# Current Status and Perspectives of Patient-Derived Xenograft Models in Cancer Research (Postprint)

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## Abstract

Cancers remain a major public health problem worldwide, which still require profound research in both the basic and preclinical fields. Patient-derived xenograft(PDX) models are created when cancerous cells or tissues from patients' primary tumors are implanted into immunodeficient mice to simulate human tumor biology in vivo, which have been extensively used in cancer research. The routes of implantation appeared to affect the outcome of PDX research, and there has been increasing applications of patient-derived orthotopic xenograft (PDOX) models. In this review, we firstly summarize the methodology to establish PDX models and then go over recent application and function of PDX models in basic cancer research on the areas of cancer characterization, initiation, proliferation, metastasis, and tumor microenvironment and in preclinical explorations of anti-cancer targets, drugs, and therapeutic strategies and finally give our perspectives on the future prospects of PDX models.

## Full Text

## Preamble

### Current Status and Perspectives of Patient-Derived Xenograft Models in Cancer Research

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## Abstract

Cancers remain a major public health problem worldwide, requiring profound research in both basic and preclinical fields. Patient-derived xenograft (PDX) models are created by implanting cancerous cells or tissues from patients'primary tumors into immunodeficient mice. These models faithfully recapitulate human tumor biology and have been extensively used in cancer research. The route of implantation appears to affect PDX research outcomes, and there has been increasing application of the more faithful patient-derived orthotopic xenograft (PDOX) models. In this review, we first summarize the methodology for establishing PDX models, then review recent applications and functions of PDX models in basic cancer research (covering cancer characterization, initiation, proliferation, metastasis, and tumor microenvironment) and in preclinical explorations of anti-cancer targets, drugs, and strategies. Finally, we offer perspectives on the future prospects of PDX models.

**Key words:** PDX models, basic research, preclinical research, cancer research, drugs

## Background

Cancers are among the leading causes of death worldwide. The Cancer Moonshot 2020 program was launched in 2016 to transform the cancer research and care ecosystem and double the rate of progress in cancer prevention, diagnosis, and treatment [**?**], though success has been achieved in reducing cancer death rates in the United States [**?**]. This program envisaged the development of precision medicine based on five critical elements—clinical bioinformatics, precision methods, disease-specific biomarkers, drug discovery and development, and precision regulations—to guide the application of precision medicine [**?**]. Novel techniques and research tools play important roles in this process.

Patient-derived xenograft (PDX) models are immunodeficient mice engrafted with patients' cancerous cells or tissues. The development of PDX models for cancer research, based on the assumption that these models faithfully resemble original tumors—especially patient-derived orthotopic xenograft (PDOX) models [**?**]—has significantly enhanced cancer research in recent years. These models for

various cancers, such as chronic lymphocytic leukemia [?], large B cell lymphoma [?], pancreatic cancer, colorectal cancer [?], gastric cancer [?, ?], colon cancer [?], high-grade serous carcinoma [?], and intrahepatic cholangiocarcinoma [?], are biologically stable and accurately reflect patients' tumors with respect to histopathology, gene expression, genetic mutations, inflammation [?], and therapeutic response. Thus, PDX models enable invaluable assessment of human tumor biology, identification of therapeutic targets, and preclinical screening and evaluation of drugs for various cancers. In this review, we summarize the methodology for establishing PDX models (Fig 1 [Figure 1: see original paper]), review recent advances in basic cancer studies and preclinical studies using PDX models (Fig 2 [Figure 2: see original paper]), and offer perspectives on the future prospects of PDX models.

## Methodology to Establish PDX Models

### Immunodeficient Mice

Immunodeficient mice engrafted with human immune systems provide powerful models for studying human immunobiology in vivo, and PDX models using these humanized mice are critical tools for investigating interactions between human immunity and various cancers. To establish a PDX model, a highly immunodeficient mouse strain is required. Several types of immunodeficient mice can be used to establish xenograft models: athymic nude mice, SCID, NOD-SCID, and recombination-activating gene 2 (Rag2)-knockout mice [?]. However, these strains are typically used to establish cancer cell line xenograft models. Primary cancerous cells or tissues require higher immunodeficiency for efficient engraftment in mice.

NOD/SCID mice with IL2rg mutations, such as NOD.Cg-PrkdcscidIl2rgtm1Wjl (NSG) [?] and NODShi.Cg-PrkdcscidIl2rgtm1Sug (NOG) mice [?], exhibit enhanced immunodeficiency and can engraft almost all types of human cancers [?]. We generated a strain of NOD/SCID/IL2rg−/− (NSI) mice, which exhibit severe immunodeficiency, lacking T, B, and NK cells, and used these mice in studies of both leukemia and solid tumors [?]. As the number of immunodeficient strains increases, the choice of mouse strains for cancer research becomes more important. We developed a method to quantitatively evaluate the immunodeficiency of various mouse strains through the tumor engraftment index (TEI) [?]. Recently, we also derived a nude strain of NOD/SCID/IL2rg−/− mice, called NSIN, by deleting foxn1 with the CRISPR/Cas9 system. The nude NSIN mice showed even higher immunodeficiency than NSI mice by TEI and may be more suitable for studies of tumors with poor engraftment efficiency (data unpublished).

### Primary Tumor Samples

For the first implantation, patient-derived tumors may be implanted into immunodeficient mice as small tumor fragments or as cell suspensions derived from

patient blood or from digestion of tumors into single-cell suspensions. The principal determinants of successful tumor engraftment into immunodeficient mice are the viability and sterility of the human tumor [**?**]. Cancer cells or tissues can be mixed with basement membrane matrix proteins (Matrigel) before injection into recipient animals, which enables tumor growth with greater efficiency of take and growth [**?**] without loss of the primary tumor phenotype [**?**]. Tumor cells can also be co-injected with additional cell types, such as fibroblasts, stromal cells, or endothelial cells, according to experimental objectives.

### Heterotopic vs. Orthotopic Implantation

Cancerous cells or tissues may be implanted heterotopically or orthotopically and monitored for tumor formation (Fig 1). Relative to orthotopic implantation, the principal benefits of heterotopic implantation include ease of cell implantation, accurate monitoring and measurement of tumor size, and reduced procedural complications. Subcutaneous and intravenous PDX models, for solid tumors and leukemia respectively, are most widely used in cancer research. Orthotopic implantation is more technically challenging, time-consuming, and often requires ultrasound examinations or exploratory laparotomies to confirm tumor presence inside the body. However, the advantage is that the external milieu is more closely preserved in orthotopic tumors, theoretically better approximating the 'natural' setting of human tumors. This method has been shown to increase the incidence of metastases associated with xenograft growth and should be considered when metastases are important experimental outcomes [**?**]. To improve engraftment efficiencies when patient-derived tumors are available in inadequate quantities, it is favorable to perform initial subcutaneous implantation into F1 mice. Once grown, the tumor may then be digested and orthotopically implanted into subsequent generations of mice.

### Induced Pluripotent Stem Cell (iPSC)-Derived PDX Models

Since many patients' primary tumors cannot engraft directly in immunodeficient mice, alternative methods are needed to establish PDX models for these patients. Primary tumor cells can be reprogrammed to iPSCs and then differentiated into the cell type of origin, which can then be used to establish PDX models. PDX models derived through an intermediate iPSC stage could be useful for the approximately one-third of patients whose primary cells cannot form PDXs [**?**]. An advantage of this method is that an intermediate iPSC stage enables genetic manipulation of the cells in vitro before transplantation to facilitate tracking or study of their effects on tumor growth in vivo.

### Next-Generation PDX Models with Humanized Mice

Recent advances in immunotherapies highlight the importance of the immune system in tumor progression and treatment, requiring PDX models with human immune systems to facilitate study of immunity-cancer interactions and preclinical assessment of cancer immune therapies. To establish human immune system-

conditioned PDX models, we first need to generate humanized mice (also known as human hemato-lymphoid chimeric mice or human immune system models). One method for generating humanized mice involves transplantation of total peripheral blood or tumor-infiltrating lymphocytes (TILs) into immunodeficient mice. These procedures are known to cause severe graft-versus-host disease (GVHD) 2–5 weeks after injection [**?**] and limit the useful investigative time window [**?**]. Another method is to transplant CD34-positive human hematopoietic stem cells (HSCs) or precursor cells isolated from umbilical cord blood, bone marrow, and peripheral blood, either alone or in combination with additional human immune tissues (e.g., human thymic tissue) into immunodeficient mice [**?**]. Transplantation with HSCs results in more complete hematopoietic reconstitution, as HSCs give rise to various lineages of human blood cells in mice. To improve the integrity of engrafted human immune systems, genetically modified immunocompromised mouse strains have been generated, such as NOG-GM3, NSG-SGM3, and MISTRG [**?**]. Next-generation PDX models based on genetically and immune cell-humanized mice, though expensive, will be widely used in future cancer research.

## PDX Models in Basic Cancer Research

Basic cancer research aims to characterize cancer biology and explore underlying mechanisms for improved understanding and prediction of cancer. PDX models provide crucial in vivo and ex vivo evidence to advance basic studies of cancer, including tumor characterization, tumorigenesis, and metastasis.

### Characterization

Given that PDX models faithfully mimic human cancers, they can be used to delineate the molecular, cellular, and sub-clonal characterizations of various cancer types. In PDX models of acute lymphoblastic leukemia (ALL), a rare unfavorable ALL subpopulation has been defined that is dormant and treatment-resistant, mimicking patients' primary cells at minimal residual disease [**?**]. PDX models of acute myeloid leukemia (AML) were used to study relationships between clonal architecture and functional heterogeneity, in which subclones showed variable engraftment potential in immunodeficient mice and xenografts were predominantly comprised of a single genetically defined subclone [**?**]. For solid tumors, intratumoral heterogeneity arises from the evolution of genetically diverse subclones during tumor progression, and PDX models are ideal tools for studying the stability, proliferation, persistence, chemotherapy tolerance, and underlying mechanisms [**?**]. PDX models revealed that tumor growth can be driven by a minor cell subpopulation, which enhances proliferation of all cells within a tumor by overcoming environmental constraints yet can be outcompeted by faster-proliferating competitors, resulting in tumor collapse [**?**].

### Tumorigenesis

PDX models are frequently used to study the cellular components involved in cancer cell initiation and proliferation. The cancer stem cell (CSC) hypothesis suggests that neoplastic clones are maintained exclusively by a rare fraction of cells with stem cell properties. Xenograft assays identified CD133+ human brain tumor initiating cells (TICs) that initiate tumors in vivo, providing insights into human brain tumor pathogenesis and strong support for the CSC hypothesis as the basis for many solid tumors [**?**]. The intrinsic molecular mechanisms of tumorigenesis are usually studied in cancer cell line xenograft (CCLX) models, in which cancer cell lines are genetically modified to consolidate in vitro studies. For example, LZAP inhibits tumor growth and vascularity, as evidenced by cancer cell line xenografts showing that decreased LZAP expression promoted tumor growth [**?**]. Knockdown of endogenous PCBP1 enhanced tumorigenesis, whereas overexpression of exogenous PCBP1 abrogated tumor formation [**?**]. Notch-Hedgehog-dependent TICs were identified in prostate cancer CCLX models [**?**], and shRNA targeting lncRNAs in castration-resistant prostate cancer cell lines strongly suppressed tumor xenograft growth in vivo [**?**]. Since it is more difficult to culture and manipulate gene expression in primary tumors compared to cancer cell lines in vitro, studies of molecular mechanisms of tumorigenesis using PDX models can adopt in vivo treatment with molecular inhibitors or agonists to monitor tumor growth. Musashi (Msi) is a critical element of pancreatic cancer progression, and Msi inhibition blocked the growth of primary patient-derived tumors [**?**]. The initiation of human neuroendocrine prostate cancer from prostate epithelial cells is driven by N-Myc and activated AKT1, as evidenced by in vivo transformation in NSG mice of prostate basal epithelial cells overexpressing N-Myc and myrAKT1 [**?**]. MiRNA-126 stabilizes B-ALL in a proliferative B cell precursor state by targeting cell cycle/apoptosis and p53 response genes, and antagonizing miRNA-126 in human B-ALL reduces disease burden in its PDX model [**?**]. Millions of somatic mutations have been found in cancers through genome sequencing, but the functional impact of most mutations is poorly understood. With PDX models, we can define impactful mutations that induce tumor formation and/or confer resistance to therapy [**?**]. The proliferation of human cancer cells can be easily defined or compared through cancer cell growth in PDX mice. Human cancer cells in PDX models increase their growth rate over time without treatment [**?**]. A method was established for identifying novel cancer targets via negative-selection RNAi screening using a human breast cancer xenograft model at an orthotopic site in mice, through which a set of metabolic genes associated with aggressive breast cancer and stemness were screened to identify those required for in vivo tumorigenesis [**?**].

### Metastasis

Metastasis is the basis of cancer lethality, yet its mechanisms are not fully understood and interventional strategies remain poorly defined. PDX models

are useful for defining cell populations and molecules associated with metastasis. Metastasis-initiating cells (MICs) have proven critical for cancer metastasis, but identifying and isolating adequate numbers of MICs from patients is difficult. PDX models serve as depositories of MICs. A PDX model of human breast cancer was used to identify and isolate MICs through a highly sensitive fluorescence-activated cell sorting (FACS)-based assay [**?**]. Circulating tumor cells (CTCs) play a critical role in tumor metastasis and have been identified and isolated from patients with several tumor types. Isolated CTCs have been used to generate PDX models of breast [**?**], pancreatic [**?**], and prostate cancer [**?**]. These PDX models are ideal for studying the tumorigenicity, phenotypic characteristics, and genetic profiles of CTCs [**?**]. Recently, both CCLX and PDX models were used to assess the effect of blocking the fatty acid receptor CD36 on cancer metastasis, revealing CD36 as an anti-metastasis target [**?**]. Additionally, the relationship between metastasis and p53 deficiency was studied in PDX models of triple-negative breast cancer [**?**].

## PDX Models in Preclinical Cancer Research

CCLX models are inadequate for preclinical development of anticancer agents because most human cancer cell lines do not accurately reflect human malignant tumors [**?**]. In contrast, PDX models better recapitulate each individual patient' s cancer pathology. Using these models for in vivo preclinical investigations yields results more predictive of subsequent activity in patients. PDX models provide in vivo platforms to study the mechanisms by which anti-tumor agents exert their effects and the cellular and molecular mechanisms of therapy resistance in cancers [**?**, **?**]. Here we briefly summarize preclinical cancer research using PDX models to identify therapeutic targets and evaluate various types of anti-cancer drugs. Representative drugs and their targets are shown in Table 1 .

### Identification of Cancer Biomarkers Through PDX Models

PDX models in preclinical cancer research aid identification of cancer-specific biomarkers that can be used for diagnosis, prognosis, and therapeutic targeting. Whole-transcriptome profiling of PDX models to identify both tumor- and stromal-specific biomarkers supports drug efficacy studies and compartment-specific biomarker discovery [**?**]. PDX models have been used to evaluate potential diagnostic agents for cancers, such as fluorescently labeled chimeric anti-CEA antibody for colon cancer detection [**?**]. The prognostic value of stem cell markers in cancers such as hepatocellular carcinoma (HCC) [**?**] has been evaluated in PDX models. For cancers such as bladder cancer, PDX models are useful both for discovering novel molecular targets and predictive biomarkers and for determining the risk of treatment failure [**?**]. Generation of paired chemonaive and chemoresistant small cell lung cancer (SCLC) PDX models led to the finding that EZH2 promotes chemoresistance by epigenetically silencing SLFN11, and EZH2 inhibition prevents acquisition of chemoresistance and

improves chemotherapeutic efficacy in SCLC [?]. NEK2 represents a strong predictor for drug resistance and poor prognosis in cancer, as targeting NEK2 by NEK2 shRNA overcame drug resistance and induced apoptosis in vitro and in a myeloma PDX model [?]. The long non-coding RNA gene SAMMSON can be targeted to sensitize melanoma to MAPK-targeting therapeutics both in vitro and in PDX models [?]. The IGF-1 receptor is universally expressed in various cancers and can be therapeutically targeted, as exemplified by an orthotopic PDX model of multiple myeloma [?].

### Identification and Evaluation of Potential Drugs

Precision medicines exert selective pressure on tumor cells that leads to preferential growth of resistant subpopulations, necessitating development of novel therapeutic generations to treat evolving cancers. A critical role for PDX models in preclinical research is identifying therapeutic targets, including specific molecules and molecular interactions. Another major role for PDX models is guiding clinical treatment of cancer patients (Fig 2). The choice of therapeutics is critical for cancer treatment and depends on both cancer type and patient characteristics. PDX models provide solutions to challenges researchers face in cancer drug research, such as positive tumor responses in mouse models that do not translate when studies are implemented in humans.

First, PDX models can help discriminate the most suitable therapy for cancer patients (Fig 2). PDX models can identify patients with cancers resistant to chemotherapy [?] and define associations between drug resistance and genetic mutations [?]. PDX models can test new treatment techniques before clinical use. For example, encapsulating BYL719 (a PI3K inhibitor) in P-selectin-targeted nanoparticles led to specific accumulation of BYL719 in the tumor milieu of a PDX model for head and neck squamous cell carcinoma [?]. Transdifferentiation-induced neural stem cells genetically engineered with optical reporters and tumoricidal genes were evaluated as effective in glioblastoma PDX models [?]. Precise fluorescence-guided surgery (FGS) has potential to greatly improve outcomes for patients with recalcitrant cancers. During development, this technique was preclinically evaluated in a PDX model of pancreatic cancer in which cancer and stromal cells were labeled with different colors [?]. A PDX model of colon cancer was also used for FGS with fluorophore-conjugated anti-CEA antibody [?]. Although tumors in PDX mice resemble surgery-derived primary tumors, secondary tumors grown in patients after surgery may evolve with additional mutations, potentially making results from PDX models unreliable for the same patient. Another noteworthy issue is the limited time window available for cancer patients, as evaluation through PDX models may require at least three months.

Second, and most importantly, PDX models are useful for preclinical drug tests that can indicate drug safety, efficacy, and dosage. PDX models have been applied to preclinical drug testing in many cancer types, including pancreatic cancer [?], NSCLC [?, ?], melanoma [?], breast cancer [?, ?], colon cancer [?], and

prostate cancer [?]. PDX model-based oncology drug development in specific cancers has been discussed comprehensively [?].

**Chemicals:** PDX models have been used to evaluate dozens of small-molecule compounds, mainly kinase inhibitors, in various cancers. Kinase inhibitors have been tested in PDX models for cholangiocarcinoma [?], chordoma [?], NSCLC [?], and gastric cancer [?]. VEGF blocker FP3 inhibited gastric cancer through an antiangiogenic mechanism in a PDX model [?]. CXCR4 is critical to T-ALL cell leukemogenicity and required for T-ALL migration, homing, and niche positioning [?], and targeting CXCR4 with small-molecule antagonists reduces tumor growth in murine T-ALL and T-ALL PDX models [?]. Inhibition of the MDM2–p53 interaction suppressed tumor growth in PDX models for non–small cell lung cancer (NSCLC) [?]. Inhibition of MTH1 selectively causes incorporation of oxidized dNTPs in cancer cells, leading to DNA damage, cytotoxicity, and therapeutic responses in patient-derived mouse xenografts [?]. Progesterone receptor antagonists show antiproliferative and proapoptotic activities in breast cancer PDX models [?]. Luteolin inhibits tumor growth in cMet-overexpressing PDX models of gastric cancer [?]. The compound trabectedin modulates gene and microRNA expression and various signaling pathways in PDX models [?]. PF-06463922, a potent and brain-penetrant ALK/ROS1 inhibitor, displayed superior potency against all known clinically acquired ALK mutations and led to regression of EML4-ALK-driven brain metastases and prolonged mouse survival [?].

**Antibodies:** PDX models allow novel antibodies to be tested before clinical application. Antibody-based therapies have been widely used in clinical treatment of cancer patients, and PDX models have been used to test antibodies for treating various cancers [?, ?]. Immune checkpoint blockade therapy (ICBT), which blocks PD-1, PD-L1, or CTLA4 with antibodies, has elicited remarkable clinical responses in certain cancer patients. We recently evaluated new PD-1/PD-L1 antibodies in NSCLC PDX models established in humanized NSI mice reconstituted with human HSCs or blood cells (unpublished). Nevertheless, intrinsic resistance to immune checkpoint inhibitors remains a daunting challenge [?]. PDX models can evaluate treatments targeting specific resistance mechanisms to sensitize ICBT-resistant tumors. Regarding other antibodies, NSCLC PDX models with genetic aberrations within EGFR, KRAS, and FGFR1 were used to evaluate responses to Gefitinib, which showed in vivo consistency with clinical trial results [?]. In a human bladder cancer PDX model, bladder cancer stem cells (CSCs) actively contributed to therapeutic resistance, which could be abrogated by a PGE2-neutralizing antibody and Celecoxib-mediated blockade of PGE2 signaling [?].

**Anti-cancer Microorganisms:** PDX models are valuable tools for careful assessment of attenuated microorganisms in cancer treatment. Salmonella typhimurium A1-R, a facultative anaerobe that can grow in both oxic viable regions and necrotic regions of tumors, has shown efficacy against osteosarcoma [?], soft-tissue sarcoma [?], and melanoma [?] in orthotopic PDX models. On-

colytic viruses are also promising for cancer treatment. Attenuated vesicular stomatitis strains AV1 and AV2 were tested in a xenograft model of ovarian cancer, effecting complete and durable cures in the majority of treated animals when delivered systemically [**?**]. Oncolytic virus Delta24-RGD [**?**] and measles virus strains [**?**] have been tested in PDX models for glioblastoma.

**Combinations:** Targeted cancer therapies often lead to resistance, which can be suppressed through combination drug therapies. Combinatory targeting of two or more onco-signaling pathways is a promising strategy for cancer therapy. PDX models are useful for defining optimal target combinations that avoid therapy resistance, as demonstrated in glioblastoma PDX models through single-cell phosphoproteomics [**?**] and other cancers. CDK4/6 inhibitors resensitize PDX tumors to HER2-targeted therapies and delay tumor recurrence [**?**]. Combination treatment with the Aurora Kinase A inhibitor MLN8237 and ABT-199 is synergistic in PDX models of MYCN-amplified neuroblastomas [**?**]. Combined CDK4/6-PI3K inhibition overcomes intrinsic and adaptive resistance, leading to tumor regressions in PIK3CA mutant breast cancer PDXs [**?**]. BRAF(V600E) mutant colon cancers may benefit from combination therapy consisting of BRAF and EGFR inhibitors, which synergize to induce apoptosis of colorectal cells and suppress colorectal tumor growth in a xenograft model [**?**]. Anti-CD47 antibody synergized with Rituximab by promoting phagocytosis to eliminate lymphoma in both disseminated and localized non-Hodgkin lymphoma (NHL) xenograft models [**?**].

**High-Throughput Drug Screening and Assessment:** A major issue in cancer drug development is the low success rate of new agents. Many compounds advance to large phase III studies that consume considerable resources but eventually fail due to low efficacy. These poor results arise partly because conventional preclinical models used to screen new agents for clinical development have poor predictive value [**?**]. Furthermore, new drugs are tested in patients without selection and response monitoring through appropriate biomarkers. In this regard, the availability of PDX models with high predictive value is of major interest. Ex vivo cultured PDX tumor cells can be used for in vitro high-throughput screening of anti-cancer drugs (Fig 2) [**?**]. PDX models theoretically can provide unlimited sources of human tumor cells for ex vivo high-throughput drug assessment. A large biobank of breast cancer PDXs that preserves morphological and molecular characteristics and intra-tumor genomic clonal architecture of originating tumors has been generated and used for high-throughput drug assessment in PDX-derived tumor cells in vitro [**?**]. The Public Repository of Xenografts (PRoXe) is a publicly available repository of well-characterized leukemia and lymphoma PDXs that can be used to characterize drug efficacy and generate transcriptional, functional, and proteomic biomarkers in both treatment-naive and relapsed/refractory disease. Randomized phase II-like studies with PRoXe are applicable to a range of therapeutic agents, especially those acting through cancer cell-intrinsic mechanisms [**?**]. PDX models are also useful for assessing drugs screened from high-throughput computational design. A novel computational design approach yielded multivalent pan-RAS inhibitors, and PDX mod-

els were used to confirm the efficacy of the identified small-molecule compound binding to KRASG12D [**?**]. Another computationally designed protein, BINDI, which binds with BHRF1 of Epstein-Barr virus, suppressed tumor growth and extended survival in a PDX model of EBV-positive human lymphoma [**?**].

**CAR T Cell Immunotherapies:** Adoptive transfer of chimeric antigen receptor (CAR) T cells has shown great promise in treating cancers, particularly B cell leukemia with CAR T cells targeting CD19. PDX models are frequently used for preclinical studies of CAR T cells [**?**]. Novel CAR designs have been frequently evaluated in PDX models. The in vivo model with NSG mice was critical to demonstrate that targeting an anti-CD19 CAR to the TRAC locus with CRISPR/Cas9 enhances tumor rejection, a strategy that averts antigen-stimulated differentiation and exhaustion [**?**]. "On-switch" CARs enable small-molecule control over CAR T cell therapeutic functions regarding timing, location, and dosage of T cell activity, thereby mitigating toxicity [**?**]. Loss of HVEM disrupts HVEM-BTLA inhibitory interaction, leading to cell-autonomous activation of B cell proliferation and promoting lymphoma development. Anti-CD19 CAR T cells producing HVEM were tested and showed improved anti-lymphoma efficacy in the PDX model [**?**]. CAR T cell immunotherapies have not generated satisfactory results in almost all types of solid tumors. PDX models for solid tumors will play essential roles in future studies to promote efficacies of CAR T cells against solid tumors.

Collectively, PDX models facilitate discovery and testing of various therapeutic regimens including small-molecule compounds, antibodies, microorganisms, and cytotoxic cells.

## Discussion

PDX models are generated by transplanting primary tumors into immunodeficient mice. PDX models are used when in vitro data require additional in vivo evidence; they can also be studied directly to yield discoveries that are followed by further in vitro research.

However, the use of these xenograft models to study human tumor biology and drug screening is limited by several factors, including replacement of human stromal components (such as cancer-associated fibroblasts, endothelial cells, immune and inflammatory cells) by murine elements, lack of a functional immune system, and absence of interactions between human stromal cells and the immune system. Development of PDX models that account for interactions between tumor, stromal, vascular, and immune cells is essential to produce a tumor microenvironment more representative of the human host. PDX models in humanized xenochimeric mice, or XactMice, engrafted with human HSPCs before tumor engraftment expressed chemical stimuli necessary to give rise to stromal and immune cells that recreated the original tumor microenvironment observed clinically [**?**]. Nonetheless, better PDX models are still needed to simulate real cancer-stromal interactions in patients.

Furthermore, new approaches to optimizing cancer drug development are required to fully achieve the goal of individualized, precision cancer therapy, and improved preclinical models that more closely reflect the genomic complexity of human cancers are needed.

Recent studies using single-cell sequencing suggest that in some PDX models, only a limited number of clones propagate in mice, indicating a selection process [**?**]. Identification of lymphocytes recognizing tumor-specific mutant neoantigens represents a major step toward future eradication of heterogeneous cancers. Only recently reported was the identification of neoantigen-specific lymphocytes in peripheral blood of melanoma patients [**?**]. However, routine detection of lymphocytes targeting neoantigens is currently limited to T cells isolated directly from cancer patients, which are often not available. This limitation might be overcome using PDX models produced by engrafting an autologous immune system.

With genetically humanized immunodeficient mice that can engraft a more integrated human immune system, we will be able to advance translational research on cancers as well as other diseases including infectious diseases and autoimmune disorders.

## Conclusions

PDX models are increasingly used in translational cancer research. These models are useful for studying cancer biology, biomarker development, drug screening, and preclinical evaluation of personalized medicine strategies. This review provides a timely overview of the key roles of PDX models in both basic and preclinical cancer research and a detailed discussion of major hurdles in the field.

## Figure Legends

**Figure 1.** Overview of methodology to establish PDX models and their uses in cancer research. Tumors from cancer patients (P0) are fragmented or digested into single-cell suspension and then transplanted (directly or with additives such as Matrigel) into immunodeficient mice (P1) for engraftment. Once grown, tumors are transplanted into secondary recipients (P2) for tumor expansion. The expanded tumors can then be cryopreserved or transplanted into P3 mice for cancer research of the tumor type of origin. Specifically, tumors can be transplanted into sites other than where the tumors originated, called heterotopic transplantation; or into corresponding anatomical sites such as brain [**?**, **?**], lung [**?**], liver [**?**], pancreas [**?**, **?**], kidney [**?**], and ovary [**?**], which is called orthotopic transplantation. Successfully established PDX models are used in cancer research, which consists of two arms: basic and preclinical. Basic and preclinical cancer research in PDX models are interconnected, as basic research can identify therapeutic targets or strategies for preclinical tests, and preclinical research can generate new basic questions.

**Figure 2.** Use of PDX models in drug screening and preclinical therapeutic evaluation. **Drug screening:** PDX models can expand tumors from patients without adequate initial tumor material for in vitro studies. The expanded tumor cells can be cultured and manipulated ex vivo and used for high-throughput screening of drugs or combinations. Identified candidate drugs and combinations can be further evaluated in PDX mice before use in patients, or directly used in patients if the drugs have already been approved. **Preclinical therapeutic evaluation:** Given that different clinical therapeutic regimens are available for cancer patients, PDX models can be used to define the best regimen for individual patients. Briefly, PDX mice from one patient are randomly divided into groups and treated with different therapeutic regimens. Through tumor assessment, the best regimen can be identified.

## Table 1

Representative potential therapeutic drugs and their targets in various types of cancers that have been assessed by xenograft models.

## Declarations

**List of Abbreviations:** Circulating tumor cells (CTCs); patient-derived xenograft (PDX); non–small cell lung cancer (NSCLC); hepatocellular carcinoma (HCC); Precise fluorescence-guided surgery (FGS)

**Ethics Approval and Consent to Participate:** All experimental protocols (SYXK(Yue)2015-0063) were performed in accordance with instruction guidelines from the China Council on Animal Care and approved by the guidelines of the Ethics Committee of Animal Experiments at Guangzhou Institutes of Biomedicine and Health (GIBH).

**Consent for Publication:** Consent to publish has been obtained from the participants.

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*Note: Figure translations are in progress. See original paper for figures.*