauer et al., 2015; Zhang et al., 2015). Most of these lymphomas were human-derived lymphomas expressing human CD45 markers, consistent with B-cell subtype lymphomas (Chen et al., 2012; Zhang et al., 2015). Further, Epstein-Barr virus (EBV) infections are highly associated with lymphomagenesis (Chen et al., 2012; Zhang et al., 2015). Tissue inflammation in parent tumors increases the risk of having a human-derived lymphoma in the PDX models, and gastric cancers exhibit a much higher rate of lymphoma proliferation than colorectal cancers partly due to higher rate of baseline inflammation (Zhang et al., 2015). A suggested mechanism of lymphoma formation is that, without immunosurveillance in immunodeficient mice, EBV can infect and transform B lymphocytes into a proliferative state (Zhang et al., 2015). Therefore, exclusion of human lymphomagenesis in PDX models requires validation of established PDX tumors in immunodeficient mice by histology and human CD34 markers (Zhang et al., 2015).

## CONCLUSION

Precision medicine for cancer treatment requires the integration of massive volumes of diverse data from genomic profiling and drug responsiveness. Recent advances in high-throughput sequencing techniques have enabled genomic analysis of individual patients' cancer. PDX models retain the histology, genomic characteristics, and even drug responsiveness of the original human patient tumors, and can be used as 'Avatars' for drug responsiveness studies. These models are valuable for diverse drug development processes including biomarker development, preclinical drug testing, co-clinical trials, and personalized drug selection. Finally, development of mutation-specified PDX collections by integrating sequencing data and avatar experiments will provide novel opportunities for therapeutic optimization, improved clinical outcomes, and precision cancer medicine.

## ACKNOWLEDGMENTS

Thanks to Stephen Sampson of JAX Scientific Program Development (SPD) for editing and review of manuscript and Zoe Reifsnnyder for assistance with Figs. 1 and 4. This work was supported by grants from a Ewha Womans University Research Grant; the National Cancer Institute under award number P30CA034196; the Korean Healthcare Technology R&D project through the Korean Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant No. HI13C2148); and the International Research & Development Program of the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (grant No. 2015K1A4A3047851).

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