

Review

Organoid-on-Chip Technologies in Precision Oncology: Bridging Patient-Specific Tumor Biology and Physiologically Relevant Drug Screening

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Abstract

The inadequacy of traditional preclinical oncology models, specifically two-dimensional (2D) monolayer cultures and murine in vivo systems, in predicting human drug responses has led to the development of patient-derived organoids (PDOs) and microfluidic organ-on-chip (OoC) technologies. These innovations represent significant recent methodological advancements in the field of cancer research. This review synthesizes the biological rationale, technical principles, and translational applications of PDO–OoC integration, with an emphasis on recent clinical validation studies, AI integration, and post-FDA Modernization Act 2.0 regulatory evolution—areas that have not been comprehensively addressed in prior reviews. We examined the predictive limitations of 2D models, organoid generation, and ToC engineering principles. The synergistic integration of organoids into chip-based systems, extended into multi-organ “Body-on-a-Chip” architectures, is presented as a unifying framework that combines patient-specific biological fidelity with dynamic microenvironmental control. We further reviewed the research applications and early clinical validation studies of high-throughput drug screening, immuno-oncology modeling, and patient-specific drug response prediction across multiple tumor types. Clinical validation studies have reported moderate correlations ($r \sim 0.4$ – 0.6) between organoid responses and outcomes, indicating partial predictive capacity. Despite this progress, clinical translation remains constrained by standardization and reproducibility deficits, biomaterial limitations (e.g., PDMS drug absorption and Matrigel batch variability), and regulatory ambiguities within the evolving FDA Modernization Act 2.0. Finally, we discuss the emerging integration of artificial intelligence, including transfer learning-based drug response prediction and real-time organoid avatar systems in clinical trials, as a pathway toward closed-loop individualized functional precision oncology. Organoid and tumor-on-chip platforms have advanced toward clinical utility, although barriers remain.



Academic Editor: Süleyman Ergün

Received: 12 August 2026

Revised: 4 September 2026

Accepted: 8 September 2026

Published: 11 September 2026

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Keywords: organoids; microphysiological systems; precision medicine; drug screening assays; antitumor; neoplasms

1. Introduction

Preclinical cancer research has long balanced biological fidelity and experimental tractability. For decades, 2D monolayer cultures and murine models have dominated, yielding basic insights but poorly predicting human outcomes and fueling high attrition rates in oncology. The rise of 3D culture and, most recently, the union of patient-derived

organoids (PDOs) with microfluidic organ-on-chip (OoC) platforms marks a highly consequential advancement in preclinical modeling. This review outlines the biological rationale, technical foundations, and translational potential of PDO–OoC systems for personalized drug screening and precision oncology research. This review addresses (1) the clinical predictive validity of PDO and ToC platforms, (2) barriers to translation, and (3) how AI accelerates adoption. This article is a focused narrative review, not a systematic review or meta-analysis. We used an iterative, topic-focused selection process aligned with the review objectives, prioritizing peer-reviewed studies published from 2020 to 2026 and retaining earlier seminal studies when needed for foundational context. We included studies evaluating patient-derived organoids (PDOs), tumor-on-chip (ToC), organoid-on-chip (OoC), or related three-dimensional/microphysiological systems; addressing drug screening, pharmacotyping, immuno-oncology, drug resistance, metastasis, biomarker discovery, pharmacokinetics or toxicity, artificial intelligence/machine learning, or regulatory translation; or reporting relevant original data, clinical validation, or methodological synthesis. We excluded irrelevant studies, non-informative engineering reports, duplicates, and studies with uninterpretable models or outcomes. Relevant official regulatory or governmental documents were retained. Selection was based on relevance, methodological or clinical validation, recency, and conceptual contribution, without formal risk-of-bias scoring. The synthesis reflects author judgment and is not exhaustive.

1.1. The Evolution of Preclinical Cancer Models: From 2D Monolayers to Complex 3D Systems

2D monolayers, established mid-century, became central for their simplicity, scalability, and cost, enabling pathway dissection and high-throughput screening with lines such as HeLa, MCF-7, and HCT116 cells. However, cells forced into a flat geometry alter their polarity, receptor expression, cytoskeleton, and mechanotransduction, and lack ECM interactions essential for proliferation, migration, and survival signaling [1]. They also omit oxygen/nutrient/waste gradients that create hypoxia and metabolic heterogeneity, driving drug resistance and cancer stemness in the tumor microenvironment (TME) [2].

Consequently, 2D screens frequently overestimate efficacy. A 3D tumor array model found that 17.6% of drugs appeared active in 2D but were resistant in 3D and eliminated 95% of 2D false positives versus patient-derived xenograft (PDX) benchmarks [3]. In colorectal cancer models, the IC₅₀ values for 5-fluorouracil, oxaliplatin, and irinotecan were markedly higher in 3D bioprinted systems than in 2D systems (31.13 μ M vs. 12.79 μ M; 26.79 μ M vs. 0.80 μ M; 16.73 μ M vs. 10.45 μ M) [4].

Three-dimensional systems, including spheroids, scaffolded cultures, and organoids, mitigate these deficiencies by re-establishing spatial organization, cell–cell and extracellular matrix interactions, and microenvironmental gradients [5]. Spheroids develop necrotic cores, hypoxic zones, and proliferative rims that mirror avascular tumor regions and clinical resistance. Organoids derived from stem cells or patient tumors preserve the architecture, histological, mutational, phenotypic, and intratumoral heterogeneity of source tissues [6].

The transition from two-dimensional (2D) to three-dimensional (3D) platforms is conceptually necessary. Two-dimensional platforms identify agents that are effective against rapidly dividing cells in unstructured, nutrient-rich environments that do not accurately represent human tumors, resulting in false positives. The adoption of 3D models is a prerequisite for improving their translational value. To anchor this conceptual transition in recent tumour-focused evidence, Table 1 summarizes representative primary studies published from 2021 to 2025.

Table 1. Representative recent tumour-focused studies using in vitro, ex vivo, and in vivo models for organoid-based and tumour-on-chip drug evaluation.

Model Type (2D/3D; Setting)	Source	Target Tumour/Organ	No. of Samples (Humans/Patients/ Technical Replicates)	Drug Used	Advantages of the Model	Limitations of the Model	Reference
In vitro studies							
3D; in vitro (PDO–microwell array)	Human lung tumour biopsies/resections; patient-derived organoids	Lung cancer	103 surgical specimens for establishment; 142 patient samples in validation cohorts; technical repeats NR	Gefitinib, afatinib, crizotinib, gemcitabine, cisplatin, pemetrexed ± combinations	Microwell-array multiplexing; rapid (<1 week) drug testing; histological/genetic fidelity; concordance with PDX and clinical response	Mainly epithelial PDOs; limited immune, stromal, vascular and perfusion context; establishment failure in a subset	[7]
3D; in vitro (microfluidic tumour-on-chip)	MCF7/NK-92/HUVEC cell system plus one patient-derived invasive ductal breast cancer line	Breast cancer; NK-cell–tumour interaction	Patient-derived line n = 1; cell-line controls; technical repeats NR	Atezolizumab, epacadostat; nutrient/pH/metabolic stress	Perfusion and controllable stress gradients; enables immune exhaustion and response readouts	Small donor number; simplified immune/TME composition; no full systemic PK or tumour heterogeneity	[8]
3D; in vitro (array-chip tumour model)	27 human cancer cell lines	Multiple tumour types	27 cell lines; replicate number not consistently reported	18 cytotoxic agents and 20 targeted agents	High-throughput, low-volume screening; CV <10%, Z' >0.7; reduced 2D false positives against PDX benchmark	Immortalized lines omit patient heterogeneity, native stroma and immunity; limited clinical generalizability	[3]
3D; in vitro (patient-derived pancreatic tumour-on-chip)	Primary pancreatic cancer cells/PDOs from one patient; human PSCs; U937 macrophage/monocyte cells	Pancreatic ductal adenocarcinoma	Patient n = 1; n = 5 chips/group for drug assay	Gemcitabine ± ATRA and liposomal clodronate (Clodrosome®)	Perfused multicellular TME; stromal and myeloid components; tests microenvironment-modulating combinations	Single-patient experiment; non-matched stromal cell lines; organoid spreading into a 2D layer with prolonged culture	[9]
3D; in vitro (intravasation-on-chip)	A549/NCI-H1975/BEAS-2B; HUVEC; THP-1-derived macrophages	Lung cancer intravasation	Cell-line models; biological/technical repeats NR	No anti-cancer drug; TGF-β1 and macrophage-conditioned medium as EMT/invasion stimuli	Direct visualization of intravasation; automated machine-learning quantification; rapid assay	No patient tissue or systemic circulation; not a validated patient-specific drug-response model	[10]

Table 1. Cont.

Model Type (2D/3D; Setting)	Source	Target Tumour/Organ	No. of Samples (Humans/Patients/ Technical Replicates)	Drug Used	Advantages of the Model	Limitations of the Model	Reference
3D; in vitro (bioprinted vascularized GBM-on-chip)	A172 glioblastoma cells; hCMEC/D3 and HUVEC endothelial cells	Glioblastoma/brain tumour	Cell-line model; technical repeats NR	No anti-cancer drug tested; simulated microgravity challenge	Vascularized, perfused brain-tumour architecture; models BBB-relevant transport and physical cues	Immortalized cells and simplified TME; no patient heterogeneity or systematic pharmacology benchmark	[11]
Ex vivo studies							
3D; ex vivo (PDO in biomimetic hydrogel)	Two primary patient-derived breast PDO lines plus one PDX-derived organoid line	Breast cancer	Three organoid lines; N = 100 spheroids across four repeated growth experiments; drug replicate structure assay-specific	Paclitaxel, eribulin, carboplatin, doxorubicin	Tunable stiffness; lower batch variability; improved flow stability vs. BME; preserves histology and drug response	Hydrogel crosslink degradation over time; reduced ER/PR expression vs. parental tissue; small line set	[12]
3D; ex vivo (PDO pharmacotyping)	Patient-derived pancreatic organoids from resection/ EUS-FNB samples	Pancreatic ductal adenocarcinoma	322 patients/samples across retrospective, pilot and prospective cohorts; four pharmacotyping replicates; IC50 measured in triplicate	FOLFIRINOX components, gemcitabine, nab-paclitaxel	Clinical-response predictive pharmacotyping; supports functional precision oncology across sample types	Organoid expansion can take weeks; possible normal ductal overgrowth; standard PDOs lack native TME	[13]
3D; ex vivo (droplet micro-organospheres)	Low-input patient-derived metastatic CRC and lung cancer biopsies	Metastatic colorectal and lung cancer	Pilot n = 8 patients; three experimental repeats	Oxaliplatin, irinotecan, nivolumab, ESK1 and 119 FDA-approved agents	Rapid low-input format (<14 days); supports multiplexed testing and retains selected stromal/immune features	Small pilot cohort; incomplete representation of native TME; limited prospective clinical validation	[14]
3D; ex vivo (paired PDO biobank)	Primary CRC and paired liver-metastasis tissues	Colorectal cancer with liver metastases	72 samples from 36 patients; 58 cultures successful; 50 PDOs from 25 patients analyzed; three independent dose-response experiments	5-FU, irinotecan/CPT11, oxaliplatin, FOLFOX, FOLFIRI	Captures intra/interpatient heterogeneity; paired primary/metastatic comparison; correlates with clinical chemotherapy response	No normal controls or native TME; potential culture selection and long-term phenotypic drift	[15]

Table 1. Cont.

Model Type (2D/3D; Setting)	Source	Target Tumour/Organ	No. of Samples (Humans/Patients/ Technical Replicates)	Drug Used	Advantages of the Model	Limitations of the Model	Reference
3D; ex vivo (organotypic tumour slice culture)	Fresh human colorectal cancer liver-metastasis slices with endogenous immune/stromal cells	CRC liver metastasis	n = 38 patient tumours; multiple slices/ treatment conditions; technical repeats NR	Anti-IL-10 antibody ± CEA-specific CAR-T cells	Preserves native 3D architecture, immune infiltrate and myeloid/T-cell interactions; measures treatment-induced cell death	Short ex vivo lifespan; no systemic circulation, pharmacokinetics or whole-organ response; tissue access is limited	[16]
3D; ex vivo (gastric PDO drug screen)	Patient-derived gastric cancer organoids from surgical tumour tissues	Gastric cancer	57 PDOs from 73 patients; 41 PDOs in six-drug screen; five concentrations in triplicate; 12 patients for clinical comparison	5-FU, oxaliplatin, cisplatin, paclitaxel, SN-38, doxorubicin	Large biobank; multi-drug response heterogeneity; PDOX and clinical concordance (11/12 patients)	Epithelial-only standard PDO context; 78% establishment rate; immune/stromal components require additional co-culture	[17]
In vivo studies							
3D; in vivo (matched PDX/PDxO validation)	Human primary/metastatic breast tumours; immunodeficient mouse PDX and PDX-derived organoids	Breast cancer	152 PDX engraftment attempts (102 primary, 50 metastatic); 16 PDxO lines ×45 compounds; usually n = 3 treated and n = 6 vehicle mice for validation	Eribulin, talazoparib, docetaxel, navitoclax, fulvestrant	Matched in vitro–in vivo platform; tests drug response and resistance; includes clinical precision-oncology case	Immunodeficient host; low ER+ engraftment; expensive and low throughput; murine stroma replaces human stroma over time	[18]
3D; in vivo (PDO/ODX and PDX comparison)	Patient-derived primary liver cancer organoids and xenografts	Primary liver cancer	52 PDO lines from 153 patients; ODX/PDX mouse numbers are experiment-specific and NR in the accessible main text	Sorafenib, regorafenib, lenvatinib and RTP combination	Preserves histopathology; compares inherent/ acquired resistance across PDO and ODX; identifies in vitro–in vivo discordance	Aggressive-cell enrichment and culture selection; immunodeficient mice; limited systemic immune context	[19]
3D; in vivo (matched tumour-on-chip/PDX validation)	PDX-derived CRC tumour spheroids from three patient-specific PDX models	Colorectal cancer	Three patients; 32-chip array with four repeats per concentration; PDX animal n not stated consistently	5-FU, oxaliplatin, irinotecan, 5-FU + oxaliplatin, 5-FU + irinotecan	Directly compares short-term perfused chip predictions with matched PDX efficacy; supports patient-specific ranking of regimens	PDX-derived rather than fresh patient tissue; immunodeficient PDX; small patient cohort and limited human TME	[20]

Table 1. Cont.

Model Type (2D/3D; Setting)	Source	Target Tumour/Organ	No. of Samples (Humans/Patients/ Technical Replicates)	Drug Used	Advantages of the Model	Limitations of the Model	Reference
3D; in vivo (large PLC organoid biobank with ODX/PDOX validation)	Multi-region patient-derived primary liver cancer organoids and xenografts	Primary liver cancer	399 tumour organoids from 144 patients; ODX/PDOX animal counts are experiment-specific and NR in the abstract/main summary	Lenvatinib, sorafenib, regorafenib and other clinically relevant agents; PKUF-01 combination candidate	Large multi-region resource; resolves intra-tumour heterogeneity; integrates pharmacogenomics, in vivo validation and patient response	Animal models lack human immunity and perfusion; multi-region culture and pharmacogenomic workflows are technically demanding	[21]

Abbreviations: BME, basement-membrane extract; CAR-T, chimeric antigen receptor T cell; CRC, colorectal cancer; GBM, glioblastoma; HUVEC, human umbilical vein endothelial cell; NR, not reported; ODX, organoid-derived xenograft; PDO, patient-derived organoid; PDxO, PDX-derived organoid; PDX, patient-derived xenograft; PLC, primary liver cancer; PSC, pancreatic stellate cell; TME, tumour microenvironment.

1.2. Limitations of Current In Vivo Models in Predicting Clinical Outcomes

Despite their regulatory status, animal models poorly predict the outcomes in humans. Between 2003 and 2014, the approval rates for oncology drugs were approximately 10.4%; however, this figure declined to 6–7% from 2011 to 2017 [22]. Notably, approximately 60% of trial failures were attributed to insufficient efficacy, whereas approximately 30% were attributed to unforeseen toxicity. These outcomes are precisely the issues that preclinical in vivo tests are designed to predict [22].

Predictive failures arise from interspecies variations in physiology, immunology, pharmacokinetics and tumor biology. Specifically, differences in murine innate populations, checkpoint expression, and tumor microenvironment cytokines from those in humans compromise the validity of immunotherapy studies conducted using these models [1]. Humanized mice partly mitigate this, but introduce graft-versus-host disease, incomplete immune reconstitution, and a lack of a human thymic niche [23]. Patient-derived xenografts (PDX) better preserve tumor biology but face 30–60% engraftment rates (lower in some cancers), high costs, and 3–6-month timelines incompatible with clinical decisions [24]. Progressive murine stromal replacement alters the TME and can shift drug responses. Standard PDX models lack functional human immunity, limiting immunotherapy evaluation [23].

The development of a single oncology drug incurs ethical and economic costs, with expenses surpassing \$2.5 billion. A significant portion of this expenditure is allocated to trials for candidates who have progressed from insufficient models to more advanced models. While animal studies provide indispensable systemic context, their poor human predictability demands complementary—and in some settings replacement—human-relevant alternatives.

1.3. Defining the Scope: The Convergence of Patient-Derived Organoids and Microfluidic Organ-on-Chip Technologies

Converging patient-derived organoids (PDOs) with microfluidic organ-on-chip (OoC) platforms is a promising approach to address these gaps. PDOs, derived directly from tumors, retain patient-specific mutations, epigenetics, transcriptional programs, phenotypes, and 3D histologic architecture, enabling the prediction of individual responses to chemotherapy and targeted therapies [25].

Although static PDO cultures capture biological fidelity, they do not replicate dynamic in vivo cues, such as shear stress, interstitial pressure, cyclic strain, and organized vascular and stromal structures. Additionally, these cultures apply drugs at constant concentrations, which differ from the variable pharmacokinetics observed in vivo [26]. Microfluidic organ-on-a-chip (OoC) devices engineer perfused, mechanically active, and structured microenvironments at sub-millimeter resolution [27]. Integrating PDOs yields tumor-on-a-chip systems that model angiogenesis, intravasation/extravasation, immune trafficking, and perfusion-driven pharmacokinetics [28]. Multi-organ Body-on-a-Chip platforms fluidically link tumors and other organoids (liver, kidney, heart, and lung) to evaluate efficacy, systemic toxicity, and combination regimens [29].

By uniting patient-specific biology with biophysical realism, PDO–OoC integration moves toward true patient avatars that capture genetic identity and dynamic context. Its potential to guide therapy selection prospectively is substantial. Figure 1 synthesizes this progression, illustrating how patient-derived organoids integrate the architectural complexity of 3D systems with patient-specific mutations, ECM networks, and intratumoral heterogeneity, achieving superior drug response prediction accuracy (~80%) compared to conventional 2D (~40%) and 3D spheroid (~60%) models.

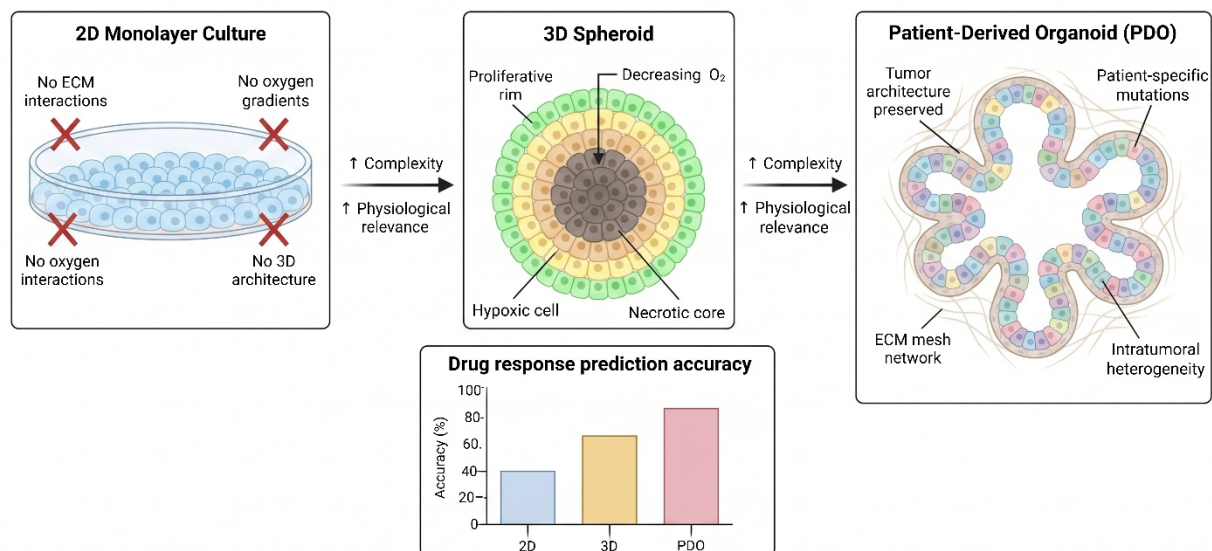


Figure 1. Patient-derived organoids integrate 3D complexity with patient-specific biology to achieve superior drug response prediction accuracy. Left panel: Two-dimensional (2D) monolayer cultures lack essential microenvironmental features, including extracellular matrix (ECM) interactions, oxygen gradients, and three-dimensional architecture. Center-left panel: Three-dimensional (3D) spheroids recapitulate spatial organization with proliferative rim, hypoxic zones, and necrotic core, increasing physiological complexity. Right panel: Patient-derived organoids (PDOs) preserve tumor architecture, patient-specific mutations, ECM mesh networks, and intratumoral heterogeneity, achieving the highest biological fidelity. Bottom panel: Comparative bar graph demonstrating drug response prediction accuracy across model systems. PDOs achieve the highest accuracy (~80%), substantially outperforming 3D spheroids (~60%) and 2D monolayer cultures (~40%), underscoring the superior predictive capacity of PDO systems for guiding clinical therapy selection.

2. Tumor Organoids in Translational Oncology

Patient-derived organoids (PDOs) have rapidly evolved from a mere curiosity to a clinically applicable tool. They maintain tumor histology, genomics, and drug sensitivity while permitting expansion, cryo-storage, and manipulation. Therefore, they represent the most accurate in vitro cancer models available [30]. This fidelity enables drug testing, biomarker discovery, mechanistic studies, and prospective personalized therapy guidance. We summarize PDO generation, biobanking, high-throughput screening (HTS), and co-culture strategies that recapitulate the tumor microenvironment (TME).

2.1. Methods of Generation: Patient-Derived Organoids from Biopsies and Resections

PDOs are established from viable tumor tissues obtained via resection or minimally invasive biopsy. Robust small biopsy protocols are crucial because most candidates have advanced, unresectable disease [30].

Standard workflows include dissociation into single cells/small clusters, ECM embedding, and culturing in media with niche-mimicking growth factors and inhibitors. Matrigel (basement membrane extract, BME) remains the dominant ECM but its animal-derived, batch-variable biochemical and mechanical properties complicate reproducibility and drug-response assays [31]. Defined alternatives, such as synthetic hydrogels, natural biomaterials, and recombinant scaffolds, improve compositional control and mechanical tunability of these materials [32].

Prince et al. (2022) showed that breast cancer PDOs thrive in a tunable nanofibrillar hydrogel (EKGel), matching BME in histopathology, transcriptomics, and drug response, while reducing batch variability and murine stromal contamination in PDX-derived cultures [12]. Lumibao et al. (2023) found BME source modulated PDAC organoid proliferation

but not drug responses or gene expression, supporting pharmacotyping robustness across matrices [33]. Transitioning to fully defined Matrigel-free systems is crucial for Good Manufacturing Practice (GMP)-compatible clinical standardization.

Growth media are organ-specific; colorectal and pancreatic PDOs often require Wnt3a/Wnt-conditioned medium, R-spondin-1, Noggin, EGF, and TGF- β /Notch inhibitors, with a composition empirically optimized by tumor type and sometimes per patient, adding complexity and variability to the success of establishment [34].

Establishment rates vary by cancer type, sample quality, and processing; colorectal cancer 70–90% in experienced centers; pancreatic ductal adenocarcinoma (PDAC) 40–60% owing to desmoplasia and low tumor cellularity [13]. EUS-FNB-derived PDAC “avatar” organoids can be generated within clinically actionable timelines to guide therapy for unresectable diseases [35]. In gastric cancer, optimized tissue sampling and media/ECM conditions increased organoid success from 25% to 88%, highlighting the value of protocol refinement for heterogeneous ECM preferences [36].

Validation encompasses histology, genomics, and transcriptomics. Kim et al. (2025) demonstrated that 3D organoids, derived from conditionally reprogrammed pancreatic cancer cells of patients, maintained the molecular profiles of the original cells [35]. These organoids displayed stage- and differentiation-associated morphologies and provided more precise predictions of clinical responses to gemcitabine/nab-paclitaxel and FOLFIRINOX than did 2D cultures. The increased IC₅₀ values were indicative of the 3D structure and penetration barriers [37].

Biopsy-compatible, Matrigel-free, and optimized protocols are prerequisites for clinical-grade standardization and regulatory approval. The next steps include protocol harmonization and rigorous QC to enable reproducible, multicenter, clinical deployment.

2.2. Biobanking: High-Throughput Screening Capabilities and Biobudgeting Challenges

Serial expansion and cryopreservation enable living tumor organoid biobanks that retain patient-specific functional properties, including drug sensitivity, unlike conventional fixed or frozen tissue repositories [38].

Large-scale PDO screens have discovered therapeutics, exemplified by the identification of MCLA-158 (EGFR $\times$ LGR5), which eliminates cancer stem cells, is active in KRAS-mutant colorectal cancer refractory to EGFR therapy, and suppresses metastatic outgrowth in epithelial tumors [39].

Prospective studies link PDO drug sensitivity with clinical outcomes: in 232 metastatic colorectal cancer patients, PDO responses correlated with biopsied and total target-lesion responses ($r = 0.41$ – 0.49 , $p < 0.011$; $r = 0.54$ – 0.60 , $p < 0.001$), and 5-FU/oxaliplatin screens showed high PPV/NPV and AUC, with associations to PFS and OS [40]. In PDAC, PDO pharmacotyping of FOLFIRINOX components predicted neoadjuvant responses, aligning with CA-19-9 declines and RECIST imaging [13]. By combining PDO drug response data with proteotranscriptomics, it was found that oxaliplatin non-responders showed a significant reliance on tRNA aminoacylation and oxidative phosphorylation. In contrast, an outstanding response to palbociclib was linked to MYC activation and increased TRiC chaperonin levels [41].

AI further extends utility: PharmaFormer, a Transformer with transfer learning from 2D cell lines fine-tuned on PDO data, markedly improved clinical drug response prediction versus cell line-only models [42].

Implementation hurdles include labor intensity, specialized expertise, and high costs of growth factors and BME. Maintaining a single PDO line can cost hundreds to thousands of dollars per month, depending on the tumor type and HTS scale [38]. Long-term passaging

risks genomic drift that can alter drug sensitivities, necessitating standardized genomic QC (e.g., periodic WES or SNP arrays) to preserve primary tumor fidelity [34].

Living biobanks function as renewable libraries of human diseases with compelling clinical and predictive value. However, equitable adoption requires cost-effective, standardized, and automated culture platforms to bridge these resource gaps.

2.3. Recapitulating the Tumor Microenvironment: Co-Culture with Immune Cells and Fibroblasts

Standard PDOs contain epithelial cancer cells but lack stromal and immune elements critical for tumor progression, therapy resistance, and immunotherapy response; therefore, faithfully modeling the immunosuppressive TME in vitro is essential [43]. Table 2 systematically summarizes the five major cellular components integrated into PDO co-culture systems, detailing their primary functions in the native tumor microenvironment and key findings from PDO co-culture studies. Cancer-associated fibroblasts confer chemotherapy resistance through immune modulation, tumor-associated macrophages determine pro-versus anti-tumor function via M1/M2 polarization, tumor-infiltrating lymphocytes enable patient-specific evaluation of checkpoint inhibitor and CAR-T efficacy, peripheral blood mononuclear cells provide an accessible source for autologous immune co-culture, and endothelial cells facilitate drug delivery and immune cell trafficking. This integrated multi-cellular approach enables comprehensive modeling of tumor-immune-stromal interactions that drive therapeutic resistance and immunotherapy response.

Table 2. Cellular components of tumor microenvironment co-culture systems in patient-derived organoid models: functions and key findings.

Co-Culture Component	Primary Function in TME	Key Findings in PDO Co-Culture Models	Reference
Cancer-Associated Fibroblasts (CAFs)	ECM remodeling, paracrine growth factor secretion, immunomodulation	Confer chemotherapy resistance; shape myeloid responses to chemotherapy-induced immunogenic signals; regulate organoid drug response by shifting stem phenotypes and protecting against chemotherapy	[44–46]
Tumor-Associated Macrophages (TAMs)	Immunosuppression, pro-tumorigenic cytokine secretion, phagocytosis	Promote tumor growth and therapy resistance; M1/M2 polarization determines pro- vs. anti-tumor function; essential for modeling immunosuppressive TME	[9,47,48]
Tumor-Infiltrating Lymphocytes (TILs)	Anti-tumor cytotoxicity, immune surveillance	Enable patient-specific evaluation of ICI and CAR-T cell efficacy in 3D context; live imaging reveals functional T-cell subsets including ‘super engagers’ informing CAR engineering	[48–50]
Peripheral Blood Mononuclear Cells (PBMCs)	Systemic immune response, NK and T cell cytotoxicity	Provide accessible source for autologous immune co-culture; enable personalized immunotherapy testing; allow study of checkpoint inhibitor responses	[8,47,51,52]
Endothelial Cells	Vascular barrier, angiogenesis, immune cell trafficking	Recapitulate tumor vasculature; required for modeling drug delivery and immune cell extravasation; enable study of transendothelial migration and vascular-channel delivery of immune cells	[37,51,53]

Cancer-associated fibroblasts (CAFs) dominate many solid tumors (notably PDAC and colorectal tumors), drive desmoplasia, impede drug delivery, and sustain tumor growth via

paracrine signals [44]. Co-culture with patient-matched CAFs reproduces mesenchymal-like stroma (fibronectin, collagen I, and TGF- β) and induces chemoresistance, which is absent in monocultures [44]. CAFs also shape early myeloid responses to chemotherapy-induced immunogenic signals, revealing their immunomodulatory roles relevant to chemo-immunotherapy design [45].

Immunocompetent models use co-culture with autologous PBMCs or TILs to study patient-specific tumor–immune interactions and checkpoint inhibitor responses [54]. Air–liquid interface cultures preserve native stromal and immune infiltrates during the initial culture, maintaining the aspects of the in situ TME [43]. Patient-derived organoid platforms have dissected iNKT–TME interactions, including CD1d-dependent recognition, α -GalCer-driven Th1 skewing, and metabolic modulation of iNKT activity, underscoring their mechanistic value in immunotherapy development [53].

A central challenge is media incompatibility: organoid media favor epithelial maintenance but impair immune viability/function, whereas immune media lack ECM/niche cues for organoids. Solutions include media optimization and spatially organized co-culture platforms, often integrated with microfluidic systems to permit shared blood circulation in the chip.

Given the centrality of the TME to immunotherapy efficacy, developing patient-derived, immunocompetent organoids that capture the full cellular complexity is both feasible and imminent, and is poised to transform personalized immunotherapy. Figure 2 synthesizes the complete PDO workflow, illustrating the integrated pathway from patient biopsy through tissue dissociation, ECM embedding with organ-specific growth factors, temporal organoid growth and maturation (Days 1–14), comprehensive molecular and histological validation (WGS/WES, H&E/IHC, RNA-seq), cryopreservation, and living biobank maintenance. The figure further demonstrates the downstream clinical applications enabled by this workflow, including high-throughput drug screening, biomarker discovery, and personalized therapy guidance—capabilities that extend to immunocompetent co-culture systems for precision oncology.

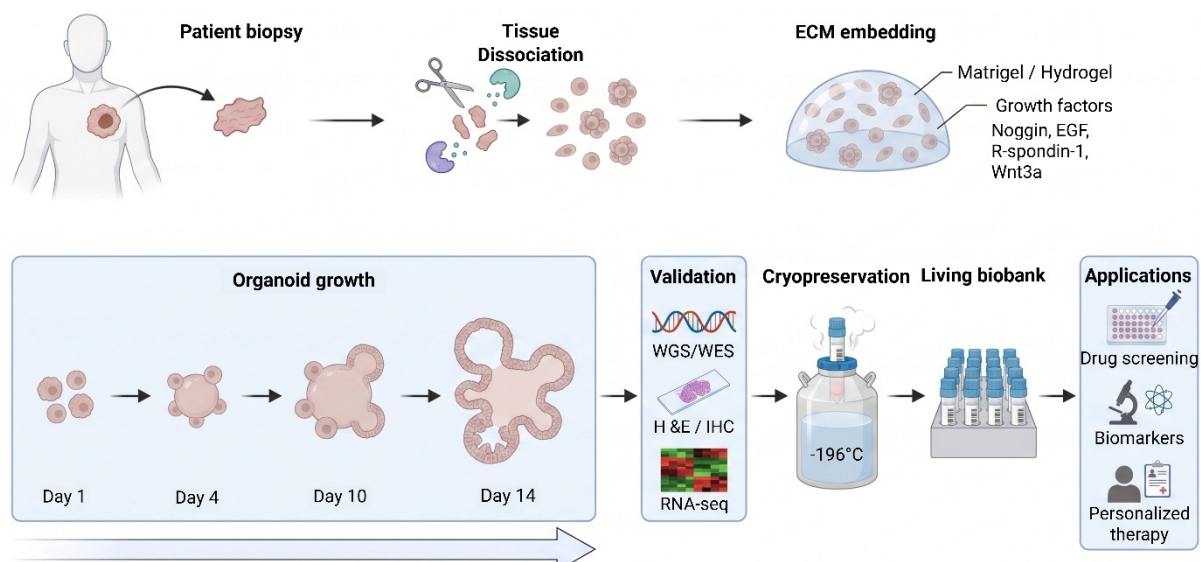


Figure 2. Complete patient-derived organoid (PDO) workflow: from biopsy to clinical applications, including generation, validation, biobanking, and personalized therapy guidance. Top row: PDO generation begins with patient biopsy or surgical resection, followed by tissue dissociation into single cells and small clusters. Dissociated tissue is embedded in extracellular matrix (ECM) scaffolds

(Matrigel or synthetic hydrogels) supplemented with organ-specific growth factors (Noggin, EGF, R-spondin-1, Wnt3a) to initiate self-organizing organoid formation. Middle panel: Temporal progression of organoid growth and maturation from Day 1 through Day 14, demonstrating increasing structural complexity and size. Right panel: Downstream validation, cryopreservation, and biobanking. Validation encompasses whole-genome sequencing (WGS), whole-exome sequencing (WES), histology and immunohistochemistry (H&E/IHC), and RNA sequencing (RNA-seq) to confirm genetic, transcriptomic, and histological fidelity to the original tumor. Validated organoids are cryopreserved at -196°C and maintained in living biobanks for long-term storage. Far right panel: Clinical and research applications including high-throughput drug screening for personalized therapy selection, biomarker discovery, and precision medicine guidance. This integrated workflow enables rapid, patient-specific functional testing and supports both monoculture and immunocompetent co-culture systems for comprehensive tumor microenvironment modeling and therapeutic decision-making.

3. Tumor-on-Chip (ToC) Platforms: Engineering the Microenvironment

Although patient-derived organoids capture tumor complexity, they remain static and are diffusion-limited. Tumor-on-Chip (ToC) platforms, a specific subtype of OoC built on microfluidics, provide controlled, dynamic microenvironments that recapitulate in vivo forces, such as fluid shear, interstitial pressure, mechanical strain, and the spatial organization of vascular and stromal compartments [55]. Integrating these parameters with relevant cells and matrices yields preclinical models that are more representative of in vivo tumors than are static systems.

3.1. Principles of Microfluidics in Cancer Research

Microfluidics manipulate fluids at the sub-millimeter scale within channels made of PDMS, PMMA, and thermoplastic elastomers [56]. Soft lithography is the standard for device fabrication, whereas 3D printing and laser cutting broaden access; syringe, peristaltic, or gravity-driven flow precisely sets the rates and shear.

Continuous perfusion stabilizes nutrients/oxygen and clears waste, avoiding metabolic artifacts of static assays that can mimic drug resistance [57]. Microfabrication-enabled compartmentalization (e.g., porous membranes or hydrogel barriers separating tumors and endothelium) permits paracrine signaling and transendothelial migration studies in defined architectures [11]. Microliter volumes conserve scarce patient samples and costly reagents, thereby enabling high-throughput screenings [29]. Integrated electrochemical, optical, and impedance (TEER) sensors provide real-time, non-destructive readouts of viability, barrier integrity, metabolism, and biomarker secretion [58].

PDMS is transparent, gas-permeable, and biocompatible; however, it absorbs hydrophobic small molecules, including many chemotherapeutics, which confound drug measurements and efficacy estimates [59]. This has motivated the development of PFPE, COC, and polyurethane alternatives with lower absorption and suitable optical and mechanical properties.

By precisely arranging cells, controlling perfusion, and integrating sensors, ToC converts static cultures into dynamic, continuously interrogable systems whose micro-scale dimensions mirror capillary and tissue architecture.

3.2. Modeling Physical Forces: Shear Stress, Interstitial Fluid Pressure, and Mechanical Strain

Tumors exhibit abnormal mechanics, including elevated vascular shear, high interstitial fluid pressure (IFP), solid stress, and a stiff extracellular matrix (ECM), which drive tumor progression, metastasis, and therapy resistance. The ToC uniquely reproduces these cues to probe behavior, delivery, and response with physiological precision [60].

Physiological shear is $\sim 1\text{--}20\text{ dyn/cm}^2$ in capillaries and $10\text{--}70$ in arteries, values precisely set by the channel geometry and flow [61]. Ortega Quesada et al. showed physiological shear reprograms estrogen receptor signaling in ER+ breast cancer, effects

absent in static culture [62]. Yankaskas et al. identified TRPM7 as a shear sensor required for cytoskeletal remodeling and intravasation under flow conditions [61]. Marrella et al. found that high shear disaggregates circulating tumor cell (CTC) clusters, highlighting cluster-mediated protection in large vessels [63].

Tumor IFP can reach 10–40 mmHg (near zero in normal tissues), creating outward convection that hinders the entry of drugs and nanoparticles [60]. Martín-Asensio et al. modeled solid stress–compressed ECM and predicted nanoparticle diffusion limits that correlated with in vivo results, providing a platform to optimize penetration [64].

Tumor ECM stiffness (5–20 kPa vs. 0.1–2 kPa in normal breast tissue) actively drives malignancies [65]. Mechanotransduction via integrin–FAK–Src, YAP/TAZ, and Piezo1/TRPV4 promotes proliferation, invasion, epithelial–mesenchymal transition (EMT), angiogenesis, and immune evasion [66]. Li et al. showed ~45 Pa ECM force activates integrin $\beta 1/3$ and cancer stemness via cytoskeleton/AIRE, whereas 450 Pa induces quiescence via DDR2/STAT1/P27—a force-dependent CSC switch [67]. Zhang et al. used GelMA hydrogels to tune PDAC stiffness, finding high rigidity accelerates progression and immunosuppression in vitro and in vivo [68].

Wang et al. reviewed mechano-organ-on-chip platforms integrating stiffness, viscoelasticity, solid stress, interstitial flow, confinement, and shear with tunable ECMs, vascular/stromal interfaces, dynamic loading, and sensor- plus AI-enabled multimodal readouts [55].

As mechanics can block drug penetration or rewire signaling, ToCs that capture physical barriers are essential for therapeutic development. Mechano-OoC offers a framework for studying the mechanics of TME interactions.

3.3. Vascularization On-Chip: Angiogenesis and Modeling the Metastatic Cascade

Angiogenesis sustains tumor growth beyond approximately 1–2 mm and enables dissemination. Vascularized ToCs recapitulate angiogenesis and the metastatic cascade—local invasion, intravasation, circulation, extravasation, and colonization—in controlled systems [69].

Endothelial cells are co-cultured in channels adjacent to tumor spheroids/organoids in 3D hydrogels, and tumor-derived VEGF, FGF, and angiopoietins drive directed sprouting. Skubal et al. used a commercial chip with renal cell carcinoma spheroids to visualize neovascularization and quantify the effects of bevacizumab within weeks, as opposed to months in animals [69]. Kim et al. built a perfused HUVEC–tumor ToC to interrogate tumor–microenvironment–drug interactions [37].

Kumar et al. combined a 3D breast tumor compartment with a flow-conditioned vascular channel, revealing tumor–endothelium anastomoses that facilitate entry and showing methylglyoxal, modeling diabetic stress, elevates intravasation via endothelial senescence and basement-membrane degradation [70]. Wong et al. created a lung intravasation-on-chip with machine learning quantification, finding EMT cues promote A549 protrusion into microvessels within 24 h [10].

Schmid et al. showed that mesenchymal A549 paraclones extravasate efficiently, whereas epithelial holoclones do not; VEGF depletion blocks paraclone extravasation [71]. Wu et al. recapitulated intravasation, CTC adhesion, and extravasation on one chip, finding adhesion favored by glycocalyx shedding and disturbed hemodynamics [72]. Brooks et al. reviewed metastasis-on-chip platforms and their applications in anti-metastatic drug screening [73].

Given the rarity and stochasticity of metastasis in vivo, vascularized ToCs provide real-time, single-cell resolution of each step and enable targeted testing of anti-metastatic strategies. ML-driven analytics are critical for improving throughput. Figure 3 integrates

the complete ToC platform architecture, illustrating the three critical physical forces (shear stress, interstitial fluid pressure, and ECM stiffness with mechanotransduction signaling), the microengineered central chip design with perfusion control via a syringe pump, endothelial vascular channels, and ECM hydrogel compartments. The figure further depicts integrated real-time sensors (electrochemical, optical/fluorescence, and TEER impedance) for continuous monitoring of viability, metabolism, and barrier integrity. Table 3 systematically summarizes microfluidic tumor-on-chip platforms for modeling each step of the metastatic cascade, including intravasation (enhanced by dicarbonyl stress and epithelial–mesenchymal transition), circulating tumor cell (CTC) circulation (with shear stress-mediated cluster disaggregation), extravasation (with mesenchymal paraclones showing superior efficiency), and angiogenesis (with tumor spheroid-induced endothelial sprouting disrupted by anti-angiogenic agents). Together, Figure 3 and Table 3 demonstrate how integrated microfluidic platforms—combining physiological forces, real-time sensing, and multicellular complexity—enable comprehensive mechanistic dissection of metastatic progression at single-cell resolution and accelerate the development of targeted anti-metastatic therapeutics.

Table 3. Microfluidic tumor-on-chip platforms for modeling metastatic cascade steps: experimental systems, mechanisms, and key findings.

Metastatic Step	Microfluidic Model	Mechanisms and Key Findings	Reference
Intravasation	Breast tumor + aortic endothelium chip	Dicarbonyl stress (modeling diabetic conditions) enhances intravasation via endothelial senescence and basement-membrane degradation	[70]
Intravasation	Lung cancer intravasation-on-a-chip	Epithelial–mesenchymal transition (EMT) facilitates tumor cell protrusion into microvessels within 24 h; machine learning enables automated quantification	[10]
CTC Circulation	Multi-channel microfluidic device	High shear stress disaggregates protective CTC clusters; cluster-mediated protection in large vessels	[63]
Extravasation	Vasculature-on-chip (lung cancer)	Mesenchymal paraclones extravasate efficiently; epithelial holoclones do not; VEGF depletion blocks paraclone extravasation	[71]
Angiogenesis	Renal cell carcinoma + HUVEC chip	Tumor spheroids induce endothelial sprouting via VEGF/FGF signaling; bevacizumab disrupts neovascularization	[69]

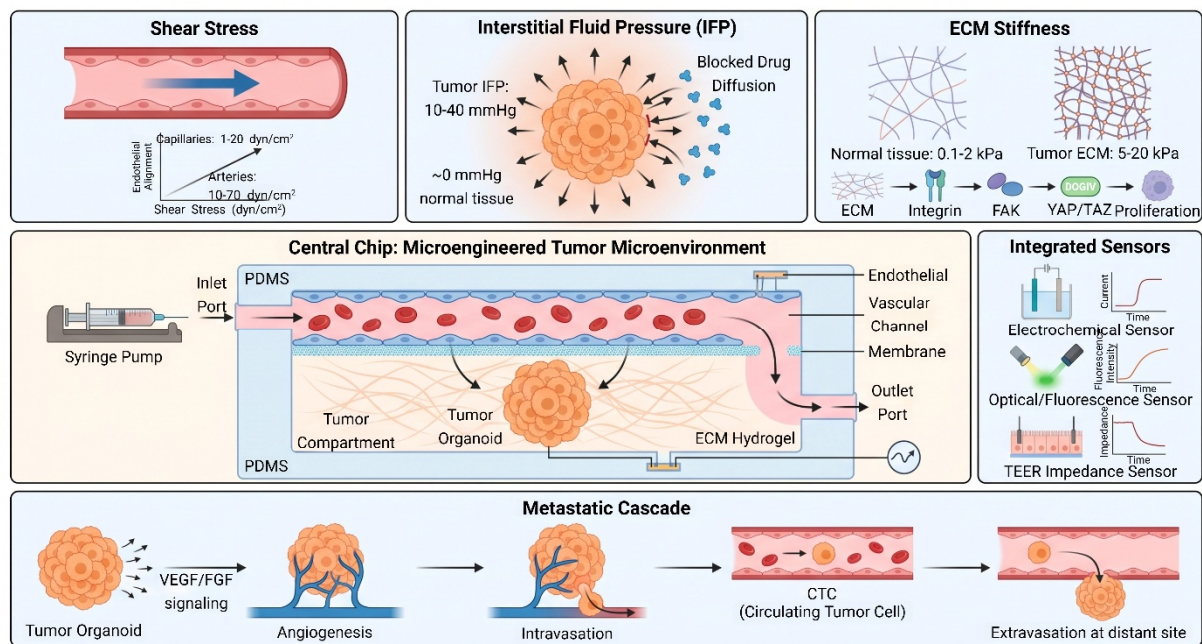


Figure 3. Tumor-on-chip (ToC) platform architecture integrating microfluidics, physical forces, vascularization, and integrated sensors for dynamic tumor microenvironment modeling. Top row panels: Three critical physical forces modeled in ToC systems. Left panel: Shear stress, showing physiological ranges in capillaries (1–20 dyn/cm²) and arteries (10–70 dyn/cm²), precisely controlled by microfluidic channel geometry and flow rates. Center panel: Interstitial fluid pressure (IFP), illustrating elevated tumor IFP (10–40 mmHg) compared to normal tissue (~0 mmHg), which blocks drug diffusion. Right panel: Extracellular matrix (ECM) stiffness, showing the progression from normal tissue (0.1–2 kPa) to tumor ECM (5–20 kPa), and the downstream mechanotransduction pathway via integrin–FAK signaling, YAP/TAZ activation, and proliferation. Center panel: Microengineered tumor microenvironment chip architecture. The device features polydimethylsiloxane (PDMS) construction with inlet and outlet ports connected to a syringe pump for precise perfusion control. The central compartment contains a tumor organoid embedded in ECM hydrogel, flanked by an endothelial vascular channel separated by a porous membrane, enabling paracrine signaling and transendothelial migration studies. Right panel: Integrated real-time sensors including electrochemical sensors for metabolite detection, optical/fluorescence sensors for biomarker quantification, and transepithelial electrical resistance (TEER) impedance sensors for barrier integrity monitoring. Bottom panel: Metastatic cascade modeling on-chip. Sequential steps include tumor organoid secretion of VEGF/FGF signaling, directed angiogenesis and sprouting, intravasation of tumor cells into the vascular channel, circulation of tumor cells as circulating tumor cells (CTCs), and extravasation at distant tissue sites. This integrated platform enables real-time, single-cell resolution visualization and quantification of each metastatic step.

4. The Synergy: Integrating Organoids into Organ-on-Chip Systems

Organoid and Organ-on-Chip (OoC) technologies each improve preclinical cancer modeling, but their integration most fully realizes their potential by coupling the patient specificity of PDOs with the controlled, dynamic microenvironments of microfluidics, yielding models that are both biologically faithful and physiologically relevant [74]. This section contrasts organoids and ToCs as stand-alone tools, outlines organoid-on-chip principles and applications, and highlights multi-organ body-on-a-chip systems for systemic pharmacology.

4.1. Why Choose? Comparing the Strengths and Limitations of Organoids and Tumor-on-Chip

PDOs and ToC platforms offer complementary strengths in terms of biological fidelity, microenvironmental realism, scalability/throughput, and clinical accessibility, which moti-

vates their integration. PDOs retain patient tumor mutations, epigenetics, transcriptional programs, and heterogeneity in 3D architecture and correlate with clinical drug responses (AUROC 0.78–0.88 in colorectal cancer, Smabers et al., 2025 [40]; predictive FOLFIRINOX response in PDAC, Seppälä et al., 2022) [13], while enabling scalable expansion, biobanking, and high-throughput screening [25]. However, standard PDOs in static BME droplets lack vascular perfusion, immune components, and mechanical cues, limiting their pharmacokinetic realism and mechanobiology capture [75]. In contrast, ToC platforms provide precise control of flow, strain, and spatial cell organization with real-time sensing of barriers, metabolism, and secreted biomarkers [76]. However, reliance on immortalized lines erodes biological fidelity and predictive power relative to patient outcomes [26], and specialized device fabrication/operation constrains the clinical adoption of these models.

Thus, organoids supply the ‘who’ (patient biology) and microfluidics the ‘where’ (physiology); used alone, each approach is incomplete. Their convergence as organoids-on-a-chip is a conceptual advance that addresses both sets of limitations. Table 4 provides a comprehensive comparison of patient-derived organoids (PDOs), tumor-on-chip (ToC) platforms, and organoid-on-chip (OoC) integration systems across seven critical parameters: biological fidelity, microenvironmental relevance, immune component integration, scalability and throughput, clinical accessibility, pharmacokinetic modeling, and regulatory acceptance. PDOs achieve high biological fidelity and clinical accessibility but remain limited in microenvironmental realism, immune integration, and pharmacokinetic modeling due to their static nature. ToC platforms excel in microenvironmental realism and dynamic flow but often sacrifice biological fidelity through reliance on immortalized cell lines. Organoid-on-chip systems integrate the strengths of both approaches, achieving high biological fidelity while providing dynamic microenvironmental conditions, feasible immune cell integration, and superior pharmacokinetic modeling, positioning them as the most comprehensive preclinical model for personalized cancer therapy.

Table 4. Comparative analysis of patient-derived organoids, tumor-on-chip platforms, and organoid-on-chip systems across biological, technical, and clinical parameters.

Parameter	Patient-Derived Organoids (PDOs)	Tumor-on-Chip (ToC)	Organoid-on-Chip (OoC Integration)
Biological Fidelity	High (patient-specific genetics, heterogeneity)	Moderate (often uses cell lines)	High (PDOs in dynamic environment)
Microenvironmental Relevance	Low (static, no flow or mechanical forces)	High (dynamic flow, mechanical cues, vascularization)	High (combines both)
Immune Component Integration	Challenging (competing media requirements)	Possible (co-culture in flow)	Feasible (microfluidics enables immune cell delivery)
Scalability/Throughput	High (multi-well plate format)	Moderate (specialized fabrication required)	Moderate-High (improving with standardization)
Clinical Accessibility	Moderate (requires specialized culture expertise)	Low (requires microfluidic expertise)	Low-Moderate (improving with commercial platforms)
Pharmacokinetic Modeling	Limited (static drug exposure)	Possible (controlled flow profiles)	High (dynamic drug delivery with PK profiles)
Regulatory Acceptance	Emerging (clinical trial data accumulating)	Emerging (FDA NAMs framework)	Emerging (most advanced non-animal model platform)

4.2. Organoid-on-Chip Approach: Combining Biological Complexity with Physiological Microenvironments

Organoid-on-chip systems culture PDOs within perfused microchannels/chambers, uniting patient-specific biology with dynamic and physiologically relevant conditions [77]. Continuous flow stabilizes nutrients/oxygen, extends viability, introduces shear stress, and enables time-varying drug delivery that mimics pharmacokinetics, which is critical for agents whose efficacy depends on their exposure profiles. Haque et al. (2022) integrated PDOs with stellate cells and macrophages to recreate a desmoplastic and immunosuppressive TME in PDAC [9]. Stroma targeting enhanced chemotherapy only in the multicellular chip, not in monoculture, validating stroma-focused strategies [9]. Adnan et al. (2026) similarly combined PDOs with fibroblasts, endothelium, and immune cells, showing tumor–stroma crosstalk, chemotherapy sensitization via stromal targeting, and the ability to evaluate checkpoint blockade—capabilities absent in standard organoid monocultures [51]. For lung cancer, Zeng et al. (2023) highlighted organoids-on-a-chip for personalized screening with improved stability, TME complexity, and throughput, positioning them for clinical translation [26]. Hu et al. (2021) used an InSMAR-chip to generate hundreds of passage-0 lung cancer organoids and deliver clinically concordant drug responses within one week, aligning with PDXs, mutations, and outcomes [7]. Under flow, vascular-channel delivery of CAR-T, NK cells, or PBMCs enables the study of recruitment, extravasation, and infiltration, which are key determinants of adoptive cell therapy efficacy in solid tumors [47]. Overall, Organoid-on-Chip provides a lab-based patient avatar: only within a dynamic, multicellular microenvironment can PDAC’s stromal barrier and its therapeutic modulation be faithfully modeled.

4.3. Multi-Organ Chips (Body-on-a-Chip) for Evaluating Systemic Drug Toxicity and Pharmacokinetics

Body-on-a-chip platforms interconnect multiple organ-specific chips via a common circulation to capture systemic PK/PD, including hepatic metabolism, renal clearance, and off-target toxicities, which are absent in single-tissue models [78]. Drugs introduced into the circuit can be metabolized by liver organoids to active species before reaching tumors and other tissues, as in Rajan et al. (2020), who used a low-cost, adhesive film-based device with liver–cardiac–lung constructs viable for 21 days to demonstrate capecitabine activation to 5-FU with downstream lung and cardiac toxicity [29]. In a six-organoid system, hepatic metabolism of ifosfamide generates chloroacetaldehyde and neurotoxicity, mirroring clinical dependence on liver biotransformation [29]. Li et al. (2025) reviewed the accelerating synergy between organoids and OoCs alongside regulatory milestones—the FDA guidance (10 April 2025) initiating animal-trial phase-out and NIH’s ORIVA launch (29 April 2025)—which are expected to speed integration into development pipelines [28]. Gracey et al. (2025) emphasized advantages of 3D organoids/MPS over 2D hepatocytes for clearance, drug-induced liver injury (DILI), and drug–drug interactions (DDIs), and the importance of physiologically based pharmacokinetic (PBPK) integration for in vitro–in vivo extrapolation [79]. Despite rapid progress in single-organ systems, multi-organ systems face hurdles in physiological scaling, universal media, and integrated analytics, which slow down translation [78]. Hsu et al. (2026) argued that near-term multi-organ chips will complement current DDI methods, supplying mechanistic, physiologically grounded parameters to PBPK models [80]. Regulatory frameworks are evolving: the FDA Modernization Act 2.0 (2022) removed mandatory animal testing for Investigational New Drugs (INDs), enabling the use of organoid/OoC data as the primary preclinical evidence [22]. In oncology, Alatawi et al. (2026) detailed the integration of PDOs and OoCs post-Modernization Act and their convergence with AI, spatial multi-omics, and liquid biopsies to accelerate precision testing [81]. Roy et al. (2026) reported benchmarking that places OoCs near

regulatory qualification thresholds (e.g., cardiac torsadogenic AUROC ≥ 0.85 ; improved renal transporter-mediated clearance prediction) [58]. Because cancer therapy is systemic, predicting its efficacy and off-target toxicity requires human-relevant and interconnected systems. If the current regulatory momentum continues and key technological barriers, including standardization, multi-organ integration, and Good Manufacturing Practice (GMP)-compatible biomaterials, are resolved, Body-on-a-Chip platforms may enter clinical drug development within the next decade, although the timeline remains uncertain.

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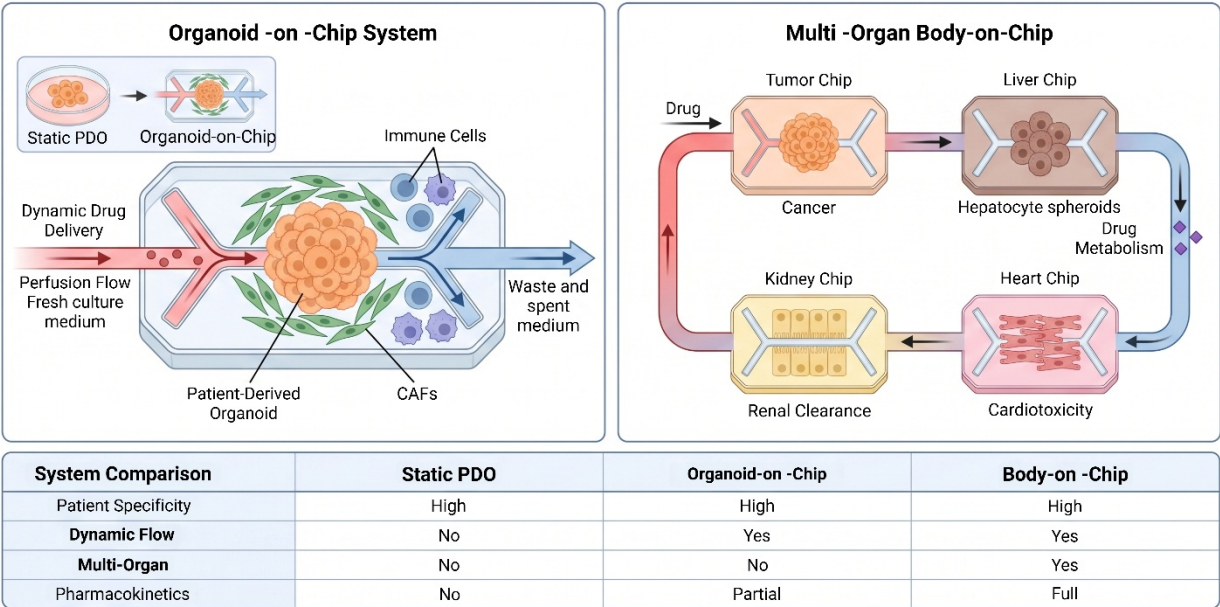


Figure 4. Integration of organoids with organ-on-chip platforms: from organoid-on-chip to multi-organ body-on-chip systems. Left panel: Organoid-on-chip system architecture. Top inset shows the transition from static patient-derived organoid (PDO) to organoid-on-chip with integrated microfluidic perfusion. The main illustration depicts a perfused microfluidic chamber containing a patient-derived organoid, cancer-associated fibroblasts (CAFs), and immune cells. Dynamic drug delivery via perfusion flow provides fresh culture medium and waste clearance, enabling physiologically realistic pharmacokinetics and mechanotransduction cues absent in static cultures. Right panel: Multi-organ body-on-chip system. Four interconnected organ chips are fluidically linked via a common circulation: tumor chip (containing cancer organoid), liver chip (for drug metabolism), kidney chip (for renal clearance), and heart chip (for cardiotoxicity assessment). Drugs introduced

into the circuit are metabolized by hepatic organoids to active or toxic metabolites before reaching tumor and other tissues, enabling systemic pharmacokinetics and off-target toxicity prediction. Bottom panel: Comparative table summarizing key features of three systems. Static PDO systems offer high patient specificity but lack dynamic flow, multi-organ integration, and full pharmacokinetics. Organoid-on-chip systems add dynamic flow and partial pharmacokinetics while maintaining patient specificity, but remain single-organ. Body-on-chip systems integrate multiple organs with full pharmacokinetic modeling, enabling comprehensive systemic drug efficacy and toxicity assessment.

5. Organoid and Organ-on-Chip Platforms as Tools for Personalized Drug Screening and Biomarker Discovery

Patient-derived organoids (PDOs) faithfully preserve primary tumor histology, genomics, and drug response phenotype [30]. Unlike 2D lines, PDOs retain intratumoral heterogeneity, enabling ex vivo screening that better predicts in vivo outcomes [18]. Integrated PDX–PDX-derived organoid pipelines have delivered high-throughput screens that reveal actionable vulnerabilities, including repurposable FDA-approved agents, within clinically compatible time frames [18]. Microfluidic organ-on-chip technology adds flow, mechanics, and endothelial–epithelial crosstalk to recapitulate TME dynamics that are inaccessible to static culture [27,82]. Bioprinting combined with label-free, time-resolved interferometric imaging enables single-organoid resolution, capturing transient responses, and early drug-resistant subclones that are obscured by endpoint assays [83]. These functional precision oncology strategies complement genomic profiling, especially when molecular alterations do not predict sensitivity [84].

Tumor biomarkers spanning genomics, epigenetics, transcriptomics, proteomics, and metabolomics now guide risk, detection, monitoring, and targeting beyond histopathology [30,85]. Pairing PDOs with single-cell and spatial multi-omics and proximity extension proteomics enables the concurrent discovery of resistance biomarkers and drug targets in the same system [86,87]. Pharmacogenomic frameworks link molecular features to responses, translating organoid drug sensitivity data into cohort-level predictive signatures [88,89]. Few-shot models trained on large screens and fine-tuned on limited patient samples extend predictions to rare and refractory contexts [90]. Multimodal integration of molecular, imaging, and clinical data is essential, as no single modality captures tumor complexity sufficiently to guide therapy [91,92].

5.1. Functional High-Throughput Screening of Chemotherapeutics and Targeted Therapies via Patient-Derived Organoid and Microphysiological System

Superhydrophobic microwell microarray chips can generate and analyze hundreds of lung cancer organoids from minimal biopsies in less than one week, enabling rapid patient-specific drug profiling [7]. Micro-organosphere formats from low-input tissues allow the assessment of tumor and immuno-oncology responses within 14 days [14]. High-throughput organ-on-chip systems with 96-device form factors, programmable flow, and integrated sensing align microphysiological sophistication with pharmaceutical-grade throughput and data capture capabilities [93].

Screening > 500 bispecific antibodies across heterogeneous colorectal PDO biobanks identified MCLA-158 (EGFR × LGR5), which eradicates cancer stem cells, is active in KRAS-mutant CRC, and suppresses metastasis in epithelial tumors [39]. Such biobank-encoded heterogeneity is advantageous over cell line HTS because it reveals agents whose efficacy maps to clinical molecular diversity [94]. Matched primary–liver metastasis CRC organoids expose inter/intra-patient heterogeneity at multi-omics resolution while generating chemosensitivity data that are predictive of clinical response [15]. High-throughput human liver organoids can predict drug-induced liver injury and capture host genetic diversity in hepatotoxicity, beyond monolayer hepatocytes [95]. Immunocompetent co-

cultures with autologous TILs, NK cells, and engineered T cells enable patient-specific evaluation of ICIs and CAR-T mechanisms in 3D TMEs [49,52]. Tumor-on-chip technology enables real-time analysis of immune exhaustion under nutrient stress, hypoxia, and waste accumulation, conditions that are absent from static cultures [8].

Deep learning platforms, such as OrganoID, automate pixel-level recognition and single-organoid tracking across high-content datasets, removing a key analytical bottleneck in organoid HTS [96]. Coupling AI analytics with organoid platforms yields closed-loop HTS that unites biological fidelity, patient specificity, and scale [97]. Microfluidic chips with electrochemical affinity biosensors enable continuous, non-destructive monitoring of pharmacodynamics and resistance trajectories beyond endpoint assays [98].

5.2. Organoid and Tumor-on-Chip Platforms for Immuno-Oncology: Modeling Immune Checkpoint Inhibitor Response and CAR-T Cell Efficacy in Solid Tumors

ICIs (CTLA-4, PD-1, PD-L1, and LAG-3) and CAR-T therapies have transformed oncology but show variable efficacy, toxicity, and resistance in solid tumors [99]. Because immunotherapies act via dynamic tumor–immune–TME interactions, organoid and tumor-on-chip platforms are uniquely suited to model them compared with 2D assays [48]. This mismatch helps to explain the gap between the preclinical promise and clinical response of ICIs and CAR-T in solid tumors [100].

Organotypic slice cultures from CRC liver metastases showed that IL-10 blockade augmented antitumor immunity alone and with CAR-T cells within intact patient-derived architectures [16]. These platforms uniquely test combinatorial and microenvironment-modulating strategies while preserving suppressive axes (cytokine, metabolic, and hypoxic) [8]. Given that key barriers to ICI efficacy arise from TME-level interactions, such microenvironment-preserving systems are essential for mechanistic dissection and better patient selection and response assessment [48].

For CAR-T in solid tumors, limited trafficking, antigen heterogeneity, and hostile TMEs underlie the inferior efficacy of hematologic cancers [101,102]. Live imaging and transcriptomics of patient-derived organoids have revealed functional T-cell subsets, including “super engagers,” informing next-generation CAR engineering [49]. These insights are timely amid strategies such as probiotic-guided CAR-Ts and in vivo CAR-T generation via targeted lipid nanoparticles [103]. Organoid/ToC platforms, which preserve patient architecture and immune trafficking, offer more predictive testing of efficacy and off-target or on-target/off-tumor risks than xenografts or monolayers [104]. Their convergence with expanding ICI and adoptive cell modalities supports the individualized prediction of responders and rational combinations to overcome resistance [105].

5.3. Organoid and Tumor-on-Chip Platforms for Patient-Specific Drug Response Prediction and Resistance Mechanism Elucidation

In metastatic CRC, a standardized PDO testing framework predicted standard-of-care responses, with liver metastasis–derived organoids performing the best [106]. Multiple CRC cohorts confirmed a high concordance with chemotherapy/radiotherapy outcomes and revealed resistance mechanisms via paired primary and metastatic biobanks [107]. In gastric cancer, organoid screens have predicted chemotherapy outcomes and have been validated in mice and humans [17]. In PDAC, organoid pharmacotyping has prospectively predicted responses in a randomized controlled trial and detected mutations missed by NGS [13]. In PDAC neoadjuvant planning, organoid profiling stratifies patients to the most active regimen pre-surgery [108]. PDOs preserved tumor features in cervical and bladder cancers while predicting sensitivity across modalities, with systematic reviews confirming a high concordance with primary tissues [109,110]. Organoids have predicted PARP inhibitor response in ovarian cancer and identified combinations overcoming resistance [111]. CTC-

derived PDAC organoids correlated with clinical responses despite minimally invasive sampling [112].

Primary liver cancer organoids linked acquired sorafenib resistance to stemness, retro-differentiation, and EMT under longitudinal drug pressure [19]. Single-cell profiling of oxaliplatin-resistant CRC organoids identified chemoresistant state targets, with ex vivo sensitivity mirroring the patient outcomes [113]. Computational lineage tracing has shown that CAFs regulate organoid drug response by shifting stem phenotypes and directly protecting them against chemotherapy [46]. CRC tumor-on-chip models matched PDX in predicting chemotherapy efficacy while being faster and more scalable [20]. Inverse-opal organ-on-chip scaffolds captured the real-time evolution of resistance during continuous exposure in liver tumor models [114]. Together, organoid and ToC platforms elucidate the genetic, epigenetic, and microenvironmental determinants of therapeutic failure beyond binary sensitivity calls [115,116].

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5.4. Artificial Intelligence for Image Analysis, Drug-Response Prediction, and Workflow Automation

In this context, artificial intelligence is best viewed as an analytical and operational layer that enhances the value of organoid and organ-on-chip data, rather than as a replacement for the experimental models themselves. In high-content screening, deep-learning methods can automate image segmentation, track individual organoids, and extract longitudinal phenotypic features, thereby reducing the subjectivity and workload associated with manual endpoint assessment. OrganoidID exemplifies this function by enabling the automated analysis of single-organoid dynamics, while broader AI-enabled organoid workflows build on these capabilities to support more standardized and scalable screening [96,97]. Beyond image analysis, machine learning can help link functional organoid readouts to clinically relevant estimates of treatment response. For instance, PharmaFormer applies transfer learning to large two-dimensional cell-line datasets and subsequently fine-tunes the resulting model using limited patient-derived organoid data, thereby addressing a key challenge in pharmacotyping: the small number and marked heterogeneity of patient samples [42]. Similarly, machine-learning-assisted image quantification has been used to standardize dynamic readouts in tumour-on-chip models, including lung cancer intravasation [10]. Together, these applications suggest that AI could strengthen

the connections among complex organoid phenotypes, molecular profiles, and treatment responses. However, the reliability of this contribution remains contingent on the quality and representativeness of the underlying datasets. Batch effects, differences in imaging systems, matrices, and chip platforms, limited cohort sizes, and inter-patient heterogeneity may introduce domain shift and limit generalizability across laboratories. Accordingly, AI-supported organoid workflows are best conceived as interpretable decision-support tools whose potential clinical value lies in complementing, rather than replacing, human judgement across diverse patient populations and clinical settings.

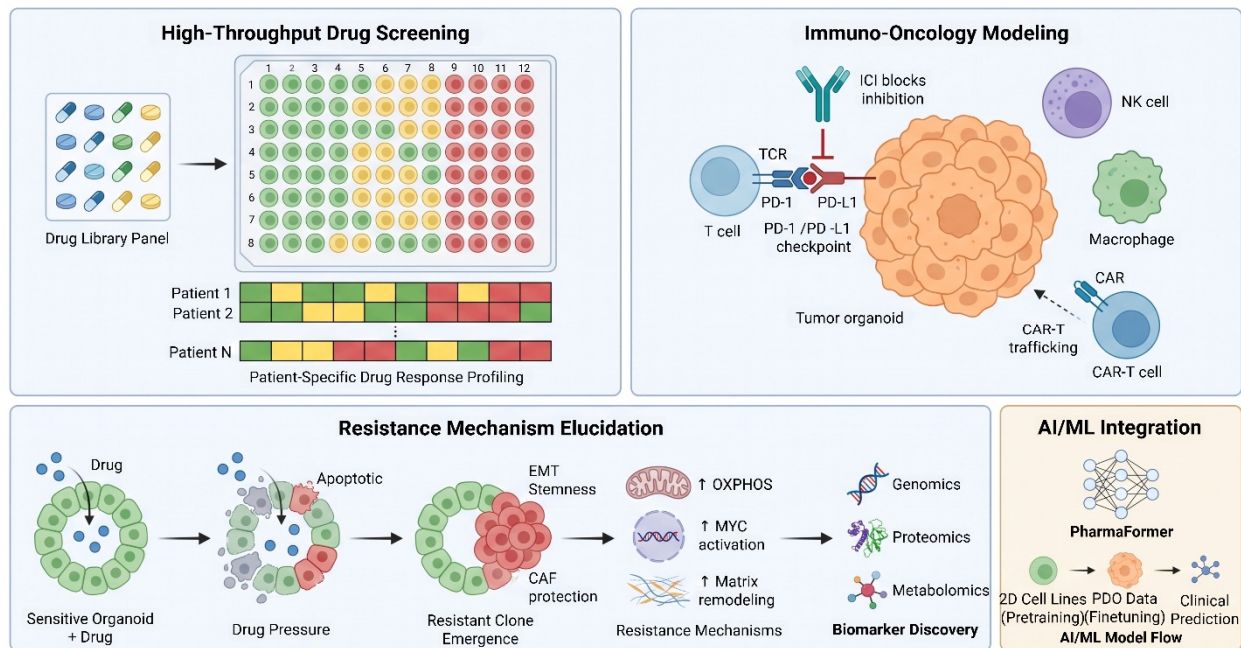


Figure 5. Clinical applications of organoid and tumor-on-chip platforms: high-throughput drug screening, immuno-oncology modeling, resistance mechanism elucidation, and AI/ML integration. Top left panel: High-throughput drug screening workflow. A drug library panel containing hundreds of chemotherapeutics and targeted agents is screened against patient-derived organoid biobanks. Multiple patient samples (Patient 1, Patient 2, Patient N) are profiled simultaneously, generating patient-specific drug response profiles represented as color-coded heatmaps (green = sensitive, yellow = intermediate, red = resistant). This approach enables rapid identification of personalized therapeutic options and discovery of repurposable FDA-approved agents. Top right panel: Immuno-oncology modeling. A tumor organoid is co-cultured with multiple immune cell types including T cells (with TCR and PD-1 receptors), NK cells, macrophages, and CAR-T cells. The diagram illustrates checkpoint inhibitor (ICI) blockade of the PD-1/PD-L1 axis and CAR-T cell trafficking and infiltration into the tumor microenvironment, enabling patient-specific evaluation of checkpoint inhibitor and adoptive cell therapy responses. Bottom left panel: Resistance mechanism elucidation. Sequential stages of drug resistance are depicted: (1) sensitive organoid exposed to drug, (2) drug pressure selects for resistant clones and induces apoptosis in sensitive cells, (3) emergence of resistant clones exhibiting epithelial–mesenchymal transition (EMT), stemness, and cancer-associated fibroblast (CAF) protection, and (4) multi-omics analysis (genomics, proteomics, metabolomics) reveals molecular mechanisms of resistance including increased oxidative phosphorylation (OXPHOS), MYC activation, and matrix remodeling. Bottom right panel: AI/ML integration. PharmaFormer, a transfer learning model, is trained on 2D cell line data, fine-tuned on patient-derived organoid (PDO) data, and applied to predict clinical drug response. This workflow demonstrates how machine learning bridges preclinical organoid platforms and clinical outcomes. Together, these four applications illustrate the comprehensive utility of organoid and ToC platforms for personalized drug selection, mechanistic understanding of resistance, and precision oncology.

6. Challenges and Bottlenecks in the Clinical Translation of Organoid and Tumor-on-Chip Platforms for Translational Oncology

Despite strong momentum, organoid and tumor-on-chip technologies still lack assay speed, reproducibility, standardization, and automation, which are interdependent deficits that remain the principal bottlenecks for clinical adoption [117]. The stochastic variability inherent in self-organizing organoid morphogenesis limits reproducibility and precise experimental control, necessitating bioengineering at the levels of cell-surface chemistry, matrix composition, and microenvironmental signaling [118]. Multicellular tumor spheroids face similar limitations; protocol heterogeneity and uncertain cross-lab comparability have curtailed their adoption despite their intermediate complexity [119]. Non-standardized tissue procurement and processing, divergent media, and reliance on animal-derived matrices leave current organoid methods insufficiently controlled; translation demands engineered next-generation systems delivering standardized, quantitative outputs that are acceptable to regulators and clinicians [120]. Until this occurs at scale, the gap between laboratory proof-of-concept and routine cross-institutional deployment will continue to persist.

6.1. Standardization, Reproducibility, and Scalability Barriers Impeding the Clinical Implementation of Organoid-Based Drug Screening Systems

Despite the promise of patient-derived tumor organoids (PDOs) and tumor-on-chip systems, their integration into practice is hindered by representativeness and assay reproducibility issues. PDOs lack a fully reconstituted TME, show variable establishment across tumor types, and often cannot deliver results fast enough for real-time decisions [30]. Intra-tumoral heterogeneity further limits any single line as a surrogate. For example, large biobanks in primary liver cancers reveal heterogeneous drug responses, such as c-Jun-mediated lenvatinib resistance, requiring scale and depth rarely feasible within clinical timelines [21]. Reconstituting the immune microenvironment remains difficult; although stromal/immune co-cultures improve fidelity, response rates vary by histology, and technical complexity increases interlaboratory variability [48]. High-throughput multi-omics adds an analytical burden, demanding advanced bioinformatics, harmonized standards, and robust statistics; data complexity, technical limitations, and ethical constraints slow cross-consortia convergence [121].

Scalability and standardization also lag behind. Organoid-organ-on-chip integration promises geometric control, mechanics, and perfusion but is hampered by batch variability, uncertainty in transfer timing, and the absence of harmonized fabrication/validation protocols across sites [122]. Similar translational bottlenecks in nanomedicine—material issues, scalable manufacturing, biological barriers, and tumor heterogeneity—still limit the extrapolation of preclinical efficacy despite >20 approved products [123]. Stimuli-responsive systems, including MOF-based formulations, face comparable hurdles in synthesis reproducibility, biocompatibility, and TME heterogeneity [124]. AI/ML can enhance stratification, elucidate resistance mechanisms, and optimize the workflows [125,126]. However, operational hurdles persist, such as data infrastructure, algorithmic generalizability across heterogeneous cohorts, inter-institutional data sharing, and integration into clinical and regulatory workflows [126].

6.2. Biomaterial Constraints as Barriers to Clinical-Grade Reproducibility in Three-Dimensional Tumor Modeling Systems

Material limitations create particularly rigid translational layers. Polydimethylsiloxane (PDMS), which is widely used in microfluidics, absorbs hydrophobic small molecules, distorting the effective drug exposure. Transitioning to thermoplastics or cyclic olefin copolymers mitigates absorption but introduces manufacturing and bonding challenges

that limit throughput and scalability [123]. Such pharmacological unreliability undermines personalized screening, where small concentration errors can invert apparent sensitivities. Matrigel remains a dominant ECM scaffold but shows profound lot-to-lot variability in composition, growth factors, and stiffness, driving uncontrolled effects on morphogenesis, proliferation, and drug response [127]. Because ECM stiffness regulates cancer behavior, immunity, and therapy efficacy, inter-batch mechanical variation adds biological noise that erodes reproducibility and comparability [128]. Moreover, the murine origin of Matrigel is incompatible with clinical-grade manufacturing, necessitating fully defined xeno-free synthetic alternatives.

Beyond PDMS and Matrigel, natural hydrogels (collagen, fibrin, and hyaluronic acid) recapitulate ECM cues but show variable gelation, mechanics, and degradation, which challenge batch-to-batch standardization [129]. Synthetic hydrogels provide greater chemical control and reproducibility; however, they often lack the complex bioactivity of tumor stroma, limiting response fidelity when microenvironmental signaling dominates outcomes [130]. Translation is further limited by the paucity of xeno-free, GMP-compliant scaffolds that meet both biological and regulatory criteria; gaps between research- and clinical-grade specifications mirror systemic issues in drug delivery and tissue engineering, where minor compositional shifts between preclinical and clinical materials alter efficacy and toxicity [131,132]. The safety and environmental risks of novel nano/biomaterials add regulatory complexity, as comprehensive biocompatibility, biodistribution, and long-term toxicology data are rarely available preclinically [133].

6.3. Regulatory Gaps and Jurisdictional Ambiguities in the Implementation of the FDA Modernization Act 2.0 for Organoid-Based Drug Screening

The FDA Modernization Act 2.0 (December 2022) eliminated mandatory animal testing and permitted organoids, organs-on-chip, microphysiological systems, and computational models as qualifying evidence for Investigational New Drugs (INDs) [22]. However, the regulatory infrastructure for evaluating these technologies is underdeveloped, and existing qualification frameworks built around small molecules and conventional biologics do not map cleanly onto context-dependent, heterogeneous organoid outputs [134]. Ambiguity regarding evidentiary sufficiency deters sponsors from investing in organoid-based claims [135]. With ~90% clinical attrition following successful preclinical evaluation, replacing inadequately validated models without robust qualification standards risks perpetuating the translational gap [136].

Modern platforms often trigger multiple overlapping FDA pathways: devices, biologics, combination products, and software as a medical device (when AI is integrated), each with distinct evidentiary standards and authorities [137]. This fragmentation prolongs timelines and discourages systematic platform qualification studies in the early stages of development [138]. Globally, divergent positions among regulators (e.g., EMA and Asia-Pacific agencies) create a fragmented landscape and asymmetric compliance burdens [139]. GMP translation is further hindered by biological variability in patient-derived organoids and the absence of standardized critical quality attributes [140]. Progress requires fit-for-purpose qualification frameworks and early prospective engagement among developers, sponsors, and regulators to define validation, inter-laboratory reproducibility, and clinical correlation standards that can realize the Act's promise in oncology [22,141].

Taken together, the convergent challenges across organoid biology, microfluidics, multi-omics, nanomedicine, and AI highlight the need for consensus standards, scalable biobanking, and clear regulatory pathways to convert these platforms into clinically actionable tools for personalized cancer therapy. Material-level constraints, such as absorption artifacts, scaffold variability, GMP incompatibility, and regulatory uncertainty, are fundamental barriers to generating reproducible decision-grade data. The momentum of

the FDA Modernization Act 2.0 must be matched by investment in fit-for-purpose qualification standards, xeno-free/good manufacturing Practice (GMP)-compatible scaffolds, and systematic clinical correlation to bridge the gap between preclinical promise and therapeutic impact.

Taken together, the convergent challenges across organoid biology, microfluidics, multi-omics, nanomedicine, and AI highlight the need for consensus standards, scalable biobanking, and clear regulatory pathways to convert these platforms into clinically actionable tools for personalized cancer therapy. Material-level constraints, such as absorption artifacts, scaffold variability, GMP incompatibility, and regulatory uncertainty, are fundamental barriers to generating reproducible decision-grade data. The momentum of the FDA Modernization Act 2.0 must be matched by investment in fit-for-purpose qualification standards, xeno-free/GMP-compatible scaffolds, and systematic clinical correlation to bridge the gap between preclinical promise and therapeutic impact. Figure 6 maps the current proof-of-concept stage of organoid and tumor-on-chip technologies against the four major bottlenecks impeding clinical translation: standardization and reproducibility deficits, biomaterial limitations (PDMS absorption and Matrigel batch variability), scalability and cost barriers, and regulatory ambiguity across overlapping FDA pathways. The figure further delineates targeted solutions for each bottleneck, including GMP-compatible synthetic hydrogels, harmonized multicenter protocols, AI/ML-driven automation and analytics, and regulatory harmonization frameworks. The timeline illustrates key regulatory milestones—FDA Modernization Act 2.0 (December 2022), FDA guidance on animal phase-out (April 2025), and NIH ORIVA launch (April 2025)—that are accelerating the path toward clinical validation and implementation of organoid and ToC platforms in precision oncology.

6.4. Ethical, Legal, and Social Implications of PDO Use in the AI Era

Patient-derived organoids (PDOs), tumor slices, organoid-on-chip platforms, and similar models are more than ordinary laboratory materials. They remain connected to the patients from whom they were obtained and may retain information about disease biology that is also relevant to biological relatives. In our view, this connection should influence every stage of their use, from collection and storage to analysis, sharing, and possible clinical application. Before a sample is used, the consent process should clearly describe the reasonably foreseeable purposes of the work, including organoid expansion, long-term biobanking, genomic and phenotypic analyses, clinical-data linkage, co-culture experiments, AI-supported analysis, international collaboration, and commercial partnerships. Participants should receive realistic information about future uses, the possibility of changing their preferences, the practical limits of withdrawal, and whether individual findings will or will not be returned.

The ethical responsibility continues after the sample has been collected. PDOs can be linked to genomic, imaging, and clinical datasets, making privacy protection a continuing obligation rather than a one-time procedural requirement. In practice, this calls for carefully coded samples, restricted access, secure storage, traceable data use, and explicit rules for connecting organoid results with clinical records. The status of the samples and their derivatives should also be agreed in advance. Responsibilities for custody, redistribution, authorship, intellectual property, commercial development, and sharing of possible benefits should be understandable to both participants and collaborating institutions. We also consider representation an important part of responsible science: if particular populations are missing from PDO biobanks or AI datasets, the resulting models may perform unevenly and may reinforce existing inequalities in precision oncology.

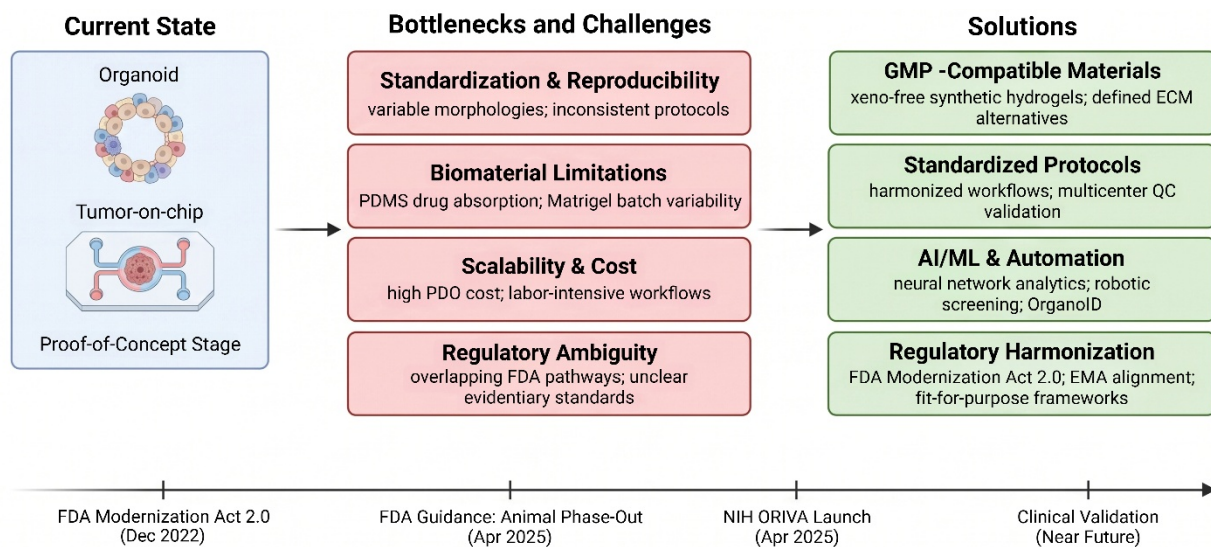


Figure 6. Current state, bottlenecks, and solutions for clinical translation of organoid and tumor-on-chip platforms. Left panel: Current proof-of-concept stage showing organoid and tumor-on-chip technologies. Center panel: Four major bottlenecks and challenges impeding clinical translation. (1) Standardization & Reproducibility: variable organoid morphologies and inconsistent protocols across laboratories limit inter-institutional comparability. (2) Biomaterial Limitations: polydimethylsiloxane (PDMS) absorption of hydrophobic drugs and Matrigel batch variability confound drug measurements and reproducibility. (3) Scalability & Cost: high costs of growth factors and biomaterials, combined with labor-intensive workflows, limit accessible adoption and high-throughput deployment. (4) Regulatory Ambiguity: overlapping FDA pathways (devices, biologics, combination products, software as a medical device) and unclear evidentiary standards create fragmented compliance burdens. Right panel: Proposed solutions addressing each bottleneck. (1) GMP-Compatible Materials: transition to xeno-free synthetic hydrogels and defined extracellular matrix alternatives that improve reproducibility and regulatory compliance. (2) Standardized Protocols: harmonized workflows with multicenter quality control validation to ensure inter-institutional consistency. (3) AI/ML & Automation: neural network analytics, robotic screening platforms, and OrganoidD deep learning for high-throughput, automated analysis. (4) Regulatory Harmonization: FDA Modernization Act 2.0 alignment, EMA coordination, and fit-for-purpose qualification frameworks. Bottom timeline: Key regulatory milestones including FDA Modernization Act 2.0 (December 2022), FDA Guidance on animal phase-out (April 2025), NIH ORIVA launch (April 2025), and anticipated clinical validation in the near future. Together, these integrated solutions position organoid and ToC platforms for clinical adoption and precision oncology implementation.

AI can make PDO research faster and more informative, but it should remain an aid to scientific and clinical reasoning rather than an independent decision-maker. The development team should be able to explain where the data came from, what the model has learned, where it is uncertain, and how consistently it performs across patient groups, laboratories, devices, and clinical settings. Independent testing, human review, careful version control, and protection against the inappropriate reuse of identifiable data are especially important when an algorithm contributes to treatment-related decisions. For national or international projects, local ethics review should be combined with clear arrangements for biosafety, sample custody, material transfer, data transfer, and compliance with the rules of every relevant jurisdiction. We do not regard these measures as unnecessary obstacles. They are the practical safeguards that can allow the promise of PDO-based precision oncology to be translated into clinical and collaborative settings without losing sight of the people whose samples make the work possible.

7. Future Perspectives and Conclusions

Organoid and tumor-on-chip research is rapidly converging with AI/ML, which is becoming an intrinsic component that accelerates analyses and enhances the predictive power of clinical oncology. Transfer learning mitigates the time- and sample-intensive nature of organoid pharmacotyping by exploiting large pharmacogenomic databases. PharmaFormer, pre-trained on two-dimensional cell line expression and drug response data and fine-tuned on limited patient-derived organoids, outperforms conventional organoid-only models in predicting clinical drug response [30]. In parallel, AI-guided automation stabilizes culture outcomes, standardizes workflows, and extracts interpretable signals from noisy and incomplete data sets, thereby improving reproducibility [142]. Miniaturized robotic screening further reduces the number of organoids per assay without losing concordance with clinical outcomes, shrinking samples and turnaround times [143]. Deep learning image cytometry and label-free time-lapse imaging now deliver automated, single-organoid readouts of response kinetics, invasive subclones, and immune–tumor dynamics at throughputs beyond those of manual or endpoint assays [96,97].

The translational goal is real-time decision support, where organoid avatars guide therapy selection, although this remains aspirational. Feasibility has been shown for glioblastoma CAR-T therapy: autologous organoids treated with the same CAR-T products as trial participants function as real-time avatars, capturing antigen loss and cytolysis in parallel with patient treatment, and revealing mechanisms as responses unfolded [50]. Achieving a closed-loop workflow—from biopsy to AI-augmented functional testing to therapy selection—at scale will require advances in materials and biofabrication for reproducibility and manufacturing, harmonized regulatory pathways under the evolving FDA Modernization Act 2.0 that recognize AI-derived organoid evidence, and generative AI digital twins to rapidly simulate patient-specific trajectories and triage options [144]. AI-driven digital pathology can add rapid and reproducible biomarker quantification directly from organoid or chip sections, shortening the time to action [145]. However, deployment must meet stringent standards for generalizability, data quality, and multi-site validation; algorithmic sophistication cannot replace rigorous prospective clinical validation for trustworthy use [146].

In conclusion, the integration of patient-derived organoids and tumor-on-chip systems with AI/ML is shifting translational oncology toward prospective, individualized functional precision medicine that can be delivered within actionable timelines. Evidence across tumor types and therapies—chemotherapy, targeted agents, checkpoint inhibitors, and adoptive cell therapies—shows meaningful predictive validity while illuminating mechanisms of resistance beyond clinical observations. The full impact depends on resolving standardization, material, scalability, and regulatory barriers, and on maturing AI-enabled analytics and automation that compress testing into the authentic therapeutic window. This will require sustained collaboration among biomaterials, bioengineering, computational biology, clinical oncology, and regulatory stakeholders. Nonetheless, the rapid rise in transfer learning models, automated miniaturized screens, and in-trial organoid avatars indicates that truly individualized, functionally validated therapy selection is a realistic goal for the next decade.

Author Contributions: M.V.B.: conceptualization, methodology, formal analysis, writing—original draft, writing—review and editing, visualization, project administration. T.D.: conceptualization, methodology, formal analysis, writing—original draft, writing—review and editing, supervision, project administration. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflicts of interest.

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