IVYSPRING INTERNATIONAL PUBLISHER

International Journal of Biological Sciences

2027; 23(1): 548-571. doi: 10.7150/ijbs.139213

Review

Patient-Derived Advanced Tumor Models for Functional Drug Screening: From Construction to Precision Oncology

Chao Jiang[1,2*], Zhouting Tuo[3*], Junjiang Ye[1*], Zhaojie Lyu[4*], Ruicheng Wu[5], Jie Wang[1], Leibo Wang[6], Dilinaer Wusiman[7,8], William C. Cho[9]✉, Koo Han Yoo[10]✉, Qi Zhang[1]✉, Dechao Feng[1,5]✉

1. Urology & Nephrology Center, Department of Urology, Zhejiang Provincial People's Hospital (Affiliated People's Hospital), Hangzhou Medical College, Hangzhou 310014, Zhejiang, China.
2. Department of Urology, The Second Affiliated Hospital of Anhui Medical University, Hefei 200032, Anhui, China.
3. Department of Urology, Tianjin Institute of Urology, The Second Hospital of Tianjin Medical University, Tianjin 300211, China.
4. Department of Urology, Institute of Precision Medicine, Shenzhen Key Laboratory of Male Reproduction and Genetics, Peking University Shenzhen Hospital, Shenzhen 518036, Guangdong, China.
5. Division of Surgery & Interventional Science, University College London, London W1W 7TS, UK.
6. Institute of Human Genetics, Jena University Hospital, Jena 07747, Germany.
7. Department of Comparative Pathobiology, College of Veterinary Medicine, Purdue University, West Lafayette, IN, 47907, USA.
8. Purdue Institute for Cancer Research, Purdue University, West Lafayette, IN, 47907, USA.
9. Department of Clinical Oncology, Queen Elizabeth Hospital, Hong Kong Special Administrative Region of China.
10. Department of Urology, School of Medicine, Kyung Hee University, Seoul 05278, South Korea.

*These authors contributed equally to this work.

✉ Corresponding authors: Dechao Feng: Urology & Nephrology Center, Department of Urology, Zhejiang Provincial People's Hospital(Affiliated People's Hospital), Hangzhou Medical College, Hangzhou, Zhejiang, China; Division of Surgery & Interventional Science, University College London, London W1W 7TS, UK. E-mail: dechao.feng@ucl.ac.uk; Tel: +4486411893; Fax: +86-28-85422451. Qi Zhang: Urology & Nephrology Center, Department of Urology, Zhejiang Provincial People's Hospital (Affiliated People's Hospital), Hangzhou Medical College, Hangzhou, Zhejiang, China. E-mail: zhangqi1@hmc.edu.cn. Koo Han Yoo: Department of Urology, School of Medicine, Kyung Hee University, Seoul 05278, South Korea. E-mail: yookoohan@khu.ac.kr. William C. Cho: Department of Clinical Oncology, Queen Elizabeth Hospital, Hong Kong SAR, China. E-mail: chocs@ha.org.hk.

© 2027 The Author(s). This is an open access article distributed under the Creative Commons Attribution 4.0 International Licence, subject to the Publisher's Terms of Use at https://ivyspring.com/terms.

Received: 2026.06.11; Accepted: 2026.08.27; Published: 2027.01.01

Abstract

Precision oncology has been largely driven by genomic profiling; however, its clinical utility remains limited by the difficulty of reliably translating molecular alterations into therapeutic responses. This gap reflects the dynamic complexity of tumor biology, including intratumoral heterogeneity, microenvironmental interactions, and adaptive resistance. Functional precision oncology (FPO) has therefore emerged as a complementary paradigm that directly measures drug response using patient-specific tumor models.

Patient-derived tumor models, particularly patient-derived organoids (PDOs) and patient-derived xenografts (PDXs), provide biologically relevant platforms that preserve key histopathological, genomic, and functional features of the original tumors. These models enable empirical evaluation of therapeutic efficacy and facilitate biomarker discovery and resistance analysis.

In this review, we summarize the construction strategies, biological characteristics, and recent technological advances of patient-derived tumor models, with a focus on PDOs and PDXs. We further discuss next-generation systems, including immune- and stroma-inclusive co-cultures, humanized xenografts, and microfluidic tumor-on-a-chip platforms, and examine their integration into medium- and high-throughput drug screening pipelines. We also critically evaluate current limitations, including lack of standardization, time and cost constraints, and insufficient clinical validation. Finally, we highlight future directions involving multi-omics and artificial intelligence, including virtual organoid and digital twin frameworks. Overall, patient-derived tumor models bridge molecular profiling and therapeutic response, advancing phenotype-validated precision oncology.

Keywords: patient-derived organoids; patient-derived xenografts; functional precision oncology; drug screening; artificial intelligence

1. Introduction

The paradigm of cancer treatment has undergone a profound shift from histology-based, one-size-fits-all regimens towards a more tailored approach known as precision oncology. This strategy aims to match

individual patients with therapies targeted against specific molecular vulnerabilities identified within their tumors[1, 2]. The cornerstone of this evolution has been the rapid advancement of high-throughput genomic, transcriptomic, and proteomic profiling technologies, which have enabled the detailed molecular characterization of tumors[2]. Consequently, several targeted agents and immunotherapies have been developed and approved based on the presence of specific biomarkers[3, 4]. However, the initial promise of genomics-led precision medicine has been tempered by significant clinical challenges, revealing a substantial gap between the identification of a genomic alteration and the predictable efficacy of a corresponding targeted therapy in individual patients[2, 5].

A primary limitation of a purely genomics-driven approach is the inherent complexity and dynamic evolution of cancer biology. Tumors are not static entities but rather evolve under therapeutic pressure, developing multiple adaptive resistance mechanisms that cannot be fully captured by a single, static molecular snapshot[3, 6]. Furthermore, the clinical utility of genomic profiling is often constrained by the limited availability of directly actionable mutations for many patients, the frequent co-occurrence of multiple driver alterations whose therapeutic implications are unclear, and the inability of genomics alone to predict the functional impact of complex molecular interactions within the tumor microenvironment (TME)[2, 7]. Genomics alone was insufficient to identify therapeutic options for the majority of patients with advanced disease in some studies[2, 7]. This critical shortfall underscores the urgent need for complementary strategies that can bridge the gap between molecular data and clinical drug efficacy, moving beyond descriptive genomics to predictive functional analysis.

In response to these limitations, the concept of FPO has gained considerable traction[5, 8]. FPO seeks to complement genomic information with direct, empirical testing of drug sensitivity using living models of a patient's tumor. This approach leverages functional assays to more precisely recapitulate patient biology and directly observe therapeutic responses, thereby providing a dynamic and integrative tool for treatment prediction[8]. The core premise is that by exposing a representative avatar of the patient's tumor to a panel of drugs ex vivo or in vivo, one can empirically identify potentially effective therapies and combinations, discover novel biomarkers, and elucidate mechanisms of resistance[2, 9]. Importantly, patient-derived tumor models are not intended to replace genomic profiling. Rather, functional drug screening extends genomics-led precision oncology by providing patient-specific phenotypic evidence that can validate, refine, or challenge therapeutic hypotheses inferred from molecular data[5].

The fidelity and predictive power of functional drug screening are intrinsically linked to the biological relevance of the tumor models used. Traditional preclinical models, such as two-dimensional (2D) cell lines and genetically engineered mouse models, have been instrumental in cancer research but are often inadequate for predicting clinical outcomes. Immortalized 2D cell lines suffer from genetic drift, loss of heterogeneity, and a lack of critical TME components, while murine models may not fully recapitulate human tumor biology and drug pharmacokinetics[3, 10, 11]. This has propelled the development and refinement of patient-derived advanced tumor models, which are established directly from a patient's tumor tissue and better preserve its original genetic, phenotypic, and microenvironmental characteristics[11-13].

The two most prominent and complementary classes of these models are PDXs and PDOs. PDX models are created by implanting fresh tumor fragments into immunocompromised or humanized mice, allowing the tumor to grow in a complex, *in vivo* setting[1, 12]. These models have demonstrated a remarkable ability to retain the principal histologic and genetic characteristics, intratumor heterogeneity, and stromal architecture of the original patient tumor across serial passages[1, 12]. Their high clinical fidelity has established PDXs as a platform for preclinical drug evaluation, biomarker discovery, and the study of tumor evolution and drug resistance mechanisms[6, 12]. Drug responses in PDX models can correlate with clinical outcomes, making them valuable for validating novel therapies[9, 14, 15]. In parallel, PDOs are three-dimensional (3D) microtissues grown *in vitro* from patient tumor cells within a supportive extracellular matrix[13, 16]. They offer a unique advantage by efficiently capturing the genomic and molecular landscape of the donor tumor while enabling scalable, high-throughput experimentation within a biologically relevant 3D architecture[13, 16]. PDOs can be established with higher success rates and shorter timelines than PDXs, making them suitable for rapid drug screening and the generation of large, clinically annotated biobanks[2, 17, 18]. Evidence supports the clinical predictive value of PDO drug responses, suggesting their utility as avatars for personalized therapy[17, 19].

Beyond these core models, the field is rapidly evolving towards next-generation and hybrid systems designed to address specific limitations. These include co-culture models that incorporate immune cells or

cancer-associated fibroblasts to better mimic the TME[13]; microfluidic organ-on-a-chip platforms that introduce perfusion and dynamic drug exposure[20, 21]; patient-derived tissue slice cultures that maintain the native tissue architecture for short-term assays[22]; and integrated models like PDX-derived organoids (PDXOs) that combine the *in vivo* selection of PDXs with the *in vitro* manipulability of organoids[17, 23]. The convergence of both foundational and next-generation patient-derived tumor models with cutting-edge technologies such as multi-omics profiling and artificial intelligence (AI) is further amplifying their potential[8, 24, 25]. AI and machine learning algorithms are being developed to integrate complex molecular data with functional drug response outcomes from these models, aiming to derive predictive signatures that can guide treatment decisions[8, 24, 25].

This review will comprehensively explore the landscape of patient-derived advanced tumor models, with a particular focus on their application in functional drug screening for precision oncology. We will delineate the concepts, establishment, and optimization of key models including PDOs and PDXs, examine emerging hybrid and next-generation systems, and critically assess their applications in drug discovery, biomarker identification, and the guidance of personalized therapy. Furthermore, we will discuss the integration of multi-omics and AI for predictive analysis, address the persistent challenges and limitations hindering clinical translation, and offer perspectives on the future trajectory of this transformative field. The overarching thesis is that the synergistic use of functionally validated, patient-specific models represents an indispensable and evolving pillar of precision oncology, one that is poised to narrow the gap between molecular diagnosis and effective therapeutic intervention. The overall operational framework through which patient-derived tumor models support functional precision oncology, from patient selection and tumor sampling to model construction, integrated analysis, and model-informed therapeutic stratification, is summarized in **Figure 1**.

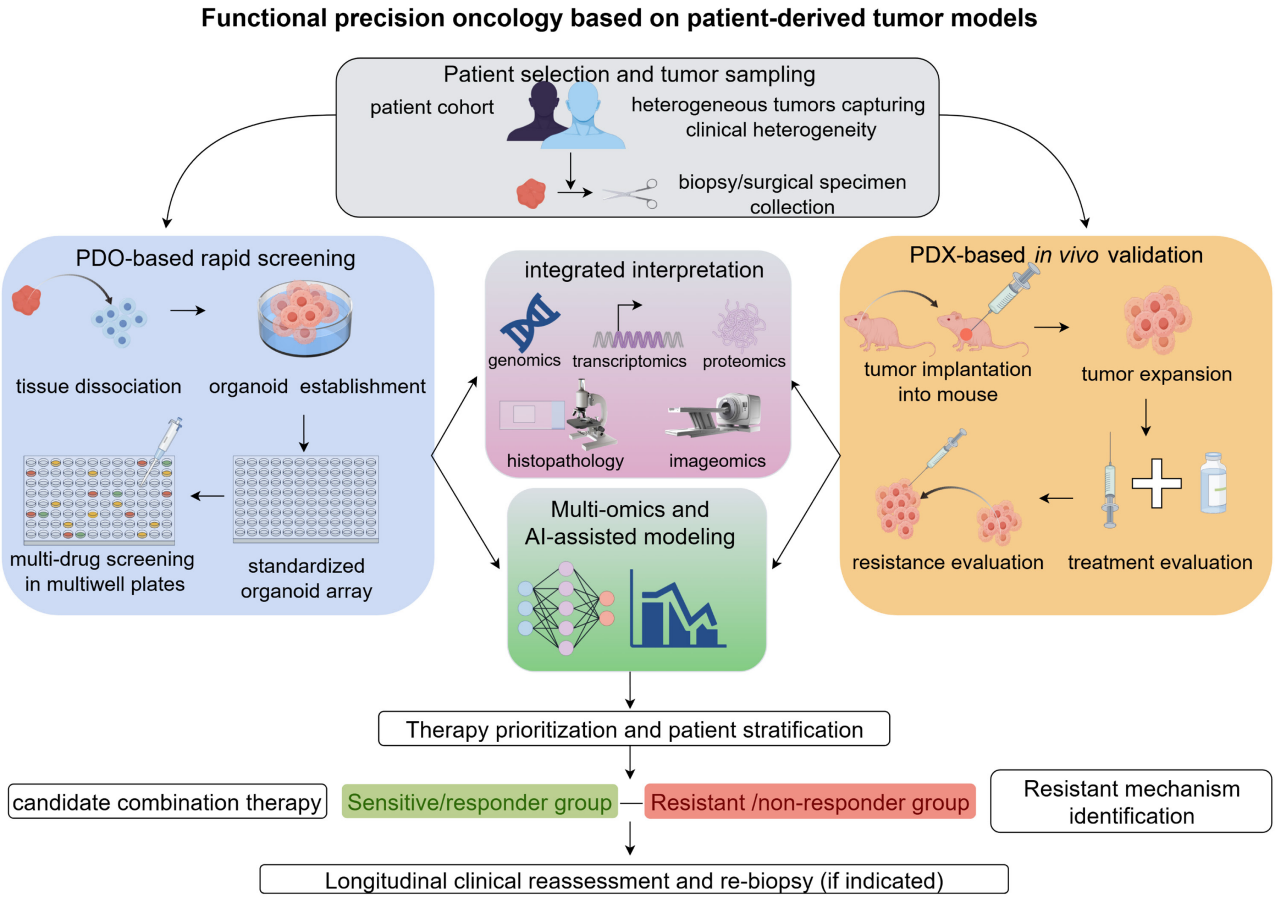

Figure 1. Operational framework of functional precision oncology based on patient-derived tumor models. Tumor samples obtained from patients are propagated into patient-derived organoids (PDOs) for rapid, high-throughput in vitro drug screening and into patient-derived xenografts (PDXs) for in vivo validation and resistance modeling. Data generated from these complementary platforms are integrated with multi-omics profiling and analyzed using artificial intelligence-assisted approaches to construct predictive models. The integrated framework supports therapeutic prioritization and model-informed stratification of patients into responders and non-responders. The feedback loop represents longitudinal clinical reassessment. Upon disease progression or suspected acquired resistance, repeat tumor sampling, when clinically indicated and feasible, may initiate a new cycle of model generation, drug screening, and treatment optimization.

2. Definitions and Classification of Patient-Derived Advanced Tumor Models

The implementation of functional precision oncology depends on experimental systems that preserve clinically relevant properties of human tumors while remaining sufficiently tractable for mechanistic studies and drug testing. In this context, patient-derived advanced tumor models refer to a spectrum of platforms established directly from patient tumor specimens, with the aim of retaining key features of the original malignancy, including genomic architecture, phenotypic diversity, and, to varying degrees, components of the TME[10-13]. Compared with long-adapted conventional cell lines, these models more faithfully reflect patient-specific tumor biology and therefore provide a more relevant framework for translational research and functional drug screening[3, 10, 11].

2.1 Definition of patient-derived tumor models

Among currently available platforms, PDOs and PDXs represent the two foundational and most widely adopted model classes. PDOs are three-dimensional *ex vivo* cultures established from patient tumor cells and maintained within a supportive extracellular matrix under lineage-appropriate growth conditions[13, 26, 27]. These cultures self-organize into multicellular structures that preserve important histological, molecular, and functional features of the parental tumor and can often be expanded, cryopreserved, and re-used for downstream analyses[13, 26, 28]. By contrast, PDXs are generated by implanting fresh patient tumor fragments or dissociated tumor cells into immunodeficient mice, thereby enabling tumor propagation in an *in vivo* context[1, 12, 29]. PDX models can preserve major histopathological characteristics, driver genomic alterations, and aspects of intratumoral heterogeneity over serial passages, which underlies their broad use in translational oncology and preclinical pharmacology[1, 12, 30-33].

Beyond these two core platforms, several additional patient-derived systems have emerged to address specific biological or translational needs. Patient-derived tumor slice cultures (PDTSCs) are short-term *ex vivo* preparations that retain native tissue architecture and multicellular organization, thereby enabling rapid functional interrogation while preserving at least part of the local TME[22]. Zebrafish patient-derived xenografts (zPDXs) offer an alternative *in vivo* host system characterized by rapid engraftment, optical transparency, and relatively high scalability, making them attractive for fast-turnaround screening and real-time visualization of tumor growth or dissemination[34-36]. In parallel, tumor-on-a-chip and related microphysiological systems incorporate patient-derived tumor cells or organoids into engineered microfluidic environments that permit controlled perfusion, concentration gradients, and multicellular co-culture, thereby increasing microenvironmental and pharmacokinetic relevance[21, 37].

Collectively, these systems constitute an advanced model ecosystem rather than a single platform. In this review, the term patient-derived advanced tumor models is used as an umbrella concept encompassing both foundational models, principally PDOs and conventional PDXs, and next-generation and hybrid models designed to improve microenvironmental fidelity, screening speed, scalability, or clinical applicability.

2.2 Classification framework and practical model selection

For conceptual clarity, patient-derived advanced tumor models can be classified along two complementary axes: biological format and functional purpose. From the perspective of biological format, they can first be divided into *in vitro / ex vivo* models and *in vivo* models. The former category includes PDOs, PDTSCs, and organoid- or tumor-on-a-chip systems, whereas the latter includes murine PDXs and alternative host xenograft platforms such as zPDXs[21, 22, 34-36]. This distinction is important because it determines not only culture conditions and experimental throughput, but also the degree to which tissue architecture, systemic physiology, stromal signaling, immune interactions, and pharmacokinetic variables can be captured.

A second and more translationally useful classification distinguishes between foundational models and next-generation and hybrid models. Foundational models include PDOs and conventional PDXs, which remain the principal workhorses of functional precision oncology because they provide complementary strengths in experimental scalability and in vivo biological relevance, respectively[6, 12, 13, 26, 29]. Next-generation and hybrid models are built to overcome specific limitations of these foundational systems. Representative examples include immune- or stroma-inclusive PDO co-cultures, humanized PDXs, microfluidic organoid-on-a-chip platforms, and intermediate systems such as PDX-derived organoids, which combine features of both *in vivo* selection and *in vitro* scalability[4, 17, 21, 23, 37, 38]. Under this framework, the field can be understood not as a competition between isolated platforms, but as an

increasingly interconnected model hierarchy in which different systems are selected according to the biological question, therapeutic context, throughput requirement, and clinical time window.

This classification also provides a practical framework for model selection, as no single patient-derived model is universally optimal. Instead, each platform offers a distinct balance among biological fidelity, microenvironmental representation, speed, scalability, and cost. PDOs are generally better suited for medium- to high-throughput functional interrogation, whereas PDXs remain more appropriate for *in vivo* pharmacology, resistance evolution, and systemic therapeutic validation[6, 12, 16, 29, 39, 40]. Emerging hybrid systems aim to bridge these domains by improving TME representation, shortening turnaround time, or enabling dynamic drug exposure in controlled settings[21, 35-37].

The principal patient-derived tumor models discussed in this review, together with their defining features, major strengths, technical constraints, representative applications, and key references, are summarized in **Table 1**.

3. Establishment and Optimization of Patient-Derived Organoid Models

The utility of PDOs in functional precision oncology is critically determined by the robustness, reproducibility, and biological fidelity of their establishment protocols. Unlike conventional *in vitro* systems, PDO generation requires the preservation of tumor-initiating cell populations while maintaining lineage-specific differentiation programs under defined three-dimensional culture conditions. Therefore, methodological variables across the entire workflow—including tissue acquisition, processing, extracellular matrix composition, and culture medium formulation—collectively influence organoid formation efficiency, expansion capacity, and downstream applicability in drug screening. In this section, we focus on the key technical determinants governing PDO establishment and optimization, including sample source and preprocessing strategies, culture system design, and validation of biological characteristics. Particular emphasis is placed on how these factors impact model fidelity, scalability, and translational relevance for functional drug screening.

Table 1. Comparative overview of major patient-derived advanced tumor models in functional precision oncology

Model	Definition	Core features	Major advantages	Major limitations / prerequisites	Typical applications
Patient-derived organoid	Three-dimensional *ex vivo* cultures established from patient tumor tissues that preserve key histopathological, molecular, and functional features of the donor tumor.	Long-term expansion; amenable to genetic manipulation; suitable for living biobank construction.	High-throughput capability; compatible with mechanistic studies; readily integrated with multi-omics analyses.	Limited native tumor microenvironment (TME); matrix batch variability; establishment success depends on tumor type and sample quality.	Drug sensitivity and resistance testing; biomarker discovery; functional validation[13, 26, 121, 163].
Patient-derived xenograft	*In vivo* models generated by implanting patient tumor tissues or cells into immunodeficient hosts.	*In vivo* tumor propagation; preservation of tumor architecture and heterogeneity; serial passaging possible.	High physiological relevance; well suited for *in vivo* therapeutic validation.	Long turnaround time; limited scalability; lack of an intact immune system in conventional models; ethical and cost constraints.	*in vivo* efficacy evaluation; combination therapy testing; resistance evolution studies [1, 6, 12, 29].
Patient-derived tumor slice culture	Short-term *ex vivo* culture system that maintains freshly obtained patient tumor slices.	Preserves spatial architecture and multicellular organization; allows rapid readout.	Rapid functional assessment; partial preservation of native tissue structure and cell–cell interactions.	Short maintenance period; limited throughput; limited inter-laboratory standardization.	Rapid response assessment; short-term mechanistic studies[22, 115].
Zebrafish patient-derived xenograft	Rapid xenograft model established by implanting patient-derived tumor cells or tissue into zebrafish embryos or larvae.	Rapid model establishment; low cell input requirement; convenient live imaging.	Short turnaround time; scalable; suitable for rapid screening.	Cross-species biological differences; limited cross-species translatability to human clinical responses; indications and standardization remain under development.	Rapid screening and stratification; individualized sensitivity testing[35, 36].
Tumor-on-a-chip / microfluidic microphysiological systems (including MOS)	Engineered microfluidic platforms that incorporate patient-derived tumor cells, organoids, or multicellular constructs in controlled microenvironments.	Dynamic perfusion or microfluidic compartmentalization; gradient generation; enhanced microenvironmental simulation; compatible with high-content imaging.	More physiologically relevant transport and microenvironmental modeling; enables dynamic response assessment.	Engineering complexity; specialized infrastructure and operational complexity; difficult standardization across platforms.	Drug transport and penetration studies; microenvironment-driven response modeling; advanced preclinical testing[21, 162].
Humanized patient-derived xenograft	PDX model established in immunodeficient mice reconstituted with human immune cells or hematopoietic stem cells.	Combines *in vivo* tumor growth with partial human immune reconstitution.	Enables immunotherapy-related studies; better reflects tumor–immune interactions than conventional PDXs.	Technically complex; costly; incomplete immune reconstitution; inter-model variability.	Immune checkpoint inhibitor testing; tumor–immune interaction studies; translational immuno-oncology[83, 84].

3.1 Sample source and pretreatment process

The process begins with the acquisition of viable tumor tissue. While surgical resection specimens have traditionally been the primary source, enabling the generation of organoid biobanks[18, 41], the clinical imperative for guiding therapy in advanced, inoperable cancers has driven the successful derivation of PDOs from minimally invasive procedures. These include endoscopic biopsies for gastric cancer[42], ultrasound-guided fine-needle biopsies for pancreatic ductal adenocarcinoma[43], and core needle biopsies for metastatic lesions[44]. The feasibility of establishing organoids from rare and challenging cancers, such as salivary gland carcinomas[45], signet ring cell carcinomas[46], and pseudomyxoma peritonei[47], further underscores the robustness of contemporary protocols. Notably, the use of liquid biopsies, specifically circulating tumor cells (CTCs), presents a promising avenue for serial monitoring and modeling of metastatic disease, although it remains a technically demanding approach[48]. Another strategically important source is patient-derived xenograft tissue, which can be used to generate PDXOs[49-51]. This approach leverages the *in vivo* passaged and expanded PDX tumor as a renewable source to create organoid models that maintain high genomic and pharmacological concordance with the original PDX, thus bridging *in vitro* high-throughput screening with *in vivo* validation platforms[49, 52]. Together, these technical considerations define the balance between model scalability and biological fidelity, which ultimately determines the predictive power of PDO-based functional drug screening.

3.2 Organoid culture system

The core of PDO culture lies in a three-dimensional extracellular matrix (ECM) scaffold supplemented with a tailored cocktail of growth factors and signaling pathway modulators designed to mimic the stem cell niche. Basement membrane extracts (BMEs), such as Matrigel, are the most widely used matrices, providing a complex mixture of ECM proteins that support organoid formation and growth[41, 45]. Research indicates that while the commercial source of the BME can affect the proliferation rate of pancreatic cancer organoids, it does not substantially shift drug dose-response curves, confirming pharmacotyping as a robust approach across different matrix lots[53]. To achieve greater definition and reproducibility, synthetic or engineered hydrogels are being developed. Hyaluronan-gelatin hydrogels have been successfully used to co-culture colorectal cancer PDOs with cancer-associated fibroblasts, providing a more physiologically relevant matrix composition[54]. The culture medium is equally critical, typically fortified with factors like WNT3A, R-spondin, Noggin, and EGF to sustain the self-renewal and proliferation of epithelial stem and progenitor cells[41, 55]. Protocol simplification is an active area of optimization; for example, WNT-free conditions have been developed for head and neck cancer PDOs, which can simplify culture and may better maintain differentiation states[56]. The meticulous balance of these components is essential to selectively support the expansion of tumor epithelial cells while suppressing the overgrowth of normal stromal components.

3.3 Biological characteristics of organoids

A fundamental prerequisite for employing PDOs in functional drug screening is the rigorous validation of their biological fidelity to the original patient tumor. Extensive molecular characterization consistently confirms that well-established PDOs recapitulate the histopathological architecture, genomic landscape, and transcriptomic profiles of their parental tumors. Histological and immunohistochemical analyses across multiple cancer types—including gastric[41], bladder[57], colorectal cancers[58], and pancreatic cancers[59]—demonstrate that PDOs preserve the key morphological features and protein marker expression patterns. Genomic fidelity is evidenced by high concordance rates of somatic mutations, copy number alterations, and gene fusions between PDOs and matched tumor tissues[45, 57, 59]. Whole-exome sequencing of gastric cancer PDOs, for example, shows they closely mirror the genetic alterations of the source tumor[41]. Transcriptomic studies further reveal that PDOs maintain the gene expression subtypes and critical pathways of the original malignancy[51, 60]. It is important to acknowledge that PDOs, like all models, undergo adaptation. Comparative multi-omic analyses reveal that tumor cells undergo chromatin remodeling influenced by their specific microenvironment, with distinct patterns observed between PDOs and PDX models[61]. While long-term PDO cultures may experience shifts in expression-based subtyping, short-term cultures better preserve the original tumor's molecular characteristics and cellular diversity, making them particularly suitable for clinical predictive applications[60]. This preservation of intratumoral and inter-metastatic heterogeneity is crucial; studies on colorectal cancer liver metastases show that PDOs from multiple sites within the same patient exhibit moderate pharmacological heterogeneity, accurately reflecting the patient's tumor landscape[62].

Despite these advances, the clinical deployment

of PDOs faces practical challenges, chiefly the variable success rate of establishment and the time required to generate sufficient material for drug testing. Success rates are influenced by factors such as tumor type, sample quality, and patient treatment history. In pancreatic cancer, take-on rates around 62% have been reported from heavily pretreated patients[59], while rates can vary widely for other malignancies[44, 45]. Research has identified predictors of success, such as optimized culture conditions, procedural experience, and specific patient characteristics[44]. Concurrently, bioengineering strategies are being aggressively pursued to increase throughput, reproducibility, and clinical relevance. Microfluidic organoid-on-a-chip platforms represent a significant leap forward, enabling high-throughput co-culture in a controlled microenvironment[28, 63, 64]. These chips can host organoids of various sizes, incorporate perfusable vascular networks, and allow for dynamic drug exposure, generating drug response data that more closely aligns with *in vivo* PDX results than static well-plate assays[63, 65]. Three-dimensional bioprinting offers another powerful tool, using patient-specific bioinks to create architecturally complex models that faithfully replicate the tumor extracellular matrix and allow for high-content drug screening[66]. Recent work has further strengthened the translational relevance of this strategy by showing that stress-relaxing granular bioprinting materials can support both precise tissue patterning and robust organoid self-organization. Using a tunable MAGIC matrix, Graham et al. demonstrated markedly improved organoid formation efficiency, inter-organoid homogeneity, and the generation of perfusable three-dimensional microphysiological systems across multiple tissue contexts, indicating that biomaterial mechanics are not merely supportive but actively shape morphogenesis and model reproducibility[67]. Furthermore, techniques like dissociating early-passage PDOs into single cells to generate uniform, miniature organoid arrays can drastically increase yield and accelerate drug testing timelines[42, 68]. For glioblastoma, four-dimensional (4D)-printed cell-culture arrays that self-transform into histological cassettes enable rapid processing and drug evaluation in patient-derived organoid-like models[69].

A major limitation of conventional PDO cultures is their focus on the epithelial compartment, often lacking the critical cellular and non-cellular components of the tumor microenvironment (TME) that govern drug response and resistance. Current optimization efforts are therefore intensely focused on developing sophisticated co-culture systems. Integrating cancer-associated fibroblasts (CAFs), derived from the same patient, into PDO models has been shown to restore biological pathways absent in PDO monoculture and influence organoid proliferation and drug sensitivity[54, 55]. For immune-oncology applications, co-culturing PDOs with autologous peripheral blood mononuclear cells (PBMCs) or specific immune cell subsets is essential. Studies have demonstrated that PDO-immune cell co-cultures can be used to predict patient responses to immune checkpoint inhibitors, such as anti-PD-1/PD-L1 therapies, in cancers like hepatocellular carcinoma and clear cell renal cell carcinoma[63, 70]. More complex systems aim to reconstitute multiple TME elements simultaneously. Microengineered platforms support the tri-culture of PDOs with mesenchymal stromal cells (which can enhance PDO growth and immune cell recruitment) and PBMCs, creating a more holistic model for immunotherapy screening[63]. Similarly, vascularized tissue-on-chip platforms co-culture endothelial cells and fibroblasts with tumor organoids to form perfusable vessel networks, improving nutrient delivery and enabling the study of size-dependent drug responses[65]. These evolving co-culture strategies, alongside advancements in 3D bioprinting and organ-on-a-chip technologies, are transforming conventional PDO platforms into next-generation and hybrid models that more accurately mirror the pathophysiological context of human tumors, thereby enhancing their predictive value for functional drug screening[71, 72].

4. Patient-Derived Xenograft Models

In contrast to *ex vivo* organoid systems, PDX models provide an *in vivo* platform that enables tumor propagation within a physiologically relevant host environment. The translational value of PDX models is largely dependent on their ability to preserve tumor architecture, maintain intratumoral heterogeneity, and recapitulate clinically relevant drug responses under systemic conditions. However, these features are highly contingent on methodological parameters during model construction and subsequent *in vivo* maintenance. This section outlines the core strategies underlying PDX model establishment, with a focus on implantation techniques, host selection, and factors influencing engraftment efficiency. We further discuss advances in humanized PDX systems and critically evaluate the strengths and limitations of PDX models in functional drug screening and precision oncology.

4.1 Construction strategies for patient-derived xenograft models

Generated through the direct implantation of fresh patient tumor fragments or cell suspensions, PDX models have demonstrated superiority in preserving the original tumor's histopathological

architecture, intratumor heterogeneity (ITH), and genomic landscapes across various stages and treatment backgrounds[1, 12, 73]. This high degree of fidelity underpins their established role as reliable preclinical models for drug screening, biomarker discovery, and the study of tumor evolution and resistance mechanisms[6, 8, 32]. The fundamental process involves transplanting tissue into immunodeficient mice, with engraftment success being a critical initial step. The choice of mouse strain is paramount; the evolution from nude mice to more severely immunocompromised strains like NOD/SCID and particularly NOD/SCID/IL2Rγnull (NSG or NOG) mice has significantly increased successful engraftment rates across multiple cancer types by reducing innate immune rejection[40, 74, 75]. The transplantation site also influences model characteristics and success. Subcutaneous implantation in the mouse flank is the most common and technically straightforward method, widely used for model establishment and drug efficacy studies[29, 76]. However, orthotopic implantation—where tumor tissue is engrafted into the murine organ corresponding to the tumor's origin—is recognized for better recapitulating the appropriate organ-specific microenvironment, often leading to more clinically relevant metastatic behavior[77, 78]. For certain cancers, such as cervical cancer, subrenal capsule implantation has been reported to yield higher engraftment rates compared to subcutaneous or orthotopic sites[75]. Success rates are not uniform and depend on multiple factors including cancer type, disease stage, prior therapy, and intrinsic tumor cell proliferation capacity[46, 74, 79-81]. Engraftment success itself can carry prognostic significance, as studies in pediatric sarcomas have found that successful PDX generation from treatment-naïve patients is associated with worse clinical outcomes, potentially identifying more aggressive disease[82].

4.2 Humanized patient-derived xenograft models

To address a major limitation of conventional PDX models—the lack of a functional human immune system for evaluating immunotherapies—significant efforts have been dedicated to developing humanized PDX (hu-PDX) models. These models combine patient-derived tumors with reconstitution or adoptive transfer of human immune components, commonly through hematopoietic stem/progenitor cells or mature immune cells, thereby enabling direct investigation of human tumor–immune interactions *in vivo*[83, 84] . Primary studies have demonstrated reconstitution of multiple human immune cell populations and functional responses to immune checkpoint blockade in such systems[83, 84].

Autologous hu-PDX models, in which the tumor and immune components are derived from the same patient, provide a particularly relevant platform for studying patient-specific tumor-immune interactions. Such models have been shown to recapitulate features of the human TME, including infiltration of diverse immune cell populations, and have been used to identify mechanisms such as VEGF-A-mediated myeloid cell activity that promotes tumor growth[84]. PDX-based systems have also enabled functional characterization of tumor-reactive lymphocytes, as demonstrated in uveal melanoma, where single-cell sequencing of PDX–TIL co-cultures identified distinct subsets of tumor-reactive lymphocytes[85]. These advanced models transform PDXs from simple drug-testing platforms into dynamic ecosystems for dissecting immunotherapy responses and resistance[12, 40].

4.3 Advantages and limitations of patient-derived xenograft models

The clinical fidelity of PDX models is their most compelling attribute, extensively validated across numerous cancer types. Histopathological and molecular analyses consistently show that PDXs retain the key genomic alterations, gene expression profiles, and proteomic signatures of their donor tumors over several passages[1, 31, 32, 86]. This genomic stability makes them excellent tools for correlating genotype with drug response. For instance, PDX models of non-small cell lung cancer (NSCLC) with EGFR mutations show expected sensitivity to EGFR tyrosine kinase inhibitors (TKIs), and the induction of resistance in these models can mirror clinical scenarios, helping uncover mechanisms like epithelial-to-mesenchymal transition (EMT) or activation of bypass signaling pathways[79, 87]. In functional drug screening, PDX models have demonstrated a strong predictive capacity for clinical outcomes. Large-scale PDX trials, sometimes termed PDX clinical trials (PCTs), where numerous models are screened against a panel of therapeutics, have identified genotype-response associations and have been proposed as a more accurate preclinical assessment tool than traditional cell line models[8, 88]. Preclinical studies using PDX models have identified potential therapeutic vulnerabilities, including responses to FOLFIRI-based combinations in colorectal signet ring cell carcinoma[46] and molecularly matched targeted therapies in biliary tract cancer[89]. This predictive power extends to the discovery of novel therapeutic strategies and drug repurposing, as seen in studies identifying multi-kinase inhibitors effective in advanced prostate cancer organoids and PDXs[90].

Despite their strengths, the fidelity of PDX models has important caveats and limitations. A significant issue is the representation of tumor heterogeneity. While PDXs preserve ITH better than cell lines, the engraftment process often imposes a selective bottleneck, where only a subset of tumor clones from the primary cancer successfully propagates in the mouse[73, 91]. This can lead to a loss of the full clonal diversity present in the patient. Multi-region sampling from the primary tumor can mitigate this by establishing several PDX lines that collectively capture more of the heterogeneity, but a single PDX model may not represent the complete genomic landscape of the original tumor[91, 92]. Furthermore, over serial passages, murine stromal cells gradually replace the human tumor stroma, altering the native TME. This stromal replacement means that while the epithelial cancer cells remain human, the supporting architecture is murine, which can affect studies of stroma-mediated drug resistance[73, 79, 85]. The time and cost associated with establishing and expanding PDX models also limit their utility for real-time clinical decision-making in aggressive cancers. Finally, ethical considerations regarding the use of animal models and patient tissue, along with the need for standardized protocols and quality control across different research centers, remain ongoing challenges[29, 93, 94]. Innovations continue to address these limitations, including the development of alternative hosts like zPDX and chick chorioallantoic membrane (CAM) models, which offer faster turnaround times and higher-throughput capabilities for drug testing[34, 95-97].

5. Next-Generation and Hybrid Patient-Derived Model Systems

Recognizing the limitations inherent to foundational patient-derived models, particularly conventional PDOs and PDXs, significant research efforts are directed toward engineering next-generation and hybrid systems. These next-generation and hybrid platforms aim to recapitulate the tumor ecosystem with greater physiological fidelity, incorporate dynamic microenvironments, and enhance throughput and scalability for functional drug testing. The conceptual evolution of tumor modeling platforms, from conventional 2D systems to PDO/PDX models, next-generation and hybrid systems, and AI-enabled digital twins, is summarized in **Figure 2**.

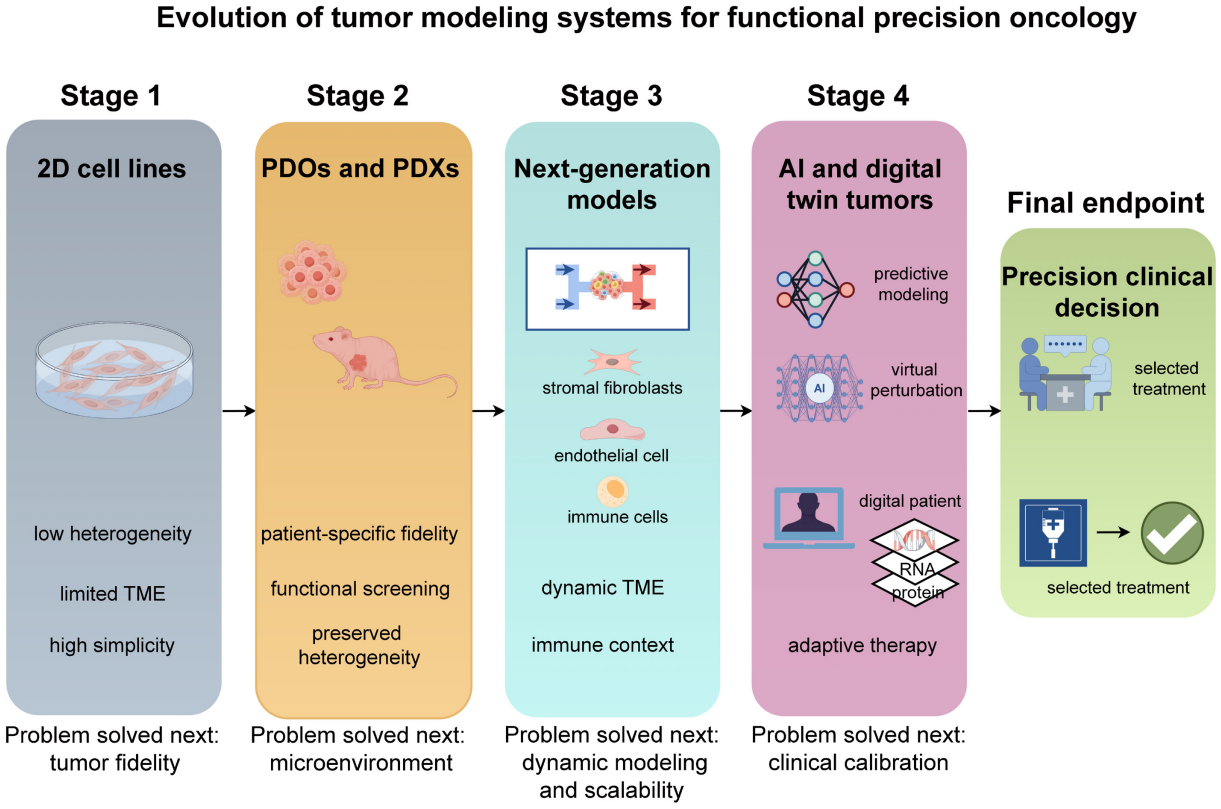

Figure 2. Evolution of tumor modeling systems for functional precision oncology. Traditional two-dimensional cell lines provide limited representation of tumor heterogeneity and microenvironmental interactions. Patient-derived organoids (PDOs) and xenografts (PDXs) improve biological fidelity and enable patient-specific functional testing. Next-generation platforms, including co-culture systems and microfluidic tumor-on-a-chip technologies, further incorporate dynamic tumor microenvironmental features. The integration of artificial intelligence and digital twin models represents the next stage, enabling predictive, adaptive, and clinically actionable therapeutic decision-making.

5.1 Organoid co-culture systems

A primary strategy involves the integration of essential TME components into organoid cultures. While traditional PDOs often lack a functional immune compartment, co-culture systems incorporating patient-derived immune cells are being developed to model immunotherapy responses. Co-culturing hepatocellular carcinoma (HCC) PDOs with PBMCs alongside mesenchymal stromal cells (MSCs) or CAFs creates an *in vitro* TME that more accurately predicts patient responses to anti-PD-L1 immunotherapy compared to PDOs alone[70]. Similarly, a malignant mesothelioma PDO-T cell co-culture system demonstrated the utility of such platforms for evaluating combined immunochemotherapy, where anti-PD-1 therapy combined with chemotherapy significantly reduced organoid viability[98]. Beyond immune cells, incorporating stromal components is crucial for modeling signaling and drug resistance mechanisms. Engineering defined 3D hydrogel systems that support the co-culture of prostate cancer PDX cells with osteoblastic cells successfully recapitulated the architecture and fibroblast growth factor-mediated signaling of bone metastatic lesions, providing a controlled platform to dissect tumor-stromal interactions[99]. For osteosarcoma, a 3D biomaterial model incorporating bone-mimicking hydroxyapatite was critical to retain *in vivo*-like signaling and drug resistance profiles observed in patient tissues and orthotopic mouse models[100].

Recent work further illustrates the value of coupling multicellular organoid systems with molecular profiling. In urothelial carcinoma, patient-derived organoid–immune co-cultures integrated with multi-omics analysis have been applied to characterize immunotherapy response and investigate mechanisms associated with therapeutic resistance[101]. Importantly, the relevant tumor microenvironment extends beyond canonical immune and stromal compartments. Peripheral nerve–cancer interactions can influence tumor progression, metastatic behavior, and therapeutic response[102], while therapy-induced cellular states such as senescence can remodel local signaling through secretory, inflammatory, and immune-modulatory programs[103]. These observations support a question-oriented strategy for next-generation co-culture models, in which selected immune, stromal, neural, or treatment-responsive cellular components are incorporated according to the biological mechanism and therapeutic modality being investigated.

5.2 Microfluidic tumor-on-a-chip platforms

Microfluidic organ-on-a-chip (OoC) technology represents another transformative advancement, enabling precise control over the cellular microenvironment, mechanical forces, and pharmacokinetic profiles. These systems integrate 3D tumor cultures within perfusable microchannels, allowing for dynamic nutrient supply, waste removal, and drug delivery that mimics vascular perfusion[37, 64]. The application of OoC platforms spans numerous cancer types. A breast cancer therapy response testing platform using a perfused microfluidic system demonstrated that 3D culture under perfusion attenuates drug responses compared to 2D cultures, offering a more physiologically relevant screening context[20]. For colorectal cancer, a multiplexed tumor OoC device used to culture PDX-derived spheroids under perfusion generated dose-dependent drug responses that correlated quantitatively with outcomes from matched *in vivo* PDX models[21]. Other designs focus on rapid prototyping and scalability, such as a novel 3D-printed, PDMS-free biochip for breast cancer studies that supports 3D cell culture and migration assays[104]. These chips also enable real-time monitoring, as demonstrated by a system combining microfluidics with luminescence imaging to track immediate and adaptive drug responses upon repeated treatment over time[105]. For bladder cancer, spheroids cultured in a customized microfluidic device showed drug sensitivity outcomes that correlated highly with both PDX models and clinical patient responses[106].

Innovations in chip design continue to enhance functionality. The Vascularized Tissue on Mesh-Assisted Platform (VT-MAP) utilizes open microfluidics to culture vascularized organoids of various sizes, revealing that drug response can be critically dependent on organoid size[65]. The importance of microenvironmental control extends beyond soluble factors and drug concentration gradients. Tumor growth and therapeutic response are also shaped by physical determinants such as solid stress, extracellular matrix stiffness, interstitial pressure, cellular traction, and vascular remodeling, highlighting the need for experimental systems capable of reproducing both mechanical and biochemical properties of the tumor microenvironment[107]. In parallel, computational studies of angiogenesis have emphasized the dynamic rearrangement of endothelial cells during vascular network formation[108]. Together, these concepts provide a mechanistic rationale for integrating controllable biomechanical cues, vascularized compartments, and computational analysis into advanced tumor-on-a-chip systems.

5.3 Alternative rapid *in vivo* hosts and hybrid model systems

Parallel to these *in vitro* innovations, alternative rapid *in vivo* hosts and hybrid patient-derived model systems are being developed to address the time, cost, throughput, and scalability limitations of conventional murine PDX models. The chick chorioallantoic membrane (CAM) and zPDX models offer rapid, scalable alternatives. The CAM model supports the engraftment of patient-derived tumor tissue fragments, with studies reporting successful xenograft growth for over a dozen tumor subtypes within 5-10 days[97]. This platform has been applied for functional precision oncology, as shown in follicular lymphoma, where tumor volume reduction upon R-CHOP treatment on the CAM predicted patient progression-free survival[96]. Zebrafish embryos, with their optical transparency and genetic tractability, provide a unique venue for real-time, longitudinal observation of tumor growth and invasion. A glioblastoma study engrafted patient-derived cells into zebrafish embryos and used AI-driven live imaging to monitor growth, finding a strong correlation in tumor initiation between zebrafish and mouse PDX counterparts[35]. This zPDX model also enabled evaluation of drug response, with effects on fish survival corresponding to established *in vitro* and *in vivo* drug sensitivity data[35]. More broadly, zebrafish have become a versatile in vivo biomedical platform because of their rapid development, optical accessibility, genetic manipulability, and compatibility with imaging and functional-omics approaches[109]. In patient-derived cancer modeling, these characteristics complement the short turnaround time and scalability of zPDXs and facilitate real-time assessment of tumor growth, dissemination, and treatment response.

A powerful trend is the strategic hybridization of different model systems to leverage their respective strengths. One approach combines the high-throughput capability of PDOs with the *in vivo* context of PDX. For example, a study on advanced breast cancer first used PDOs for initial drug screening, then validated the identified candidate drug combination in a mini-PDX model, creating a rapid and precise tiered screening platform[110]. The mini-PDX assay itself, often based on microfluidic enrichment of tumor cells followed by short-term *in vivo* implantation, has shown clinical utility. In non-small cell lung cancer, chemotherapy guided by a microfluidic-based mini-PDX assay significantly improved patient overall survival and progression-free survival compared to conventional chemotherapy[111]. Another hybrid strategy involves deriving stable, conditionally reprogrammed cell lines (CR-PDX) from PDX tumors. These cells maintain the genetic drivers and drug response of the parental PDX, can be expanded *in vitro* for high-throughput 2D screening, and can be re-implanted *in vivo*[112]. Similarly, engineering matched *in vitro* and *in vivo* models from the same PDX source ensures consistency. A 3D macroporous sponge system was used to culture organoids from a panel of HCC-PDX lines, and genomic, transcriptomic, and drug response profiles showed strong concordance with the matched *in vivo* PDX tumors[113]. This concept extends to biomaterial-based 3D models designed explicitly for PDX cells, such as hydrogel-encapsulated prostate cancer PDX cells that maintained viability and exhibited drug sensitivity distinct from conventional cell lines[114]. Recent bioengineered hydrogel-based approaches have further improved *ex vivo* preservation of patient-derived tumor slices, enabling prolonged maintenance of tumor, stromal, and immune compartments and supporting co-clinical drug evaluation[115].

The integration of advanced fabrication techniques and AI is pushing the boundaries of these hybrid systems. 4D bioprinting, using shape-memory polymers, has been employed to create arrays that self-transform from 3D cell-culture inserts into histological cassettes, enabling rapid processing and drug evaluation in glioblastoma patient-derived models[69]. AI and machine learning are increasingly critical for analyzing the complex, high-dimensional data generated by these sophisticated platforms. AI can automate phenotyping, classify therapeutic responses from imaging data, and enable real-time control of microfluidic environments[116]. At the analytical level, novel computational models like scAdaDrug utilize multi-source domain adaptation to predict drug response at the single-cell level by integrating data from cell lines, PDX models, and clinical cohorts[117]. Together, these next-generation and hybrid systems—spanning enhanced co-cultures, dynamic microfluidic chips, rapid alternative *in vivo* hosts, and intelligent data integration—are constructing a more nuanced and powerful toolkit. They bridge the gap between simplified *in vitro* assays and complex, time-consuming *in vivo* studies, thereby accelerating the path towards more predictive functional drug screening and personalized therapeutic strategies.

6. Application of Patient-Derived Models in Functional Drug Screening and Precision Therapy

Compared to de novo drug development, drug repurposing offers superior efficiency. The screening of effective drugs/compounds is particularly crucial in innovative drug development and application. As

organoid technology enables the relatively rapid generation and long-term maintenance of 3D tumor tissue structures that closely resemble the native state *ex vivo*, while also preserving the gene expression profile characteristics of tumors[118, 119], patient-derived cancer organoids demonstrate significant application potential in drug and target screening research. By evaluating and screening the therapeutic effects of single drugs or drug combinations on tumor organoids, candidate drugs potentially effective against a patient's tumor can be rapidly identified, thereby playing a major role in promoting the clinical translation of drugs.

6.1 Establishment of a medium- to high-throughput drug screening platform

PDOs provide a scalable experimental platform for functional drug screening, enabling the systematic evaluation of therapeutic responses across a range of compounds and combinations. Compared with conventional two-dimensional systems, PDO-based platforms preserve key structural and molecular characteristics of tumors while allowing for controlled experimental manipulation and relatively high-throughput testing[118, 119].

A typical PDO-based screening workflow involves the generation of standardized organoid cultures, followed by controlled drug exposure and quantitative assessment of treatment response. A schematic overview of the standardized PDO establishment and functional drug screening pipeline, including sample processing, organoid establishment and standardization, drug exposure, response detection, and downstream analysis, is shown in **Figure 3**. To minimize variability, organoids are often dissociated into uniform cell clusters and re-embedded under defined conditions to ensure consistent drug penetration and growth kinetics[120, 121]. Drug response is then evaluated using viability-based readouts, such as ATP quantification, in combination with molecular analyses including transcriptomic profiling and pathway enrichment[122].

However, despite these technical advances, several methodological constraints must be considered when interpreting PDO-based drug screening results. First, drug exposure *in vitro* does not fully recapitulate pharmacokinetic and pharmacodynamic processes *in vivo*, including metabolism, distribution, and systemic toxicity. Second, the absence or simplification of the TME in conventional PDO cultures can significantly influence drug sensitivity, particularly for therapies targeting stromal or immune components[123]. Third, variability in organoid size, matrix composition, and culture conditions can introduce experimental noise, potentially affecting reproducibility across laboratories.

To address these limitations, emerging strategies—including microfluidic platforms, co-culture systems, and machine learning-assisted optimization—are being developed to improve standardization, throughput, and physiological relevance[124-127]. These innovations highlight a key principle: while PDO-based platforms enable efficient functional screening, their predictive accuracy is highly dependent on experimental design and model context.

6.2 Drug efficacy prediction and clinical relevance

A central question in functional precision oncology is the extent to which drug responses observed in patient-derived models can predict clinical outcomes. While numerous studies have demonstrated concordance between PDO-based drug sensitivity and patient responses, the strength and consistency of this association vary substantially across study designs, tumor types, and treatment classes.

From an evidence hierarchy perspective, current data can be broadly categorized into case reports, retrospective analyses, and prospective cohort studies. Early clinical observations, including individual case reports, have shown that PDO-guided therapy selection can lead to meaningful clinical responses, such as partial remission or prolonged disease stabilization[128]. Although these findings are encouraging, they remain inherently limited by small sample size and potential selection bias.

More robust evidence is provided by retrospective and meta-analytical studies. A systematic review encompassing multiple tumor types reported an overall sensitivity of 0.81 and specificity of 0.74 for PDO-based drug response prediction[129]. However, substantial heterogeneity was observed across studies, reflecting differences in experimental protocols, treatment regimens, and patient populations. Importantly, most available data are derived from a limited number of cancer types, particularly colorectal cancer, which restricts the generalizability of these findings. Accordingly, predictive performance established for a particular tumor type or therapeutic class should not be assumed to generalize directly to other malignancies or treatment modalities.

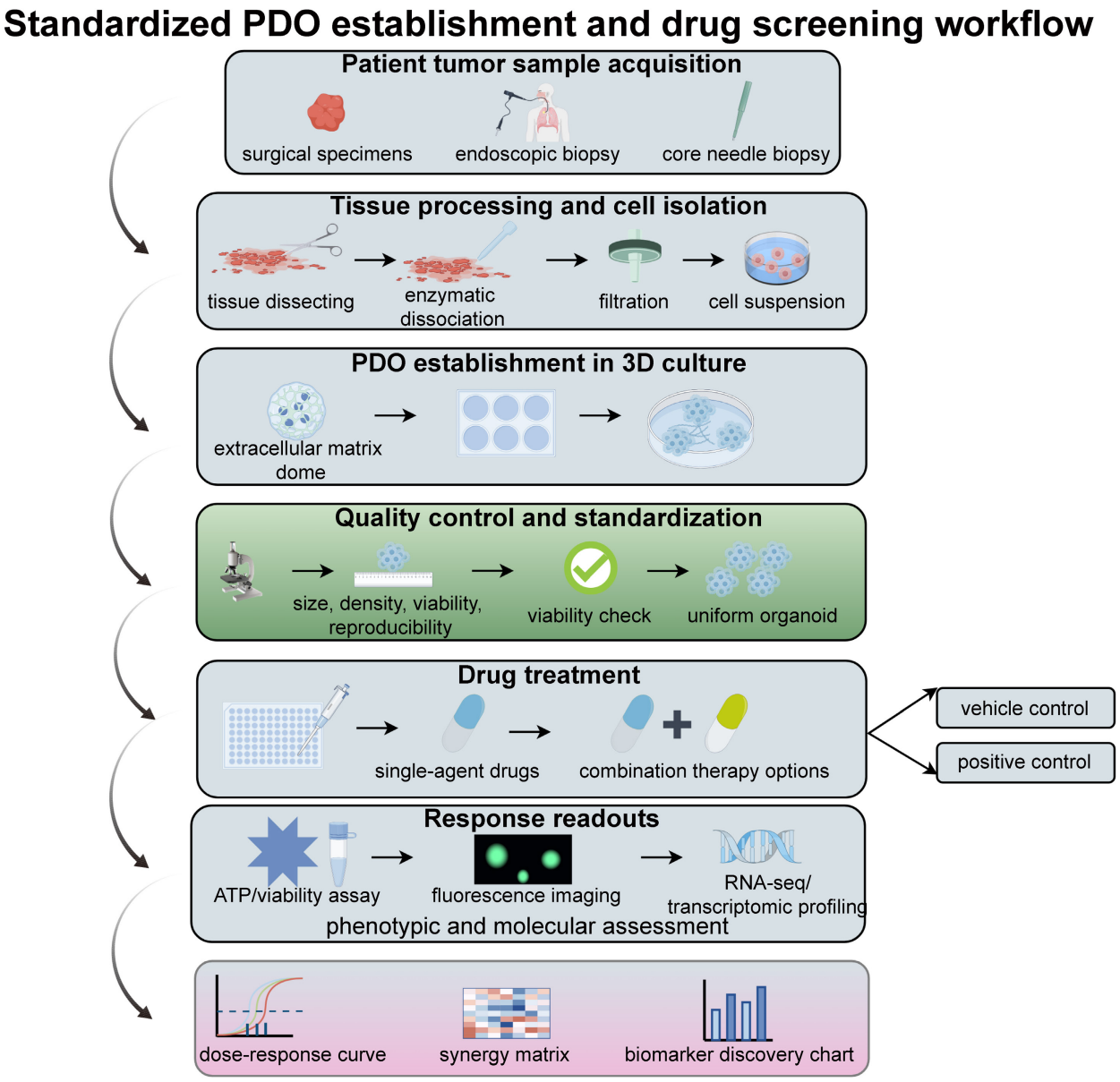

Figure 3. PDO construction and functional drug screening workflow. Patient tumor tissues are initially dissociated and subjected to cell isolation, followed by organoid establishment in an extracellular matrix-supported three-dimensional culture system to preserve patient-specific tumor characteristics. Quality control and standardization of organoid size, density, and viability improve assay reproducibility, while high-throughput single-agent and combination screening increases scalability for therapeutic prioritization. Subsequent phenotypic and molecular readouts, including viability assays, imaging analysis, and transcriptomic profiling, provide increasingly comprehensive characterization of treatment response. Integration with AI-assisted analytical frameworks further enables quantitative response modeling and predictive biomarker identification. The workflow illustrates the progressive development from patient-specific tumor preservation and standardized organoid generation to scalable drug screening, multimodal response characterization, and computationally assisted predictive analysis.

In a prospective cohort of patients with metastatic colorectal cancer, PDO drug sensitivity was significantly correlated with changes in target lesion size (R = 0.54–0.60). For 5-fluorouracil- and oxaliplatin-based treatment, PDO testing achieved an area under the receiver operating characteristic curve (AUROC) of 0.88 (95% CI: 0.71–1.00) for predicting responses across all target lesions[44]. Notably, PDO resistance predictions were associated with shorter progression-free survival and overall survival, highlighting their potential prognostic value[44]. Similarly, in osteosarcoma, PDO-based drug sensitivity testing showed high concordance with treatment response following neoadjuvant therapy, with predictive accuracies exceeding 80%[130]. Despite these advances, several critical factors limit the universal predictive power of PDO models. First, intrapatient heterogeneity represents a major challenge. Organoids derived from different tumor regions or metastatic sites within the same patient can exhibit markedly divergent drug responses, with up to tenfold differences in IC50 values reported in ovarian cancer[131]. Second, model establishment success rates vary widely across tumor types and clinical

contexts, ranging from approximately 30% to 90%, and can be significantly lower in heavily pretreated or rare malignancies[129, 132]. Third, PDO models inherently lack systemic physiological factors, including immune responses and drug metabolism, which are essential determinants of treatment efficacy in patients. Taken together, these findings indicate that PDO-based drug screening provides valuable but context-dependent predictive information, with the current level of evidence remaining heterogeneous across tumor types, treatment classes, assay platforms, and clinical study designs. Rather than serving as a standalone clinical decision tool, PDOs are best viewed as a complementary platform that integrates with genomic profiling, clinical parameters, and additional model systems to improve therapeutic stratification.

6.3 Biomarker discovery and drug resistance mechanism research

Molecular biomarkers associated with drug sensitivity

PDOs provide a uniquely patient-specific and functionally tractable platform for biomarker discovery, particularly when integrated with multi-omics profiling strategies[133]. By integrating multi-omics sequencing and analytical methods, PDOs can dynamically and systematically identify biomarkers associated with treatment response and drug resistance. For example, by comparatively analyzing PDOs from the same patient before and after treatment, or comparing sequencing results of drug-sensitive versus drug-resistant PDOs or corresponding tumor tissues, key molecular biomarker changes related to drug response can be captured[134, 135].

Merrill et al.[60] successfully established and validated 65 short-term patient-derived organoid models from 106 bladder cancer tumor samples and performed systematic genomic, transcriptomic, and proteomic sequencing for each PDO and its corresponding tumor sample. The study conducted *ex vivo* screening of various drugs or drug combinations based on PDOs. By integrating multi-omics features with drug response data, the research untangled multi-omics biomarkers associated with gemcitabine efficacy: at the genomic level, alterations in TP53 or RB1 genes were significantly correlated with increased sensitivity to gemcitabine, while KDM6A mutations were associated with drug resistance; at the transcriptomic level, basal/squamous and neuronal subtypes were more sensitive to gemcitabine; at the proteomic level, the expression levels of 68 proteins were significantly correlated with gemcitabine response[60]. Finally, these biomarkers discovered based on PDO models were validated in an independent TCGA BLCA cohort, demonstrating consistent molecular characteristics[60]. Key gene sets screened from transcriptomic data of PDOs with different treatment responses can be used to construct predictive models to better distinguish populations with varying drug responses[136].

Importantly, clinically relevant therapeutic vulnerabilities are not restricted to canonical genomic alterations. Emerging studies have identified additional layers of targetable cancer biology, including cytoskeletal dependencies, as illustrated by therapeutic modulation of R11–actin interactions and downstream cytoskeletal remodeling in bladder cancer[137], and stress-adaptive ribonucleoprotein granule networks regulated by protein modifications[138]. In parallel, increasing attention is being directed toward noncanonical proteins as previously under-recognized sources of diagnostic biomarkers and therapeutic targets[139]. These developments broaden the scope of biomarker discovery beyond DNA-level alterations and highlight an important role for patient-derived functional models in determining whether mechanistically plausible targets translate into selective and reproducible therapeutic phenotypes.

Construction and application of acquired drug resistance models

For in-depth mechanistic studies of drug resistance, acquired drug-resistant PDO models can be constructed through various methods such as gene editing or long-term drug exposure. The development of acquired drug-resistant PDO models addresses the scarcity of longitudinal clinical patient samples, enabling the identification of key resistance genes and validation of their causal functions. Additionally, these models can be used for high-throughput drug screening to systematically identify combination therapies capable of reversing drug resistance, thereby enhancing their predictive value in personalized treatment.

Steiner et al.[140] established PDOs from the postoperative tumor tissue of a treatment-naïve PDAC patient harboring a rare BRAF R506_K507ins VLR mutation. These PDOs retained the BRAF mutation and exhibited sensitivity to gemcitabine and trametinib, consistent with the patient's clinical response. By continuously treating the PDOs with gradually increasing concentrations of trametinib over 32 weeks, five resistant PDO lines were obtained. Transcriptomic analysis revealed that trametinib-resistant PDOs were significantly enriched in genes of the WNT/β-catenin pathway. At the protein level, nuclear positivity of β-catenin was observed in both trametinib-resistant PDOs and the patient's liver

metastasis, suggesting WNT pathway activation. Functional experiments confirmed that β-catenin overexpression could confer partial drug resistance to parental cells. High-throughput drug screening identified that resistant PDOs were highly sensitive to the AURKA inhibitor alisertib and the WNT inhibitor LGK-974[140]. This study was the first to untangle WNT/β-catenin signaling activation as a key mechanism of MEK inhibitor resistance in BRAF-mutant PDAC and suggested alisertib and LGK-974 as potential therapeutic strategies. Huang et al.[141] established hepatocellular carcinoma PDOs and subsequently generated acquired lenvatinib-resistant patient-derived organoid xenografts (ALR PDOXs). Unlike PDX-derived organoids (PDXOs), which are established from PDX tumor tissues, PDOXs are generated by transplanting PDO-derived material into immunodeficient mice. Using a kinase-focused CRISPR–Cas9 screening strategy in these organoid-derived xenograft models, the investigators identified NEK7 as a critical mediator of acquired lenvatinib resistance. This study combined acquired drug-resistant organoid transplantation models with CRISPR screening to enable functional screening of resistance genes in an *in vivo* environment, overcoming the limitations of traditional cell models in simulating tumor heterogeneity.

Therapeutic resistance likewise arises through mechanisms that may be incompletely represented by baseline genomic profiling alone. In prostate cancer, persistent intratumoral androgen signaling and genetic variation represent interconnected mechanisms contributing to treatment resistance and castration-resistant disease[142]. At the cellular level, alternative stress-response and cell-death programs may provide additional therapeutic opportunities; paraptosis, for example, has been proposed as a means of exploiting endoplasmic reticulum, oxidative, and mitochondrial stress to bypass apoptosis-associated resistance in urological malignancies[143]. Context-dependent signaling networks add another layer of complexity, as exemplified by the noncanonical NFκB pathway, which regulates cellular survival, growth, differentiation, and immune-associated states[144]. Patient-derived resistant models are therefore valuable not only for identifying recurrent genomic alterations but also for functionally testing dynamic pathway dependencies and alternative cell-state vulnerabilities that emerge under therapeutic pressure.

7. Integration of Complex Algorithms and Organoids

The clinical translation of organoids is constrained by several bottlenecks, including the lack of standardized and real-time monitoring in cultivation protocols, the subjectivity and inefficiency of traditional data analysis, and the difficulty in integrating multimodal data, including spatial features[145]. AI, a branch of computer science, iteratively learns from complex data through algorithms to simulate human cognitive functions such as visual perception and decision-making, thereby automating decision-making and prediction[146]. The integration of AI with organoids represents an emerging direction in organoid research, enabling the construction of more complex and accurate models of human organ function and disease. This provides powerful tools for drug discovery, disease diagnosis, and therapeutic development.

7.1 Algorithmic analysis for predicting drug response

In drug screening and efficacy prediction, the interaction between algorithmic analysis and organoid systems enables the use of complex algorithms such as artificial intelligence to analyze the intricate data generated by organoids. This approach identifies novel therapeutic targets and biomarkers, allowing for more precise and accurate prediction of drug responses. Due to the high dimensionality and limited sample size of PDO model data, which can easily lead to overfitting, traditional machine learning struggles to extract robust features. Kong et al.[147] proposed a method combining network analysis and machine learning to identify robust biomarkers. This study first screened pathways adjacent to drug targets using a PPI network, then trained machine learning regression models with PDO gene expression and drug response data, selecting the pathway with the highest predictive performance as the biomarker. Results showed that the "activation of BH3-only proteins" pathway could serve as a predictive biomarker for 5FU in colorectal cancer, while the "amino acid synthesis and interconversion" pathway could predict cisplatin response in bladder cancer. This method outperformed traditional machine learning and feature-agnostic approaches, maintaining accuracy in external validation[147].

Additionally, multi-paradigm computational models can be used to directly predict patient responses to specific drugs. Unlike traditional biomarker methods, which treat drugs as "labels" rather than input features and emphasize interpretability and biological mechanisms, end-to-end prediction models such as PharmaFormer incorporate drug structures as part of the model input, prioritizing predictive performance and model generalization. However, these models often exhibit "black-box" characteristics, which may limit

mechanistic interpretation, clinician confidence, and regulatory acceptance. Their clinical translation will therefore require explainable modeling strategies, explicit uncertainty quantification, and prospective calibration against observed clinical outcomes. Zhou et al.[148] utilized gene expression, drug response, and drug structure data from the GDSC database to construct the PharmaFormer pre-training model based on a custom Transformer architecture and transfer learning strategy, aiming to predict drug responses in tumors. The model was fine-tuned using drug response datasets from colorectal, bladder, and hepatocellular carcinoma PDOs, resulting in a PDO-optimized model. Finally, it was applied to RNA-seq data from corresponding tumor patients in the TCGA database to predict their responses to specific drugs. The study found that the PharmaFormer model outperformed classical machine learning algorithms such as Support Vector Regression (SVR), Multilayer Perceptron (MLP), Random Forest (RF), k-Nearest Neighbors (KNN), and Ridge Regression in predictive accuracy. Moreover, PDO data significantly enhanced the pre-training model's predictive performance across different tumor types and drugs[148]. Another study incorporated *ex vivo* drug sensitivity data from PDOs into a Quantitative Systems Pharmacology (QSP) model. By adjusting individualized parameters, a virtual MET-amplified NSCLC patient model based on PDOs was constructed, successfully simulating the inter-individual heterogeneity in response to dose-response curves observed in PDOs[149]. This virtual patient model can be used to predict the clinical efficacy of different drug combination therapies at various doses, providing a reference for personalized treatment design. Beyond improving response prediction from existing datasets, these computational advances are also beginning to support dynamic *in silico* modeling of tumor evolution and perturbation response, thereby extending organoid-based analysis toward digital twin and virtual organoid frameworks.

7.2 Digital twin tumors, virtual organoids, and *in silico* perturbation

As organoid systems become increasingly complex and physiologically relevant, their broader application in large-scale drug screening and routine clinical decision-making remains constrained by high cultivation costs, limited scalability, batch-to-batch variation, observational incompleteness, and ethical oversight requirements. In parallel, although AI-assisted organoid research has improved image analysis and multi-omics integration, most current approaches still depend on static datasets rather than continuously updated patient-specific digital models. These limitations have driven growing interest in digital twin tumors and virtual organoids (VOs), which aim to integrate multimodal data into dynamic and predictive representations of tumor behavior[150, 151].

VOs can be conceptualized as computational counterparts of biological organoid systems, built by integrating imaging, clinical, genomic, transcriptomic, proteomic, spatial, and other phenotypic data across multiple scales. In principle, such models could simulate tumor evolution, forecast treatment response, and support personalized therapeutic decision-making. Among the emerging functionalities of this framework, virtual knockout or *in silico* perturbation may be one of the most translationally relevant, because it moves digital tumor modeling beyond descriptive reconstruction toward functional hypothesis generation. By computationally estimating the phenotypic, transcriptional, and network-level consequences of perturbing specific genes, pathways, or cellular states before wet-lab testing, these approaches may help prioritize the most biologically plausible candidates for subsequent experimental evaluation. This capability is particularly attractive in precision oncology, where virtual perturbation may help prioritize context-specific therapeutic targets, identify actionable dependencies, and nominate rational drug combinations for subsequent experimental validation in PDOs, PDXs, or other model systems[152, 153].

Recent studies have begun to establish the technical basis for this direction. Squidiff is a diffusion model-based generative framework developed to predict high-resolution transcriptomic changes across diverse cell types and experimental conditions. Its performance across cell differentiation, gene perturbation, and drug-response prediction illustrates the potential of diffusion-based models to forecast cellular responses to perturbations and support rapid hypothesis generation[152]. In hepatocellular carcinoma, Xia et al. integrated large-scale single-cell transcriptomic datasets spanning normal liver, primary tumors, portal vein tumor thrombi, and metastatic lymph nodes with spatial transcriptomic profiling. The authors subsequently performed Geneformer-based *in silico* perturbation and virtual knockout screening across three biologically relevant cell-state transitions[153]. Together, these studies illustrate two complementary applications of computational perturbation modeling: diffusion-based prediction of transcriptomic responses across perturbation conditions and foundation model-based virtual knockout for prioritizing context-specific therapeutic dependencies. However, the resulting predictions remain hypotheses that require direct genetic, pharmacological, and functional validation in

patient-derived experimental models.

At a higher systems level, agent-based virtual tumor models have been used to reconstruct tumor–immune interactions and to reveal how spatial microenvironmental heterogeneity shapes immunotherapy efficacy[154]. Early preclinical efforts have begun to explore transformer-based virtual organoid frameworks for integrating multi-scale biological data into predictive digital tumor systems. For example, ODFormer has been reported in a preprint as a virtual organoid framework for predicting personalized therapeutic responses in pancreatic cancer[155]. However, these findings remain preliminary and require peer-reviewed evaluation, independent validation, and prospective assessment before their potential clinical utility can be established. Taken together, these emerging studies and frameworks indicate that digital twins and VOs may eventually provide a computational layer that complements patient-derived biological models by linking multimodal tumor characterization with perturbation-aware therapeutic prediction.

Despite these advances, the translational readiness of digital twins, virtual organoids, and *in silico* perturbation strategies should not be overstated. Their performance remains highly dependent on the quality, scale, and standardization of multimodal training data, while data fragmentation across institutions, platforms, and assay types continues to limit interoperability and generalizability across tumor types and clinical contexts[151, 156]. Addressing this challenge will require harmonized data-generation pipelines, common metadata standards, interoperable multimodal repositories, and, ideally, federated or multicenter learning frameworks that reduce data-island effects without compromising patient privacy.

A second major challenge is model interpretability[156]. Although end-to-end architectures such as PharmaFormer can achieve strong predictive performance, their "black-box" nature limits mechanistic insight, clinician confidence, and regulatory acceptability[148]. Future progress will likely depend on combining predictive performance with explainable or hybrid modeling strategies, including pathway-constrained learning, causal inference, explicit uncertainty quantification, and transparent linkage between model outputs and biologically testable hypotheses. Such approaches will be necessary not only to clarify how predictions are generated, but also to communicate the confidence and limitations associated with individual patient-level predictions. In this regard, virtual knockout is particularly valuable not as a replacement for functional experimentation, but as a hypothesis-prioritization tool that can focus downstream genetic or pharmacological validation in PDOs or PDXs on the most plausible perturbations and therapeutic combinations.

Equally important is the question of validation and calibration. At present, most virtual organoid and digital twin studies remain exploratory and retrospective. Their predictions must first be benchmarked against experimentally observed perturbation responses in patient-derived systems and subsequently prospectively calibrated against clinical outcomes, including objective response, progression-free survival, overall survival, and resistance trajectories. Such calibration should assess not only discriminatory performance but also whether predicted probabilities correspond to observed outcome frequencies across clinically relevant patient subgroups[145, 149]. A plausible translational pathway would therefore involve stepwise validation across matched PDO/PDX datasets, clinically annotated patient cohorts, and ultimately interventional trials testing whether model-informed treatment selection improves outcomes beyond standard-of-care approaches.

Taken together, digital twin tumors, virtual organoids, and virtual knockout strategies represent a promising extension of functional precision oncology, but their current role is best understood as complementary rather than definitive. At present, their greatest value lies in hypothesis prioritization, target nomination, and rational preselection of perturbations or drug combinations before experimental validation in patient-derived biological systems. Future progress will depend on standardized multimodal data infrastructures, improved model interpretability, and rigorous prospective validation pipelines linking computational prediction with PDO/PDX testing and clinical outcome data.

8. Challenges and Future Directions

Despite the rapid advancement of patient-derived tumor models, their translation from experimental platforms to routine clinical decision-making tools remains constrained by multiple interconnected challenges. These barriers can be broadly categorized into three domains: technical and operational limitations, regulatory and ethical considerations, and the need for robust clinical validation. Addressing these challenges is essential for realizing the full potential of functional precision oncology. The major barriers to clinical translation and the corresponding roadmap for implementation of patient-derived tumor models are summarized in **Figure 4**.

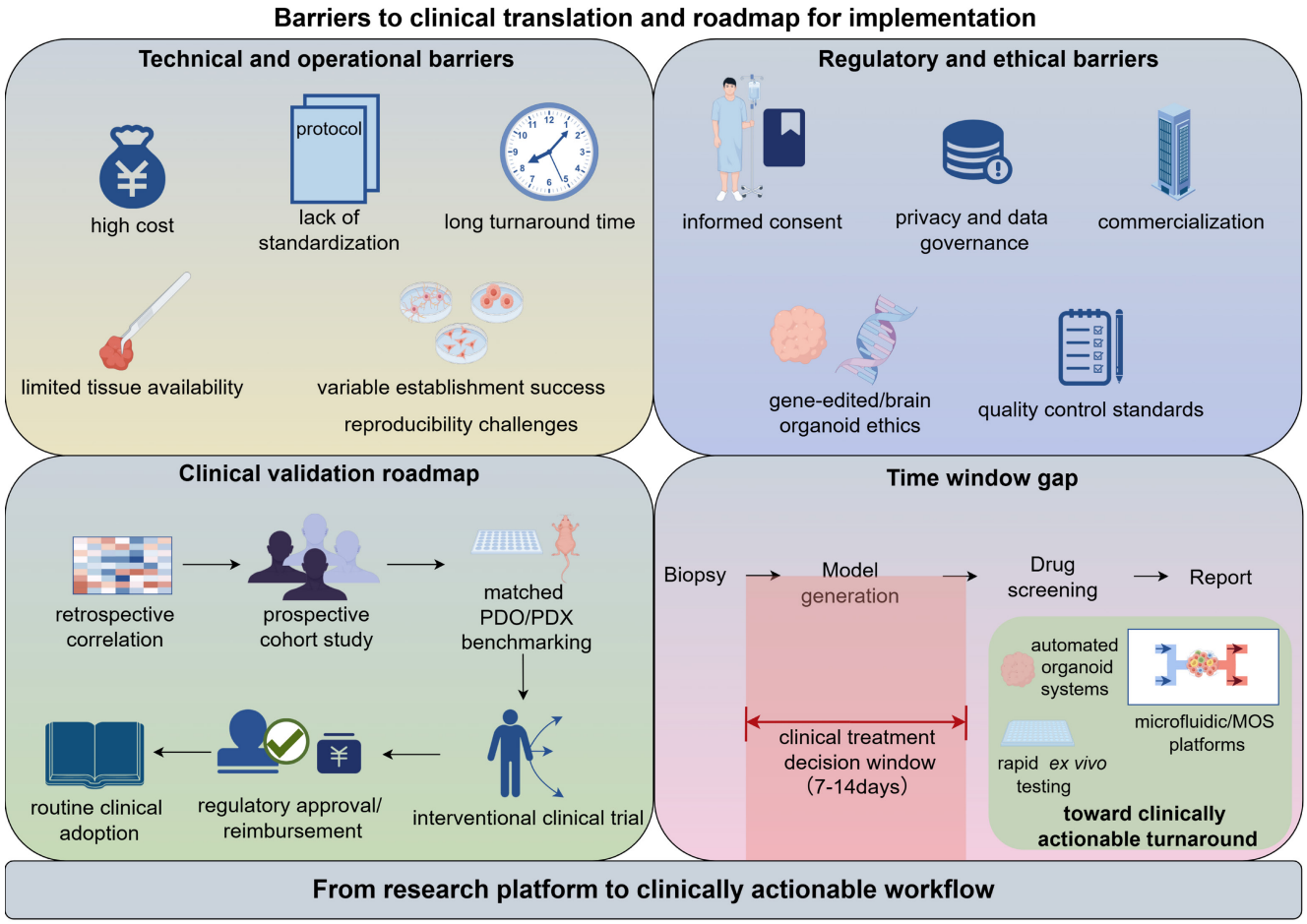

Figure 4. Barriers to clinical translation and roadmap for implementation of patient-derived tumor models. Clinical application of patient-derived tumor models is limited by interconnected technical, operational, regulatory, ethical, and validation-related barriers. Key challenges include variability in model establishment, lack of standardization, high cost, prolonged turnaround time, and limited tissue availability. Particular emphasis is placed on the time-window gap between model establishment and functional testing and the shorter clinical interval available for therapeutic decision-making, which represents a major constraint on real-time implementation. Translation into routine care will require shortened assay timelines, stepwise validation across retrospective and prospective studies, integration into clinically actionable workflows, and the establishment of regulatory and reimbursement pathways.

8.1 Technical and operational barriers

The clinical translation of PDO platforms is fundamentally constrained by a set of interconnected technical and operational challenges, including lack of standardization, variable success rates, extended processing timelines, and high economic burden. These factors collectively limit scalability, reproducibility, and real-world clinical applicability.

A primary obstacle lies in the absence of standardized protocols for PDO construction and drug screening. Current methodologies vary substantially across laboratories in terms of tissue processing, enzymatic digestion, culture media composition, and quality control criteria, resulting in significant inter-study variability and limited reproducibility[124]. Reported PDO establishment success rates range widely from 31% to 90%, depending on tumor type, sample quality, and technical expertise[129]. In certain advanced malignancies, such as metastatic prostate cancer, success rates can be as low as 16%[132]. Tumor cellularity has been identified as a key determinant of successful organoid establishment, while additional factors—including tissue source, histological subtype, disease stage, and specific culture conditions such as the use of ROCK inhibitors (Y−27632)—further influence outcomes[129, 157, 158].

Efforts to address these limitations have led to the development of standardized and optimized protocols. For example, Kang et al. proposed a comprehensive workflow encompassing specimen acquisition, processing, culture, cryopreservation, and drug screening, applicable across multiple specimen types including surgical resections, biopsies, and body fluids[159]. Similarly, disease-specific protocols have been established for colorectal and other tumor types, improving reproducibility and experimental consistency[160].

In parallel, the integration of automation technologies has significantly enhanced scalability and standardization. Microfluidic systems combined with 3D bioprinting enable controlled organoid generation

with improved uniformity and fidelity to patient tumors[161]. These platforms also allow precise regulation of drug exposure dynamics, better simulating *in vivo* pharmacokinetics compared to conventional static assays[118].

Despite these advances, time constraints remain a critical bottleneck for clinical implementation. In a representative colorectal cancer study, the median time required to generate PDOs suitable for downstream applications was approximately 61 days, followed by additional expansion time prior to drug screening[44]. This timeline is incompatible with clinical decision-making windows, which typically require treatment selection within 10–14 days for aggressive cancers[162]. This mismatch between model turnaround time and the clinically actionable treatment window constitutes a major translational bottleneck and is highlighted schematically in Figure 4.

Emerging technologies aim to address this gap. Automated organoid culture systems can accelerate growth kinetics, achieving structurally mature organoids within one week[161], while microfluidic-based platforms such as micro-organosphere (MOS) systems enable high-throughput drug sensitivity testing directly from biopsy samples within two weeks[162]. Importantly, these systems preserve key tumor microenvironment components, including stromal and immune cells, thereby enhancing biological relevance compared to conventional organoid cultures.

In addition to technical and temporal limitations, economic burden and clinical accessibility represent major barriers to widespread adoption. PDO culture requires costly biomaterials, including extracellular matrix substitutes and growth factor-enriched media, as well as specialized infrastructure and technical expertise[163]. Furthermore, limited biopsy material must be distributed among multiple diagnostic procedures, reducing the availability of tissue for functional assays. These constraints restrict PDO-based approaches to specialized research centers and limit their scalability in routine clinical practice.

Collectively, overcoming these technical and operational barriers will require coordinated efforts to standardize protocols, reduce costs, improve automation, and shorten processing timelines. Advances in microfluidics, bioprinting, and integrated screening platforms hold particular promise for bridging the gap between experimental systems and clinically actionable workflows, thereby enabling the transition of PDO-based functional testing from research settings to routine clinical decision-making.

8.2 Regulatory and ethical challenges

With the recent FDA announcement promoting organoid technology as an alternative to animal model testing, the number of organoid studies has surged. This is expected to lead to more data and resource-sharing platforms dedicated to organoid research in the future[164].

The development and application of patient-derived tumor models raise a series of ethical and regulatory challenges, primarily related to the acquisition, use, and governance of human biological materials. Organoid research relies on patient-derived tissues, necessitating strict adherence to ethical principles including informed consent, data privacy protection, and transparency regarding potential commercial applications[165-167]. However, globally unified regulatory frameworks specific to organoid research remain lacking, and existing guidelines vary significantly across countries, posing challenges for international collaboration and standardization[165]. Current ethical oversight is largely guided by established frameworks for stem cell research and biobanking, such as the International Society for Stem Cell Research (ISSCR) guidelines[166]. These emphasize donor autonomy, data security, and responsible use of biological materials. Additional considerations arise in specific contexts, including the use of gene-edited organoids, brain organoids, and human–animal chimeric systems such as PDX models, which may require more stringent ethical evaluation[167, 168]. Quality control represents another critical dimension of regulatory compliance. Standardized procedures are required throughout the entire workflow, including tissue acquisition, culture conditions, genetic stability monitoring, and post-cryopreservation validation[169]. The integration of automated platforms and artificial intelligence-based tools, such as MOrgAna and Phindr3D, has improved the efficiency and reproducibility of organoid quality assessment[170, 171]. Nevertheless, establishing universally accepted quality standards remains an ongoing challenge for clinical translation.

8.3 Clinical validation and translational barriers

Beyond technical feasibility and ethical considerations, the most critical barrier to clinical implementation is the lack of high-level evidence demonstrating that model-guided therapy improves patient outcomes. While patient-derived models have shown promising predictive performance in retrospective and early prospective studies, large-scale randomized controlled trials (RCTs) directly comparing model-guided strategies with standard-of-care treatments remain limited[44, 129].

In addition, the integration of organoid-based platforms into clinical workflows presents logistical challenges. These include coordination between tissue acquisition, model generation, data analysis, and therapeutic decision-making, all within a clinically actionable timeframe. The previously described time-window gap between model turnaround and the clinically actionable treatment interval further complicates this process, particularly for patients with rapidly progressing disease.

From a regulatory perspective, functional testing platforms face a higher evidentiary threshold compared to genomic biomarkers. Approval requires not only analytical validity but also demonstration of clinical utility, namely that their application leads to improved patient outcomes. This creates additional barriers for regulatory approval and reimbursement.

Taken together, these factors indicate that patient-derived tumor models are currently best positioned as complementary tools that enhance, rather than replace, existing clinical decision-making frameworks. Future progress will depend on the generation of high-quality prospective evidence, the development of streamlined clinical workflows, and the establishment of clear regulatory pathways for functional precision oncology.

9. Conclusions and Perspectives

Patient-derived advanced tumor models, particularly PDOs and PDXs, are redefining the conceptual framework of precision oncology. Beyond serving as preclinical tools, these models introduce a fundamentally different paradigm: the direct measurement of therapeutic response in patient-specific biological systems. In this context, functional precision oncology can be viewed as an emerging third pillar of cancer treatment, complementing genomics and clinical pathology by providing phenotype-based validation of therapeutic hypotheses.

The central advantage of patient-derived models lies in their capacity to bridge the long-standing gap between molecular alteration and drug efficacy. While genomics identifies potential vulnerabilities, it often fails to predict treatment response due to tumor heterogeneity, adaptive resistance, and microenvironmental complexity. Functional models address this limitation by enabling empirical drug testing in systems that preserve key features of patient tumors, thereby transforming precision oncology from a predominantly inference-based discipline into a validation-driven framework.

Despite rapid progress, these models have not yet reached the level of maturity required for routine clinical deployment. Their current role remains largely confined to research settings and exploratory clinical applications. Several barriers continue to impede translation, including lack of standardization, limited scalability, high economic cost, and, critically, insufficient prospective clinical validation. Moreover, biological constraints—such as incomplete reconstruction of the tumor microenvironment and clonal selection during model establishment—may affect predictive fidelity in specific contexts.

The next phase of the field will depend on overcoming these limitations through coordinated technological and clinical advances. Key priorities include the development of standardized and interoperable workflows, the integration of automated and microfluidic platforms to reduce turnaround time, and the execution of large-scale prospective trials to establish clinical utility. Equally important is the establishment of regulatory and reimbursement frameworks that enable the incorporation of functional testing into routine oncology practice.

Looking forward, the convergence of patient-derived models with multi-omics profiling and artificial intelligence is poised to further transform the field. Integrative computational approaches, including digital twin tumors and virtual organoid systems, offer the potential to unify static molecular data with dynamic functional responses, enabling real-time and patient-specific therapeutic optimization. Such frameworks may ultimately shift clinical decision-making from reactive treatment selection to predictive and adaptive intervention strategies.

In summary, patient-derived tumor models represent a critical transition in oncology—from genotype-guided inference to phenotype-validated precision medicine. While significant challenges remain, continued advances in standardization, scalability, and clinical validation are expected to establish these models as indispensable components of next-generation cancer care.

Acknowledgments

The authors acknowledge the assistance provided by Figdraw (www.figdraw.com) and Chengdu Basebiotech Biotechnology Co., Ltd. in figure illustration and data processing. This work was supported by National Health Commission of the People's Republic of China - Zhejiang Province Jointly Constructed Project, Zhejiang Province Medical and Health Science and Technology Plan (No. WKJ-ZJ-2517), and Clinical-Basic Joint Research Project of Zhejiang Provincial People's Hospital Affiliated with Hangzhou Medical College (No.C-2025-YXLH09), Shenzhen Medical Research Fund (A2503095), National Natural Science Foundation of China (82472831, 82073182), Natural Science Foundation of Shenzhen Science and Technology Innovation

Committee (JCYJ20220531093800001), Basic and Applied Basic Research Funding of Guangdong Province (2023A1515220164), Natural Science Foundation of Guangdong Province (2024A1515010330).

Author contributions

CJ, ZTT, JJY, and ZJL jointly conceived the study, performed data analysis, interpreted the data, and drafted the manuscript. RCW, JW, LBW, and DW were responsible for data analysis and interpretation. WCC, KHY, QZ, and DCF supervised the research and participated in data interpretation. All authors reviewed and revised the manuscript.

AI statement

The authors utilized ChatGPT solely for language editing and refinement, including grammatical correction, sentence structure optimization, and improvements in clarity and readability. All scientific concepts, literature interpretation, critical analyses, and conclusions were independently developed and verified by the authors. The authors have carefully reviewed and approved the final manuscript and take full responsibility for its scientific accuracy, originality, and academic integrity.

Competing Interests

The authors have declared that no competing interest exists.

References

1. Hidalgo M, Amant F, Biankin AV, Budinská E, Byrne AT, Caldas C, et al. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discov. 2014; 4: 998–1013.
2. Pauli C, Hopkins BD, Prandi D, Shaw R, Fedrizzi T, Sboner A, et al. Personalized In Vitro and In Vivo Cancer Models to Guide Precision Medicine. Cancer Discov. 2017; 7: 462–77.
3. Genta S, Coburn B, Cescon DW, Spreafico A. Patient-derived cancer models: Valuable platforms for anticancer drug testing. Front Oncol. 2022; 12: 976065.
4. Zhang W, Zheng X. Patient-derived xenografts or organoids in the discovery of traditional and self-assembled drug for tumor immunotherapy. Front Oncol. 2023; 13: 1122322.
5. Napoli GC, Figg WD, Chau CH. Functional Drug Screening in the Era of Precision Medicine. Front Med (Lausanne). 2022; 9: 912641.
6. Zanella ER, Grassi E, Trusolino L. Towards precision oncology with patient-derived xenografts. Nat Rev Clin Oncol. 2022; 19: 719–32.
7. Lau LMS, Mayoh C, Xie J, Barahona P, MacKenzie KL, Wong M, et al. In vitro and in vivo drug screens of tumor cells identify novel therapies for high-risk child cancer. EMBO Mol Med. 2022; 14: e14608.
8. Blanchard Z, Brown EA, Ghazaryan A, Welm AL. PDX models for functional precision oncology and discovery science. Nat Rev Cancer. 2025; 25: 153–66.
9. Tan D, An J, Gong M, Wang H, Li H, Meng H, et al. Screening of an individualized treatment strategy for an advanced gallbladder cancer using patient-derived tumor xenograft and organoid models. Front Oncol. 2022; 12: 1043479.
10. Hou X, Du C, Lu L, Yuan S, Zhan M, You P, et al. Opportunities and challenges of patient-derived models in cancer research: patient-derived xenografts, patient-derived organoid and patient-derived cells. World J Surg Oncol. 2022; 20: 37.
11. Idrisova KF, Simon HU, Gomzikova MO. Role of Patient-Derived Models of Cancer in Translational Oncology. Cancers (Basel). 2022; 15.
12. Liu Y, Wu W, Cai C, Zhang H, Shen H, Han Y. Patient-derived xenograft models in cancer therapy: technologies and applications. Signal Transduct Target Ther. 2023; 8: 160.
13. Yang R, Yu Y. Patient-derived organoids in translational oncology and drug screening. Cancer Lett. 2023; 562: 216180.
14. Luo M, He Y, Xie B, Li S, Gan F, Zhang S, et al. Establishment and characterization of an ovarian yolk sac tumor patient-derived xenograft model. Pediatr Surg Int. 2021; 37: 1031–40.
15. Henderson YC, Mohamed ASR, Maniakas A, Chen Y, Powell RT, Peng S, et al. A High-throughput Approach to Identify Effective Systemic Agents for the Treatment of Anaplastic Thyroid Carcinoma. J Clin Endocrinol Metab. 2021; 106: 2962–78.
16. Liu C, Qin T, Huang Y, Li Y, Chen G, Sun C. Drug screening model meets cancer organoid technology. Transl Oncol. 2020; 13: 100840.
17. Ryu S, Kim HS, Lee S, Yoon SH, Baek M, Park AY, et al. Anti-cancer drug sensitivity testing and preclinical evaluation of the anti-cancer potential of WEE1 inhibitor in triple-negative breast cancer patient-derived organoids and xenograft models. Breast Cancer Res. 2025; 27: 113.
18. Luo Z, Wang B, Luo F, Guo Y, Jiang N, Wei J, et al. Establishment of a large-scale patient-derived high-risk colorectal adenoma organoid biobank for high-throughput and high-content drug screening. BMC Med. 2023; 21: 336.
19. Maru Y, Kohno M, Suzuka K, Odaka A, Masuda M, Araki A, et al. Establishment and characterization of multiple patient-derived organoids from a case of advanced endometrial cancer. Hum Cell. 2024; 37: 840–53.
20. Lanz HL, Saleh A, Kramer B, Cairns J, Ng CP, Yu J, et al. Therapy response testing of breast cancer in a 3D high-throughput perfused microfluidic platform. BMC Cancer. 2017; 17: 709.
21. Ong LJY, Chia S, Wong SQR, Zhang X, Chua H, Loo JM, et al. A comparative study of tumour-on-chip models with patient-derived xenografts for predicting chemotherapy efficacy in colorectal cancer patients. Front Bioeng Biotechnol. 2022; 10: 952726.
22. Manasterski PJ, Danks MR, Thomson JP, Muir M, Lee M, Dawson JC, et al. Genomics-led approach to drug testing in models of undifferentiated pleomorphic sarcoma. Mol Oncol. 2025; 19: 3223–42.
23. Takada K, Aizawa Y, Sano D, Okuda R, Sekine K, Ueno Y, et al. Establishment of PDX-derived salivary adenoid cystic carcinoma cell lines using organoid culture method. Int J Cancer. 2021; 148: 193–202.
24. Kim Y, Bismeijer T, Zwart W, Wessels LFA, Vis DJ. Genomic data integration by WON-PARAFAC identifies interpretable factors for predicting drug-sensitivity in vivo. Nat Commun. 2019; 10: 5034.
25. Yu G, Fan Q. Deep learning-driven drug response prediction and mechanistic insights in cancer genomics. Sci Rep. 2025; 15: 20824.
26. Tong L, Cui W, Zhang B, Fonseca P, Zhao Q, Zhang P, et al. Patient-derived organoids in precision cancer medicine. Med. 2024; 5: 1351–77.
27. Nie X, Liang Z, Li K, Yu H, Huang Y, Ye L, et al. Novel organoid model in drug screening: Past, present, and future. Liver Res. 2021; 5: 72–8.
28. Zhou Z, Cong L, Cong X. Patient-Derived Organoids in Precision Medicine: Drug Screening, Organoid-on-a-Chip and Living Organoid Biobank. Front Oncol. 2021; 11: 762184.
29. Abdolahi S, Ghazvinian Z, Muhammadnejad S, Saleh M, Asadzadeh Aghdaei H, Baghaei K. Patient-derived xenograft (PDX) models, applications and challenges in cancer research. J Transl Med. 2022; 20: 206.
30. Lai Y, Wei X, Lin S, Qin L, Cheng L, Li P. Current status and perspectives of patient-derived xenograft models in cancer research. J Hematol Oncol. 2017; 10: 106.
31. Chen X, Shen C, Wei Z, Zhang R, Wang Y, Jiang L, et al. Patient-derived non-small cell lung cancer xenograft mirrors complex tumor heterogeneity. Cancer Biol Med. 2021; 18: 184–98.
32. Gu A, Li J, Li MY, Liu Y. Patient-derived xenograft model in cancer: establishment and applications. MedComm (2020). 2025; 6: e70059.
33. Jung J, Seol HS, Chang S. The Generation and Application of Patient-Derived Xenograft Model for Cancer Research. Cancer Res Treat. 2018; 50: 1–10.
34. Wang W, Li Y, Lin K, Wang X, Tu Y, Zhuo Z. Progress in building clinically relevant patient-derived tumor xenograft models for cancer research. Animal Model Exp Med. 2023; 6: 381–98.
35. Almstedt E, Rosén E, Gloger M, Stockgard R, Hekmati N, Koltowska K, et al. Real-time evaluation of glioblastoma growth in patient-specific zebrafish xenografts. Neuro Oncol. 2022; 24: 726–38.
36. Gatzweiler C, Ridinger J, Herter S, Gerloff XF, ElHarouni D, Berker Y, et al. Functional Therapeutic Target Validation Using Pediatric Zebrafish Xenograft Models. Cancers (Basel). 2022; 14.
37. Lipreri MV, Totaro MT, Baldini N, Avnet S. From Spheroids to Tumor-on-a-Chip for Cancer Modeling and Therapeutic Testing. Micromachines (Basel). 2025; 16.
38. Hakuno SK, Michiels E, Kuhlemaijer EB, Rooman I, Hawinkels L, Slingerland M. Multicellular Modelling of Difficult-to-Treat Gastrointestinal Cancers: Current Possibilities and Challenges. Int J Mol Sci. 2022; 23.
39. Cao J, Chan WC, Chow MSS. Use of conditional reprogramming cell, patient derived xenograft and organoid for drug screening for individualized prostate cancer therapy: Current and future perspectives (Review). Int J Oncol. 2022; 60.
40. Zeng M, Ruan Z, Tang J, Liu M, Hu C, Fan P, et al. Generation, evolution, interfering factors, applications, and challenges of patient-derived xenograft models in immunodeficient mice. Cancer Cell Int. 2023; 23: 120.
41. Zu M, Hao X, Ning J, Zhou X, Gong Y, Lang Y, et al. Patient-derived organoid culture of gastric cancer for disease modeling and drug sensitivity testing. Biomed Pharmacother. 2023; 163: 114751.
42. McDonald HG, Harper MM, Hill K, Gao A, Solomon AL, Bailey CJ, et al. Creation of EGD-Derived Gastric Cancer Organoids to Predict Treatment Responses. Cancers (Basel). 2023; 15.

43. Kim H, Jang J, Choi JH, Song JH, Lee SH, Park J, et al. Establishment of a patient-specific avatar organoid model derived from EUS-guided fine-needle biopsy for timely clinical application in pancreatic ductal adenocarcinoma (with video). Gastrointest Endosc. 2024; 100: 85–96.e9.
44. Smabers LP, Wensink GE, Verissimo CS, Doorn M, Yang T, Voskuilen T, et al. Patient-Derived Organoids Predict Treatment Response in Metastatic Colorectal Cancer. Clin Cancer Res. 2025; 31: 5015–26.
45. Lassche G, van Boxtel W, Aalders TW, van Hooij O, van Engen-van Grunsven ACH, Verhaegh GW, et al. Development and characterization of patient-derived salivary gland cancer organoid cultures. Oral Oncol. 2022; 135: 106186.
46. Chaudhary N, La Ferlita A, Choudhary BS, Jog E, Kazi M, Yahya S, et al. Patient-Derived Organoids and Xenografts Uncover Therapeutic Vulnerabilities in Colorectal Signet Ring Cell Carcinomas. Clin Cancer Res. 2025; 31: 1359–73.
47. Martínez-Quintanilla J, Cabot D, Sabia D, Arqués O, Vergés J, Chicote I, et al. Precision Oncology and Systemic Targeted Therapy in Pseudomyxoma Peritonei. Clin Cancer Res. 2024; 30: 4082–99.
48. Praharaj PP, Bhutia SK, Nagrath S, Bitting RL, Deep G. Circulating tumor cell-derived organoids: Current challenges and promises in medical research and precision medicine. Biochim Biophys Acta Rev Cancer. 2018; 1869: 117–27.
49. Xu X, Kumari R, Zhou J, Chen J, Mao B, Wang J, et al. A living biobank of matched pairs of patient-derived xenografts and organoids for cancer pharmacology. PLoS One. 2023; 18: e0279821.
50. Nikeghbal P, Zamanian D, Burke D, Steinkamp MP. Organoid models established from primary tumors and patient-derived xenograft tumors reflect platinum sensitivity of ovarian cancer patients. BMC Cancer. 2025; 25: 1459.
51. Nelson SR, Zhang C, Roche S, O'Neill F, Swan N, Luo Y, et al. Modelling of pancreatic cancer biology: transcriptomic signature for 3D PDX-derived organoids and primary cell line organoid development. Sci Rep. 2020; 10: 2778.
52. Pham NA, Radulovich N, Ibrahimov E, Martins-Filho SN, Li Q, Pintilie M, et al. Patient-derived tumor xenograft and organoid models established from resected pancreatic, duodenal and biliary cancers. Sci Rep. 2021; 11: 10619.
53. Lumibao JC, Okhovat SR, Peck KL, Lin X, Lande K, Yomtoubian S, et al. The effect of extracellular matrix on the precision medicine utility of pancreatic cancer patient-derived organoids. JCI Insight. 2024; 9.
54. Luo X, Fong ELS, Zhu C, Lin QXX, Xiong M, Li A, et al. Hydrogel-based colorectal cancer organoid co-culture models. Acta Biomater. 2021; 132: 461–72.
55. Xu R, Zhou X, Wang S, Trinkle C. Tumor organoid models in precision medicine and investigating cancer-stromal interactions. Pharmacol Ther. 2021; 218: 107668.
56. Li W, Nishino M, Reed E, Akshinthala D, Pasha HA, Anderson ES, et al. Head and neck tumor organoid grown under simplified media conditions model tumor biology and chemoradiation responses. Sci Rep. 2025; 15: 24221.
57. Minoli M, Cantore T, Hanhart D, Kiener M, Fedrizzi T, La Manna F, et al. Bladder cancer organoids as a functional system to model different disease stages and therapy response. Nat Commun. 2023; 14: 2214.
58. Hao M, Cao Z, Wang Z, Xin J, Kong B, Xu J, et al. Patient-Derived Organoid Model in the Prediction of Chemotherapeutic Drug Response in Colorectal Cancer. ACS Biomater Sci Eng. 2022; 8: 3515–25.
59. Boilève A, Cartry J, Goudarzi N, Bedja S, Mathieu JRR, Bani MA, et al. Organoids for Functional Precision Medicine in Advanced Pancreatic Cancer. Gastroenterology. 2024; 167: 961–76.e13.
60. Merrill NM, Kaffenberger SD, Bao L, Vandecan N, Goo L, Apfel A, et al. Integrative Drug Screening and Multiomic Characterization of Patient-derived Bladder Cancer Organoids Reveal Novel Molecular Correlates of Gemcitabine Response. Eur Urol. 2024; 86: 434–44.
61. Xiang K, Wang E, Mantyh J, Rupprecht G, Negrete M, Sanati G, et al. Chromatin Remodeling in Patient-Derived Colorectal Cancer Models. Adv Sci (Weinh). 2024; 11: e2303379.
62. Bruun J, Kryeziu K, Eide PW, Moosavi SH, Eilertsen IA, Langerud J, et al. Patient-Derived Organoids from Multiple Colorectal Cancer Liver Metastases Reveal Moderate Intra-patient Pharmacotranscriptomic Heterogeneity. Clin Cancer Res. 2020; 26: 4107–19.
63. Zou Z, Lin Z, Wu C, Tan J, Zhang J, Peng Y, et al. Micro-Engineered Organoid-on-a-Chip Based on Mesenchymal Stromal Cells to Predict Immunotherapy Responses of HCC Patients. Adv Sci (Weinh). 2023; 10: e2302640.
64. Lim J, Ching H, Yoon JK, Jeon NL, Kim Y. Microvascularized tumor organoids-on-chips: advancing preclinical drug screening with pathophysiological relevance. Nano Converg. 2021; 8: 12.
65. Lee J, Jung S, Hong HK, Jo H, Rhee S, Jeong YL, et al. Vascularized tissue on mesh-assisted platform (VT-MAP): a novel approach for diverse organoid size culture and tailored cancer drug response analysis. Lab Chip. 2024; 24: 2208–23.
66. Du L, Zheng Z, Zhang K, Sun H, Lu Y, Sun M, et al. Exploring personalized prediction of clinical chemotherapy efficacy and revealing tumor heterogeneity using patient-derived 3D bioprinting gastric cancer models. Mol Cancer. 2025; 24: 259.
67. Graham AJ, Khoo MWL, Srivastava V, Viragova S, Kim H, Parekh K, et al. Stress-relaxing granular bioprinting materials enable complex and uniform organoid self-organization. Nat Mater. 2026.
68. Gao M, Harper MM, Lin M, Qasem SA, Patel RA, Mardini SH, et al. Development of a Single-Cell Technique to Increase Yield and Use of Gastrointestinal Cancer Organoids for Personalized Medicine Application. J Am Coll Surg. 2021; 232: 504–14.
69. Chadwick M, Yang C, Liu L, Gamboa CM, Jara K, Lee H, et al. Rapid Processing and Drug Evaluation in Glioblastoma Patient-Derived Organoid Models with 4D Bioprinted Arrays. iScience. 2020; 23: 101365.
70. Xue Y, Wang B, Tao Y, Xia J, Yuan K, Zheng J, et al. Patient-derived organoids potentiate precision medicine in advanced clear cell renal cell carcinoma. Precis Clin Med. 2022; 5: pbac028.
71. Yang R, Wang S, Li Z, Yin C, Huang W, Huang W. Patient-derived organoid co-culture systems as next-generation models for bladder cancer stem cell research. Cancer Lett. 2025; 625: 217793.
72. Morceau F, El-Khoury V, Lee K, Berna MJ, Kwon YJ. Pancreatic Cancer Organoids: Modeling Disease and Guiding Therapy. Cancers (Basel). 2025; 17.
73. Shi J, Li Y, Jia R, Fan X. The fidelity of cancer cells in PDX models: Characteristics, mechanism and clinical significance. Int J Cancer. 2020; 146: 2078–88.
74. Okada S, Vaeteewoottacharn K, Kariya R. Application of Highly Immunocompromised Mice for the Establishment of Patient-Derived Xenograft (PDX) Models. Cells. 2019; 8.
75. Tanaka T, Nishie R, Ueda S, Miyamoto S, Hashida S, Konishi H, et al. Patient-Derived Xenograft Models in Cervical Cancer: A Systematic Review. Int J Mol Sci. 2021; 22.
76. Schuch LF, Silveira FM, Wagner VP, Borgato GB, Rocha GZ, Castilho RM, et al. Head and neck cancer patient-derived xenograft models - A systematic review. Crit Rev Oncol Hematol. 2020; 155: 103087.
77. Liu X, Xin Z, Wang K. Patient-derived xenograft model in colorectal cancer basic and translational research. Animal Model Exp Med. 2023; 6: 26–40.
78. Hoffman RM. Patient-derived orthotopic xenografts: better mimic of metastasis than subcutaneous xenografts. Nat Rev Cancer. 2015; 15: 451–2.
79. Kita K, Fukuda K, Takahashi H, Tanimoto A, Nishiyama A, Arai S, et al. Patient-derived xenograft models of non-small cell lung cancer for evaluating targeted drug sensitivity and resistance. Cancer Sci. 2019; 110: 3215–24.
80. Prasetyanti PR, van Hooff SR, van Herwaarden T, de Vries N, Kalloe K, Rodermond H, et al. Capturing colorectal cancer inter-tumor heterogeneity in patient-derived xenograft (PDX) models. Int J Cancer. 2019; 144: 366–71.
81. Yu X, Chen Y, Lu J, He K, Chen Y, Ding Y, et al. Patient-derived xenograft models for gastrointestinal tumors: A single-center retrospective study. Front Oncol. 2022; 12: 985154.
82. Castillo-Ecija H, Pascual-Pasto G, Perez-Jaume S, Resa-Pares C, Vila-Ubach M, Monterrubio C, et al. Prognostic value of patient-derived xenograft engraftment in pediatric sarcomas. J Pathol Clin Res. 2021; 7: 338–49.
83. Meraz IM, Majidi M, Meng F, Shao R, Ha MJ, Neri S, et al. An Improved Patient-Derived Xenograft Humanized Mouse Model for Evaluation of Lung Cancer Immune Responses. Cancer Immunol Res. 2019; 7: 1267–79.
84. Chiorazzi M, Martinek J, Krasnick B, Zheng Y, Robbins KJ, Qu R, et al. Autologous humanized PDX modeling for immuno-oncology recapitulates features of the human tumor microenvironment. J Immunother Cancer. 2023; 11.
85. Jin J, Yoshimura K, Sewastjanow-Silva M, Song S, Ajani JA. Challenges and Prospects of Patient-Derived Xenografts for Cancer Research. Cancers (Basel). 2023; 15.
86. Rogojina A, Klesse LJ, Butler E, Kim J, Zhang H, Xiao X, et al. Comprehensive characterization of patient-derived xenograft models of pediatric leukemia. iScience. 2023; 26: 108171.
87. Wang X, Zhu J, Li L, Zhao Q, Huang Y, Wen C, et al. Utility of patient-derived xenografts to evaluate drug sensitivity and select optimal treatments for individual non-small-cell lung cancer patients. Mol Med. 2024; 30: 209.
88. Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med. 2015; 21: 1318–25.
89. DiPeri TP, Evans KW, Scott S, Zheng X, Varadarajan K, Kwong LN, et al. Utilizing Patient-Derived Xenografts to Model Precision Oncology for Biliary Tract Cancer. Clin Cancer Res. 2025; 31: 387–402.
90. Karkampouna S, La Manna F, Benjak A, Kiener M, De Menna M, Zoni E, et al. Patient-derived xenografts and organoids model therapy response in prostate cancer. Nat Commun. 2021; 12: 1117.
91. Hynds RE, Huebner A, Pearce DR, Hill MS, Akarca AU, Moore DA, et al. Representation of genomic intratumor heterogeneity in multi-region non-small cell lung cancer patient-derived xenograft models. Nat Commun. 2024; 15: 4653.
92. Qin T, Hu Z, Zhang L, Lu F, Xiao R, Liu Y, et al. Genomic profiling of a multi-lineage and multi-passage patient-derived xenograft biobank reflects heterogeneity of ovarian cancer. Cell Rep Med. 2024; 5: 101631.
93. Tsumura R, Koga Y, Hamada A, Kuwata T, Sasaki H, Doi T, et al. Report of the use of patient-derived xenograft models in the development of anticancer drugs in Japan. Cancer Sci. 2020; 111: 3386–94.
94. Liu M, Yang X. Patient-derived xenograft models: Current status, challenges, and innovations in cancer research. Genes Dis. 2025; 12: 101520.
95. Li X, Li M. The application of zebrafish patient-derived xenograft tumor models in the development of antitumor agents. Med Res Rev. 2023; 43: 212–36.
96. Zala M, Lipinski B, Costechareyre C, Jarrosson L, Teinturier R, Julia E, et al. Functional precision oncology for follicular lymphoma with patient-derived xenograft in avian embryos. Leukemia. 2024; 38: 430–4.
97. DeBord LC, Pathak RR, Villaneuva M, Liu HC, Harrington DA, Yu W, et al. The chick chorioallantoic membrane (CAM) as a versatile patient-derived

xenograft (PDX) platform for precision medicine and preclinical research. Am J Cancer Res. 2018; 8: 1642–60.
98. Liu Y, Yang Y, An S, Wan H, Zhang M, Yin R, et al. A novel malignant mesothelioma organoids-T cell co-culture platform for personalized immunochemotherapy testing. Sci Rep. 2025; 15: 41305.
99. Fong EL, Wan X, Yang J, Morgado M, Mikos AG, Harrington DA, et al. A 3D in vitro model of patient-derived prostate cancer xenograft for controlled interrogation of in vivo tumor-stromal interactions. Biomaterials. 2016; 77: 164–72.
100. González Díaz EC, Lee AG, Sayles LC, Feria C, Sweet-Cordero EA, Yang F. A 3D Osteosarcoma Model with Bone-Mimicking Cues Reveals a Critical Role of Bone Mineral and Informs Drug Discovery. Adv Healthc Mater. 2022; 11: e2200768.
101. Jiang S, Song Y, Peng Y, Yan R, Niu Y, Chen B, et al. Patient-derived organoid-immune co-cultures integrated with multi-omics reveal immunotherapy resistance mechanisms in urothelial carcinoma. Imeta. Australia: © 2026 The Author(s). iMeta published by John Wiley & Sons Australia, Ltd on behalf of iMeta Science.; 2026. p. e70130.
102. Shao F, Wang J, Li A, Wu R, Chen J, Tuo Z, et al. Peripheral Nerve-Cancer Interactions in the Tumor Microenvironment: A Three-Dimensional Framework Integrating Mechanisms, Modulators, and Therapeutic Strategies. Research (Wash D C). 2026; 9: 1221.
103. Jiang C, Tuo Z, Wusiman D, Wang J, Yao H, Cho WC, et al. The Implications of Radiotherapy-Induced Cellular Senescence for Cancer Treatment and Tumor Microenvironment Modulation. Int J Biol Sci. 2026; 22: 3488–508.
104. Wootten S, Komarnisky RM, Ravi K, Manoharan TJM, Nikkhah M. Advancing tumor-on-a-chip technologies: a 3D-printed, PDMS-free biochip for breast cancer studies. Biofabrication. 2025; 18.
105. Zuieva A, Can S, Boelke F, Reuter S, Schattscheider S, Töpfer E, et al. Real-time monitoring of immediate drug response and adaptation upon repeated treatment in a microfluidic chip system. Arch Toxicol. Germany: © 2022. The Author(s). 2022. p. 1483–7.
106. Xiong Q, Liu T, Ying Y, Yu X, Wang Z, Gao H, et al. Establishment of bladder cancer spheroids and cultured in microfluidic platform for predicting drug response. Bioeng Transl Med. 2024; 9: e10624.
107. Moradi Kashkooli F, Mirala F, M HHT, Alirahimi M, Souri M, Golzaryan A, et al. Mechanical Forces in Tumor Growth and Treatment: Perspectives From Biology, Physics, Engineering, and Mathematical Modeling. WIREs Mech Dis. 2025; 17: e70000.
108. Stepanova D, Byrne HM, Maini PK, Alarcón T. Computational modeling of angiogenesis: The importance of cell rearrangements during vascular growth. WIREs Mech Dis. 2024; 16: e1634.
109. Mohanty B, Mohan M, Nanjappa DP, Shenoy RD, Hosmane GB, Chakraborty G, et al. Biomedical Models: Use of Zebrafish as a Multi-Utility In Vivo Tool Box. WIREs Mech Dis. 2025; 17: e70002.
110. Cui Y, Ran R, Da Y, Zhang H, Jiang M, Qi X, et al. The combination of breast cancer PDO and mini-PDX platform for drug screening and individualized treatment. J Cell Mol Med. 2024; 28: e18374.
111. Wang X, Sun Y, Xu Y, Wen D, An N, Leng X, et al. Mini-patient-derived xenograft assay based on microfluidic technology promises to be an effective tool for screening individualized chemotherapy regimens for advanced non-small cell lung cancer. Cell Biol Int. 2021; 45: 1887–96.
112. Borodovsky A, McQuiston TJ, Stetson D, Ahmed A, Whitston D, Zhang J, et al. Generation of stable PDX derived cell lines using conditional reprogramming. Mol Cancer. England; 2017. p. 177.
113. Fong ELS, Toh TB, Lin QXX, Liu Z, Hooi L, Mohd Abdul Rashid MB, et al. Generation of matched patient-derived xenograft in vitro-in vivo models using 3D macroporous hydrogels for the study of liver cancer. Biomaterials. 2018; 159: 229–40.
114. Fong EL, Martinez M, Yang J, Mikos AG, Navone NM, Harrington DA, et al. Hydrogel-based 3D model of patient-derived prostate xenograft tumors suitable for drug screening. Mol Pharm. 2014; 11: 2040–50.
115. Adine C, Fernando K, Ho NCW, Quah HS, Ho SSW, Wu KZ, et al. Bioengineered hydrogels enhance ex vivo preservation of patient-derived tumor explants for drug evaluation. Biomaterials. 2024; 305: 122460.
116. Ibrahim MS, Kim M. Artificial Intelligence-Aided Microfluidic Cell Culture Systems. Biosensors (Basel). 2025; 16.
117. Liu H, Duan W, Luo J. Predicting Single-Cell Drug Sensitivity Utilizing Adaptive Weighted Features for Multi-Source Domain Adaptation. IEEE J Biomed Health Inform. 2025; 29: 6043–54.
118. Khorsandi D, Yang JW, Foster S, Khosravi S, Hosseinzadeh Kouchehbaghi N, Zarei F, et al. Patient-Derived Organoids as Therapy Screening Platforms in Cancer Patients. Adv Healthc Mater. 2024; 13: e2302331.
119. Navarro P, Grazioso TP, Barquín A, Barba M, Yagüe M, Millán C, et al. Multicenter study correlating molecular characteristics and clinical outcomes of cancer cases with patient-derived organoids. J Exp Clin Cancer Res. 2025; 44: 182.
120. Prince E, Cruickshank J, Ba-Alawi W, Hodgson K, Haight J, Tobin C, et al. Biomimetic hydrogel supports initiation and growth of patient-derived breast tumor organoids. Nat Commun. 2022; 13: 1466.
121. Driehuis E, Kretzschmar K, Clevers H. Establishment of patient-derived cancer organoids for drug-screening applications. Nat Protoc. 2020; 15: 3380–409.
122. Mao Y, Wang W, Yang J, Zhou X, Lu Y, Gao J, et al. Drug repurposing screening and mechanism analysis based on human colorectal cancer organoids. Protein Cell. 2024; 15: 285–304.
123. Benboubker V, Ramzy GM, Jacobs S, Nowak-Sliwinska P. Challenges in validation of combination treatment strategies for CRC using patient-derived organoids. J Exp Clin Cancer Res. 2024; 43: 259.
124. Zuo J, Fang Y, Wang R, Liang S. High-throughput solutions in tumor organoids: from culture to drug screening. Stem Cells. 2025; 43.
125. Li L, Chen Y, Wang H, An G, Wu H, Huang W. A high-throughput, open-space and reusable microfluidic chip for combinational drug screening on tumor spheroids. Lab Chip. 2021; 21: 3924–32.
126. Liao J, Zhang Z, Zhu G, Liu D, Tu Z, Zhu W, et al. Dual-channel six-step linear concentration gradient microfluidic chip for orthogonal combination drug screening in head and neck tumor cells. Lab Chip. 2025; 25: 6490–503.
127. Yakavets I, Kheiri S, Cruickshank J, Hickman RJ, Rakhshani F, Aldeghi M, et al. Machine learning-assisted exploration of multidrug-drug administration regimens for organoid arrays. Sci Adv. 2025; 11: eadt1851.
128. Qin L, Wang S, Li L, Qin H. Patient-derived organoid facilitating personalized medicine in non-small cell lung cancer: two case reports. Front Oncol. 2025; 15: 1674897.
129. Wensink GE, Elias SG, Mullenders J, Koopman M, Boj SF, Kranenburg OW, et al. Patient-derived organoids as a predictive biomarker for treatment response in cancer patients. NPJ Precis Oncol. 2021; 5: 30.
130. Nie JH, Wan CY, Li H, Zhou JL, Zhong GQ, Yang T, et al. Personalized prediction of chemotherapy efficacy in osteosarcoma through patient-derived organoids: correlation with survival and tumor proliferation potential. J Exp Clin Cancer Res. 2025; 45: 16.
131. de Witte CJ, Espejo Valle-Inclan J, Hami N, Lõhmussaar K, Kopper O, Vreuls CPH, et al. Patient-Derived Ovarian Cancer Organoids Mimic Clinical Response and Exhibit Heterogeneous Inter- and Intrapatient Drug Responses. Cell Rep. 2020; 31: 107762.
132. Puca L, Bareja R, Prandi D, Shaw R, Benelli M, Karthaus WR, et al. Patient derived organoids to model rare prostate cancer phenotypes. Nat Commun. 2018; 9: 2404.
133. Singh H, Mijakovic I, Singh P. Advances in precision oncology using patient-derived organoids and functional biomaterials. Front Cell Dev Biol. 2025; 13: 1670328.
134. Tsai KK, Huang SS, Northey JJ, Liao WY, Hsu CC, Cheng LH, et al. Screening of organoids derived from patients with breast cancer implicates the repressor NCOR2 in cytotoxic stress response and antitumor immunity. Nat Cancer. 2022; 3: 734–52.
135. Li P, Huang M, Li M, Li G, Ma Y, Zhao Y, et al. Combining molecular characteristics and therapeutic analysis of PDOs predict clinical responses and guide PDAC personalized treatment. J Exp Clin Cancer Res. 2025; 44: 72.
136. Ren X, Huang M, Weng W, Xie Y, Wu Y, Zhu S, et al. Personalized drug screening in patient-derived organoids of biliary tract cancer and its clinical application. Cell Rep Med. 2023; 4: 101277.
137. Liu Z, Zhou C, Xu W, Zhang D, Zheng B, Zhang F, et al. Polyarginine Peptide R11-Actin Interaction Induces a Domino Effect on Cytoskeleton Remodeling to Suppress Bladder Cancer Metastasis. Research (Wash D C). 2026; 9: 1109.
138. Li D, Yu Q, Wu R, Yoo KH, Wusiman D, Wang J, et al. Harnessing Ribonucleoprotein Granule Biology for Cancer Therapy: The Central Role of Protein Modifications. Research (Wash D C). 2026; 9: 1133.
139. Rajinikanth N, Chauhan R, Prabakaran S. Harnessing Noncanonical Proteins for Next-Generation Drug Discovery and Diagnosis. WIREs Mech Dis. 2025; 17: e70001.
140. Steiner S, Pliego-Mendieta A, Haberecker M, Hussung S, Kollár A, Fritsch R, et al. Ex vivo modeling of acquired drug resistance in BRAF - mutated pancreatic cancer organoids uncovers individual therapeutic vulnerabilities. Cancer Lett. 2024; 584: 216650.
141. Huang Q, Wang W, Ge Q, Xu D, Dong R, Chang R, et al. NEK7-induced phosphorylation of EGFR on serine 1070 drives the acquired lenvatinib resistance in hepatocellular carcinoma. Hepatology. 2025.
142. Ye J, Xie Y, Wang J, Wu R, Li D, Yoo KH, et al. Intratumoral Androgens and Genetic Variants Driving Therapy Resistance in Prostate Cancer. Research (Wash D C). 2026; 2026: 1128.
143. Wang J, Zhang Q, Zhou C, Lyu Z, Feng D. Paraptosis Turning Cellular Stress into Therapeutic Weapon for Urological Diseases. Research (Wash D C). 2026; 9: 1156.
144. Rodriguez BN, Huang H, Chia JJ, Hoffmann A. The noncanonical NFκB pathway: Regulatory mechanisms in health and disease. WIREs Mech Dis. 2024; 16: e1646.
145. Bai L, Wu Y, Li G, Zhang W, Zhang H, Su J. AI-enabled organoids: Construction, analysis, and application. Bioact Mater. 2024; 31: 525–48.
146. Hamet P, Tremblay J. Artificial intelligence in medicine. Metabolism. 2017; 69s: S36–s40.
147. Kong J, Lee H, Kim D, Han SK, Ha D, Shin K, et al. Network-based machine learning in colorectal and bladder organoid models predicts anti-cancer drug efficacy in patients. Nat Commun. 2020; 11: 5485.
148. Zhou Y, Dai Q, Xu Y, Wu S, Cheng M, Zhao B. PharmaFormer predicts clinical drug responses through transfer learning guided by patient derived organoid. NPJ Precis Oncol. 2025; 9: 282.
149. Hu J, Zhao Y, Rao Q, Li G, Jiao Z, Wang H, et al. Combining mechanistic quantitative systems pharmacology modeling and patient-derived organoid testing in MET-aberrant non-small cell lung cancer for high-throughput combination efficacy analysis and personalized treatment design. Front Pharmacol. 2025; 16: 1685468.

150. Bai L, Su J. Artificial Intelligence Virtual Organoids (AIVOs). Bioact Mater. 2026; 59: 45–68.
151. Asghar US, Chung C. Application of digital twins for personalized oncology. Nat Rev Cancer. 2025; 25: 823–5.
152. He S, Zhu Y, Tavakol DN, Ye H, Lao YH, Zhu Z, et al. Squidiff: predicting cellular development and responses to perturbations using a diffusion model. Nat Methods. 2026; 23: 65–77.
153. Xia P, Shuang S, Fu D, Liu L, Yang D, Guo Y, et al. Large-scale single-cell analysis and in silico perturbation reveal dynamic evolution of HCC: from initiation to therapeutic targeting. NPJ Precis Oncol. 2026; 10.
154. Wang Y, Bergman DR, Trujillo E, Fernald AA, Li L, Pearson AT, et al. Agent-Based Modeling of Virtual Tumors Reveals the Critical Influence of Microenvironmental Complexity on Immunotherapy Efficacy. Cancers (Basel). 2024; 16.
155. Xu J, Yang X, Li Y, Wang H, Li Y, Tang S, et al. ODFormer: a Virtual Organoid for Predicting Personalized Therapeutic Responses in Pancreatic Cancer. bioRxiv. 2025: 2025.07.08.663664.
156. De Domenico M, Allegri L, Caldarelli G, d'Andrea V, Di Camillo B, Rocha LM, et al. Challenges and opportunities for digital twins in precision medicine from a complex systems perspective. NPJ Digit Med. 2025; 8: 37.
157. Vlachogiannis G, Hedayat S, Vatsiou A, Jamin Y, Fernández-Mateos J, Khan K, et al. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. Science. 2018; 359: 920–6.
158. Jiang KL, Wang XX, Liu XJ, Guo LK, Chen YQ, Jia QL, et al. Success rate of current human-derived gastric cancer organoids establishment and influencing factors: A systematic review and meta-analysis. World J Gastrointest Oncol. 2024; 16: 1626–46.
159. Kang S, Lee MR, Choi W, Kong SY, Kim YH. Protocol for generation and utilization of patient-derived organoids from multimodal specimen. STAR Protoc. 2025; 6: 104039.
160. Devasahayam Arokia Balaya R, Heydari Z, Sarkar G, Cruz Garcia EM, de Hoyos-Vega JM, Krueger E, et al. A Practical Guide to Developing and Troubleshooting Patient-Derived "Mini-Gut" Colorectal Organoids for Clinical Research. Methods Protoc. 2025; 8.
161. Jiang S, Zhao H, Zhang W, Wang J, Liu Y, Cao Y, et al. An Automated Organoid Platform with Inter-organoid Homogeneity and Inter-patient Heterogeneity. Cell Rep Med. 2020; 1: 100161.
162. Ding S, Hsu C, Wang Z, Natesh NR, Millen R, Negrete M, et al. Patient-derived micro-organospheres enable clinical precision oncology. Cell Stem Cell. 2022; 29: 905–17.e6.
163. Zhao Z, Chen X, Dowbaj AM, Sljukic A, Bratlie K, Lin L, et al. Organoids. Nat Rev Methods Primers. 2022; 2.
164. Carratt SA, Zuch de Zafra CL, Oziolor E, Rana P, Vansell NR, Mangipudy R, et al. An industry perspective on the FDA Modernization Act 2.0/3.0: potential next steps for sponsors to reduce animal use in drug development. Toxicol Sci. 2025; 203: 28–34.
165. Zhou LB, Lei YT, Han XX. Charting bioethical frontiers: China's human organoid guidelines in a global context. Mil Med Res. England; 2025. p. 64.
166. Lovell-Badge R, Anthony E, Barker RA, Bubela T, Brivanlou AH, Carpenter M, et al. ISSCR Guidelines for Stem Cell Research and Clinical Translation: The 2021 update. Stem Cell Reports. 2021; 16: 1398–408.
167. Mollaki V. Ethical Challenges in Organoid Use. BioTech (Basel). 2021; 10.
168. Hartung T, Morales Pantoja IE, Smirnova L. Brain organoids and organoid intelligence from ethical, legal, and social points of view. Front Artif Intell. 2023; 6: 1307613.
169. Lee H, Yang S, Lee KJ, Kim SN, Jeong JS, Kim KY, et al. Standardization and quality assessment for human intestinal organoids. Front Cell Dev Biol. 2024; 12: 1383893.
170. Gritti N, Lim JL, Anlaş K, Pandya M, Aalderink G, Martínez-Ara G, et al. MOrgAna: accessible quantitative analysis of organoids with machine learning. Development. 2021; 148.
171. Mergenthaler P, Hariharan S, Pemberton JM, Lourenco C, Penn LZ, Andrews DW. Rapid 3D phenotypic analysis of neurons and organoids using data-driven cell segmentation-free machine learning. PLoS Comput Biol. 2021; 17: e1008630.