

REVIEW

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The application of organoids in treatment decision-making for digestive system cancers: progress and challenges

Yufei Wang^{1†}, Limin Zhang^{2,3†}, Louis Zizhao Wang^{4,5†}, Yang Cao^{6,7}, Lulu Huang^{6,8}, Gautam Sethi^{9,10}, Xiaoguang Chen^{1*}, Lingzhi Wang^{6,9,10*} and Boon-Cher Goh^{6,9,10,11*}

Abstract

Digestive system cancers—including gastric, liver, colorectal, esophageal, and pancreatic malignancies—remain leading causes of cancer death, with treatment resistance posing major challenges in advanced disease. Patient-derived cancer organoids (PDCOs), 3D mini-tumors grown from patient biopsies, have revolutionized personalized oncology by faithfully replicating tumor biology and enabling predictive drug testing for chemotherapy, radiotherapy, targeted therapy, and immunotherapy. While demonstrating good predictive accuracy, current limitations include incomplete tumor microenvironments, variable establishment rates, and lengthy processing times. Emerging technologies like AI, organ-on-chip systems, and 3D bioprinting are addressing these challenges, while clinical trials explore applications in neoadjuvant therapy and real-time treatment guidance. This Review highlights key advances in PDCO technology and its transformative potential for treatment decision-making in digestive system cancers, bridging laboratory research with clinical care to enable truly personalized therapeutic strategies tailored to individual tumor biology.

Keywords Digestive system cancers, Organoids, Tumor microenvironment, Drug screening, Precision oncology, Personalized medicine

[†]Yufei Wang, Limin Zhang and Louis Zizhao Wang contributed equally to this work.

*Correspondence:

Xiaoguang Chen
chen_xg@yangtzeu.edu.cn

Lingzhi Wang
csiwl@nus.edu.sg

Boon-Cher Goh
phcgbc@nus.edu.sg

¹School of Basic Medicine, Health Science Center, Yangtze University, Jingzhou 434023, China

²Jingzhou Hospital of Traditional Chinese Medicine, Jingzhou 434000, China

³The Third Clinical Medical College of Yangtze University, Jingzhou 434000, China

⁴Department of Gastroenterology & Hepatology, Changi General Hospital, Singapore 529889, Singapore

⁵Singhealth Duke-NUS Academic Medical Centre, Singapore 168582, Singapore

⁶Cancer Science Institute of Singapore, National University of Singapore, Singapore 117599, Singapore

⁷Department of Laboratory Medicine, The First Hospital of Jilin University, Jilin 130031, China

⁸Department of Radiation Oncology, First Affiliated Hospital of Guangxi Medical University, Shuang Yong Road 22, Nanning, Guangxi 530021, China

⁹Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117600, Singapore

¹⁰Yong Loo Lin School of Medicine, NUS Center for Cancer Research (N2CR), National University of Singapore, Singapore 117599, Singapore

¹¹Department of Haematology-Oncology, National University Cancer Institute, Singapore 119228, Singapore



Introduction

Cancers of the digestive system—including gastric, liver, colorectal, esophageal, and pancreatic cancers—represent a major global health challenge. According to Global Cancer Statistics 2022, colorectal, liver, and stomach cancers are among the top five causes of cancer-related death worldwide, collectively accounting for a significant portion of the 9.7 million cancer deaths reported annually [1]. These malignancies arise from a complex interplay of factors, including inherited genetic mutations such as *APC* and *TP53*, as well as modifiable environmental and lifestyle influences like diet, alterations in the gut microbiome, chronic inflammation, and infections by viruses or bacteria [2–9]. Despite advances in screening, diagnostics, and therapy, many patients with digestive system cancers are still diagnosed at late stages, where curative treatments are limited and outcomes remain poor [10]. Historically, treatment has been guided by histological subtype and staging. However, it became evident that patients with similar clinical features often respond differently to the same treatment. This recognition has propelled the shift toward *personalized medicine*—an approach that tailors treatment based on the unique molecular and functional characteristics of an individual's cancer.

One of the earliest breakthroughs in this field was the identification of specific genetic mutations that predict response or resistance to targeted therapy. For instance, *KRAS* mutations in colorectal cancer serve as a negative predictor for response to anti-EGFR therapies [11], while amplification of *HER2* in gastric cancer led to the approval of trastuzumab, one of the first targeted treatments in gastrointestinal (GI) oncology [12, 13]. The advent of next-generation sequencing (NGS) has further accelerated biomarker discovery, uncovering a broad spectrum of genetic and epigenetic alterations across various types of digestive system cancers [14]. Alongside genomic profiling, functional precision oncology tools have emerged to better predict therapeutic responses. Among these, patient-derived organoids (PDOs) stand out as highly promising. These three-dimensional (3D) cultures are grown from patient tumor biopsies and can faithfully replicate the cellular structure, genetic mutations, and drug sensitivity of the original tumor [15]. Unlike traditional two-dimensional (2D) cell lines, PDOs can be generated in just a few weeks and used to test a variety of treatment options *ex vivo*. They are particularly valuable in cancers with limited standard treatments—such as pancreatic and cholangiocarcinoma—where they may help guide off-label therapy decisions or enrollment into clinical trials.

Patient-derived cancer organoids (PDCOs) preserve not only the genetic features of the tumor but also important aspects of the tumor microenvironment, such as

stromal architecture, immune cell infiltration, and extracellular matrix interactions [16, 17]. This allows them to more accurately model drug responses, resistance mechanisms, and even predict efficacy of immunotherapies. For instance, PDCOs have been used to investigate immune checkpoint pathways like programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), helping identify patients who are most likely to benefit from immunomodulatory treatments [18, 19]. This level of personalized *ex vivo* testing provides clinicians with a valuable bridge between laboratory research and clinical care—allowing for more precise, effective, and safer treatment strategies.

In this review, we explore the progress and challenges of using PDCOs in treatment decision-making for digestive system cancers. We begin with a historical and technical overview of organoid development, then examine how PDCOs are applied to study treatment efficacy, resistance, and toxicity across various digestive system cancers. We also assess their emerging clinical applications in precision oncology. Finally, we highlight current limitations and suggest strategies to improve their clinical translation in the future.

Origin and development of organoids

Organoid technology: definition and classification

The development of organoid technology marks a major advancement in biomedical research, offering the ability to 3D *in vitro* tissue models that mimic the architectural and functional characteristics of human organs [20]. Organoids can be derived from pluripotent stem cells, adult stem or progenitor cells, or even differentiated cells from both healthy and diseased tissues [21]. When generated from patient samples, these models are referred to as PDOs. More specifically, when derived from tumor tissues—such as those obtained through surgical resection or biopsy—they are termed PDCOs (or PDTOs) [22, 23]. PDCOs exhibit strong self-organizing properties and accurately recapitulate the histological, genetic, and molecular characteristics of their parent tumors. This high fidelity makes them valuable tools for disease modeling and personalized cancer therapy.

Historical evolution of organoid research

The concept of cellular self-organization, which underpins modern organoid research, can be traced back to Wilson's 1907 experiment with siliceous sponges. He demonstrated that when dissociated into individual cells, sponge tissues could re-aggregate and self-organize under certain conditions, eventually regenerating into functional sponge structures [24]. The term “organoid” was later introduced in 1946 by Smith and Cochrane to describe organ-like elements found in teratomas [25].

Through the mid-20th century, developmental biologists showed that dissociated embryonic cells in species like amphibians and birds could reconstitute rudimentary tissue structures [26]. However, it was the isolation and characterization of pluripotent and adult tissue-specific stem cells that truly catalyzed the emergence of modern organoid systems [27–29].

Establishment and cultivation of PDCOs

PDCOs are typically established by enzymatically or mechanically dissociating tumor tissues into single cells or small clusters, which are then embedded in a supportive extracellular matrix such as Matrigel [30, 31]. These are cultured in media containing specific growth factors and signaling molecules, tailored to the tissue of origin. Notably, the introduction of Matrigel into gastric cancer organoid cultures increased the establishment rate from ~50% to nearly 78% [32, 33]. Alternative systems—such as the air–liquid interface (ALI) [18] and microfluidic chip platforms [34]—improve gas exchange and enable co-culture with immune or stromal components, further enhancing organoid viability and complexity. 3D

bioprinting [35] now allows for precise spatial organization, while genome-editing tools like CRISPR/Cas9 [36] enable direct manipulation of oncogenic pathways in organoids.

Advantages over traditional models

Compared to conventional 2D cell lines or in vivo models, PDCOs offer a balance of physiological relevance and scalability. Established cell lines such as HCT116 or HepG2 are easily maintained but often poorly reflect tumor heterogeneity and in vivo behavior [37–39]. Patient-derived xenografts (PDXs) and genetically engineered mouse models (GEMMs) offer more physiological insights but are limited by cost, long development times, and species-specific differences [40–42]. PDCOs preserve key characteristics of the original tumor—including histology and mutational landscapes—and can be expanded rapidly, often within 4–6 weeks [22, 43]. Their compatibility with high-throughput drug screening further supports their utility in translational research and precision oncology. Table 1 provides a comparative summary of PDCOs and other commonly used tumor models in cancer research.

Table 1 Performance characteristics of tumor models: a comparative analysis

Module	2D Cell Lines	GEMM	PDX	PDCO
Type	Ex Vivo	In Vivo	In Vivo	Ex Vivo or In Vitro
Cell Structure	2D	3D	3D	3D
Cost	Low	Very high	High	Moderate
Culture Conditions	Simple	Complex	Complex	Standard
Modeling cycle	1–3 Days	6–24 months	3–6 months	4–6 weeks
Genetic Stability of Original Tissue or Disease	May undergo passage-related variation	Stable	Stable	Stable
Tumor Heterogeneity	May lose some tumor features	Engineered	Preserved	Preserved
Disease Diversity Simulation	NA	YES	YES	YES
Variability in Implantation Rates	NA	NA	YES	NA
Species differences	NA	YES	YES	NA
TME Simulation	NA	YES	YES	YES
Scalability	High	NA	Low	Moderate
Standardization	High	Low	Low	Moderate

Note: GEMM: Genetically engineered mouse model. PDX: Patient-derived xenograft model. PDCO: Patient-derived cancer organoid. TME: Tumor microenvironment. 2D: Two-dimensional. 3D: Three-dimensional. NA: Not Applicable

Integration with AI and emerging technologies

Despite their promise, variability in PDCOs generation and characterization remains a significant barrier. Artificial intelligence (AI)-based tools are increasingly being employed to automate workflows and enhance reproducibility. As an important branch of AI, machine learning can assist in real-time monitoring, image-based analysis, and quality control by identifying features such as necrosis, proliferation, and morphological irregularities [44, 45]. For example, the SiQ-3D platform enables real-time visualization of T-cell-mediated tumor cell killing within PDCOs, helping predict responses to immune checkpoint blockade [46]. The OrBITS platform allows integrated imaging and analysis for medium-throughput drug screening in pancreatic cancer organoids. These innovations streamline organoid research and enhance clinical translatability [47].

Milestones in digestive system cancer organoid development

Although organoid technology has been explored for several decades, its broad application in digestive system cancer research gained momentum more recently. A major breakthrough occurred when Hans Clevers and colleagues established the first long-term culture system for intestinal organoids [48]. In 2011, they further advanced the field by generating tumor organoids from patient-derived colorectal adenomas, colorectal adenocarcinomas, and Barrett’s esophagus tissues [49]. By supplementing the culture media with growth factors like

FGF10, they successfully maintained organoids for over three months, marking a pivotal milestone in cancer-focused organoid research.

In 2015, Boj et al. [50] reported the development of pancreatic organoids from both human and mouse ductal cells. These models provided insight into the progression from precancerous lesions to invasive pancreatic cancer and served as a valuable platform for testing therapeutic agents. In 2017, Broutier et al. [51] established patient-derived liver cancer organoids—also known as “tumoroids”—that accurately mimic the histological, genetic, and molecular characteristics of major liver cancer types, including hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), and mixed HCC/CCA. These organoids retained key tumor-specific markers such as AFP and EpCAM, as well as common driver mutations like CTNNB1 and KRAS^{G12D}. As a result, they serve as reliable models for studying disease mechanisms and evaluating drug responses. Notably, these tumoroids also reproduced metastatic traits, making them a promising, scalable tool for personalized drug screening. This advancement represents a significant step toward precision oncology by linking tumor biology to clinical decision-making. Future improvements may include incorporating immune and stromal elements to better replicate the tumor microenvironment

(TME) and enhance predictive accuracy. By 2018, Nanki et al. [52] demonstrated that specific genetic alterations in patient-derived gastric cancer (GC) organoids directly influence their dependence on niche growth factors. For example, mutations in CDH1/TP53 and RNF43/ZNRF3 rendered the organoids independent of R-spondin and Wnt signaling, respectively, while ERBB amplifications allowed them to grow without EGF or FGF10. These genotype-phenotype relationships also predicted drug responses: tumors with RNF43 mutations showed sensitivity to Wnt pathway inhibitors, while ERBB-amplified organoids responded to HER2-targeted therapies (Fig. 1). These findings were validated in both organoid and xenograft models. By linking tumor genetics to niche factor requirements, this study supports the use of GC-organoids as a powerful tool for guiding personalized treatment decisions based on individual molecular profiles, advancing the field of functional precision oncology. Around the same time, Li et al. [53] developed PDCOs from esophageal adenocarcinoma tissues. Impressively, approximately 90% of the organoids survived beyond six months in culture. Medium-throughput drug sensitivity assays conducted on these models demonstrated their value in guiding personalized cancer therapy. Figure 2 provides a visual summary of these key milestones in the evolution of digestive system cancer organoid models.

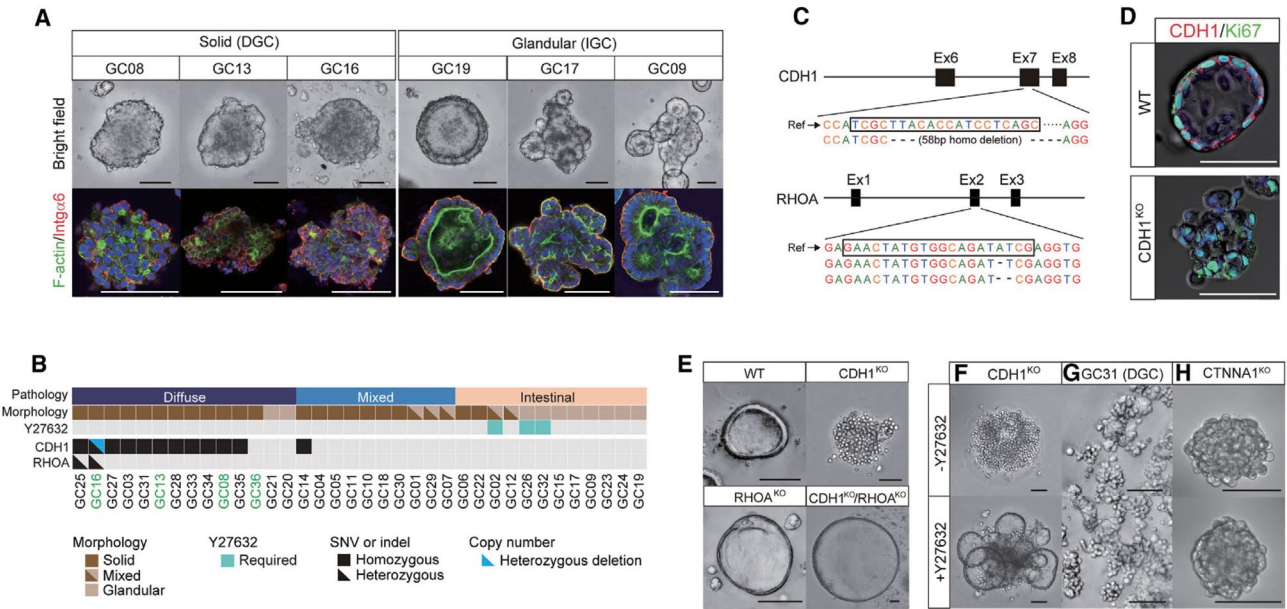


Fig. 1 Genetic mutations mediating morphological transformation of GC organoids. **A** Representative bright-field (top) and confocal (bottom) images of solid (left) and glandular organoid morphologies (right) with F-actin (green) and integrin- $\alpha 6$ (red) staining. **B** Pathological and morphological subtypes, and Y-27,632 requirement status of GC organoids, associated with CDH1 and RHOA alterations. GS-GC organoids are highlighted in green characters. **C** Sanger sequencing confirmation of CDH1 and RHOA KO in single KO organoids. Black frames show sgRNA targets. **D** CDH1 (red) and Ki67 (green) immunostaining validating CDH1 KO and the viability of CDH1KO organoids, respectively. **E** DGC-like morphological transformation by CDH1 KO and the retention of the cystic structure by RHOA single-KO and CDH1/RHOA DKO. **F–H** ROCK inhibitor (Y-27632) treatment on CDH1KO gastric organoids (F), DGC organoids (G) and CTNNA1KO gastric organoids (H). GC: Gastric cancer. GS: Genomically stable. KO: Knockout. DKO: Double knockout. DGC: Diffuse type GC. Reprinted from Cell, Vol 174/4, Nanki et al. Divergent Routes toward Wnt and R-spondin Niche Independency during Human Gastric Carcinogenesis, Pages No.5, Copyright 2018, with permission from Elsevier.

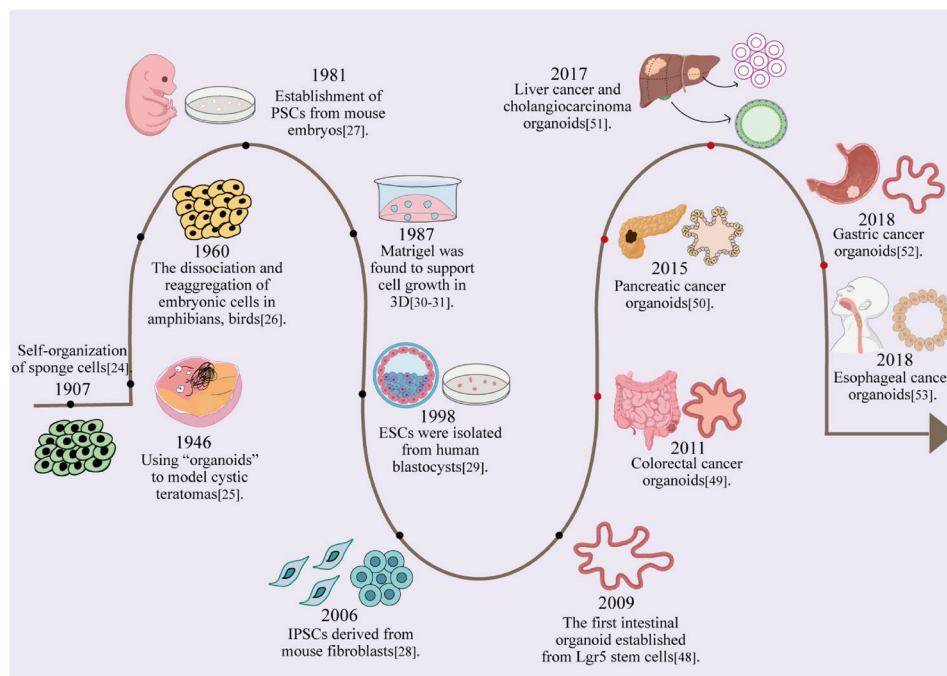


Fig. 2 Milestones in Organoid Development and Digestive System Cancer Organoid Models. Note: *PSCs*: Pluripotent Stem Cells. *3D*: Three-dimensions. *ESCs*: Embryonic Stem Cells. *iPSCs*: Induced Pluripotent Stem Cells. This figure was created with Adobe Illustrator

PDCO-Guided precision cancer therapy

Surgical removal remains the mainstay of treatment for early-stage digestive system cancers. However, for patients with more advanced disease, chemotherapy and radiotherapy can significantly improve outcomes [54–56]. Although chemotherapy, radiotherapy, and their combination are commonly used, these treatments can damage healthy tissues, cause significant side effects, and lack personalization. Targeted therapies [57, 58] offer more precise treatment by focusing on tumors with specific mutations like EGFR or ALK. However, these mutations are found in only a small group of patients, limiting the broader use of targeted drugs. Immunotherapy has revolutionized cancer treatment in recent years and improved survival for many patients [59, 60], but challenges remain. Only a minority of patients respond well, and treatment can lead to immune-related side effects. Moreover, both targeted and immune therapies are often expensive and not affordable for many families. Given these limitations, there is a growing need for better models to guide personalized treatment. PDCOs are small, lab-grown tumor models that closely resemble a patient's original cancer in terms of genetics, structure, and behavior [43]. These “mini-tumors” can be used to test how an individual's cancer is likely to respond to various treatments—such as chemotherapy, radiotherapy, targeted therapy, immunotherapy, or their combinations—before those therapies are actually given. Acting like “patient avatars,” PDCOs can be combined with genetic testing, multi-omics

data, and AI to help guide treatment choices, fine-tune drug dosages, and support more personalized and effective clinical decision-making [46, 47]. In this section, we examine how PDCOs are advancing personalized treatment strategies for digestive system cancers, including standard approaches such as chemotherapy, radiotherapy, targeted therapy, and immunotherapy, as well as emerging modalities like microbiome-based interventions and photodynamic therapy. Figure 3 summarizes the use of PDCOs in different therapeutic approaches. PDCOs serve as “patient avatars” to personalize treatment by replicating tumor biology and microenvironment. The figure highlights their role in bridging lab research and clinical decision-making, enabling precision oncology through functional drug testing and biomarker discovery. Emerging technologies (e.g., AI, 3D bioprinting) further enhance PDCO utility.

Chemotherapy

Chemotherapy continues to serve as a fundamental treatment modality for most digestive system malignancies, utilizing cytotoxic agents including DNA-damaging compounds and antimetabolites to inhibit tumor proliferation or induce apoptotic cell death [61]. Commonly used agents include fluoropyrimidines (e.g., 5-Fluorouracil analogs), platinum compounds, irinotecan, and taxanes. However, clinical experience has shown marked inter-patient variability in response to standardized chemotherapy regimens. As a result, many patients suffer from

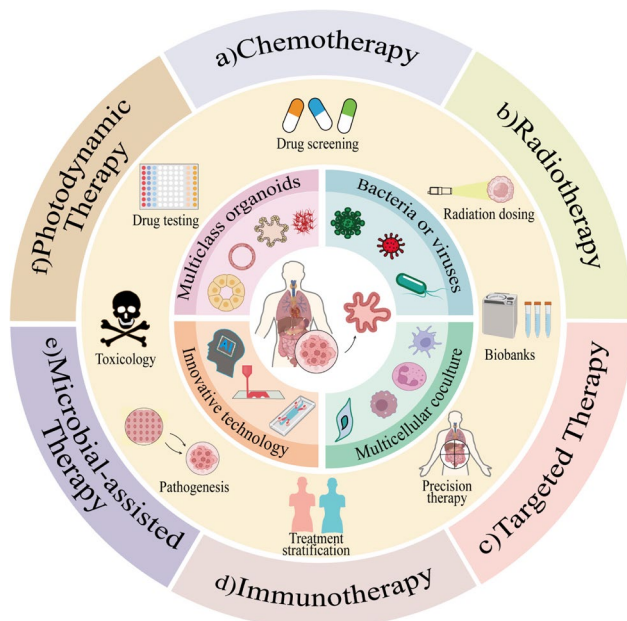


Fig. 3 Therapeutic Applications of PDCOs in Digestive System Malignancies. **a** Chemotherapy: PDCOs are used for drug screening and testing to predict patient responses to cytotoxic agents like 5-FU and oxaliplatin. **b** Radiotherapy: Organoids assess tumor radiosensitivity and optimize radiation dosing. **c** Targeted Therapy: PDCOs identify effective targeted drugs (e.g., KRAS inhibitors) and resistance mechanisms. **d** Immunotherapy: Co-cultures with immune cells evaluate checkpoint inhibitors (e.g., anti-PD-1) and CAR-T/NK therapies. **e** Microbial-Assisted Therapy: Probiotics and microbiota interactions are tested for anti-tumor effects. **f** Photodynamic Therapy (PDT): Organoids model light-activated treatments combined with drugs or immunotherapy. This figure was created with Adobe Illustrator

treatment-related toxicities without deriving significant therapeutic benefit [62]. PDCOs offer a promising solution by directly assessing the tumor's sensitivity to specific chemotherapeutic agents. This functional approach may help tailor individualized treatment plans and optimize therapeutic outcomes.

For instance, Zhao et al. [33] developed a high-fidelity biobank of 57 PDCOs from GC patients, achieving a 78% success rate in organoid establishment. These PDCOs closely retained the histological and molecular features of the original tumors, allowing for individualized drug testing. Importantly, the organoids accurately predicted patient responses to common chemotherapy agents such as 5-fluorouracil (5-FU) and oxaliplatin, with a clinical concordance rate of 91.7%. RNA sequencing further identified gene expression patterns associated with treatment response—such as higher expression of tumor suppressor genes in sensitive PDOs and proliferation-related genes in resistant ones—providing potential biomarkers for predicting drug efficacy ($AUC > 0.8$). In addition, co-culture experiments with cancer-associated fibroblasts (CAFs) and T cells revealed that CAFs could induce drug resistance, while microsatellite instability-high (MSI-H)

tumors showed strong T cell-mediated cytotoxicity [33]. These findings demonstrate the value of PDCOs in modeling the TME. Notably, drug screening could be completed in under two weeks and was validated in both xenograft models and actual patient outcomes. Overall, this work highlights the clinical potential of GC-PDCOs to guide personalized therapy, predict treatment response, and uncover mechanisms of resistance—bridging the gap between bench research and bedside decision-making in precision oncology.

In a separate clinical investigation, Cartry et al. demonstrates the feasibility of using PDCOs from colorectal cancer (CRC) patients, including core needle biopsies (61.5% success). The PDCOs maintained a 94% genomic similarity to the original tumor and generated chemo-gram (always presented in the form of heatmaps and dose-response curves) for 25 drug tests within 6 weeks. Effective drugs were identified for 92% of cases, including non-standard agents, and predictions matched clinical responses with 75% accuracy [63]. Fewer drug “hits” correlated with greater tumor burden and prior treatments. PDCOs show promise in guiding personalized treatment, identifying off-label options, and informing trial design—especially for refractory CRC patients without actionable mutations. Larger studies are needed to validate outcomes. A particularly illustrative case involved a patient with metastatic CRC to the liver who had previously undergone 11 lines of unsuccessful therapy. In the PDCO-based drug sensitivity screen, oxaliplatin emerged as the most effective agent. Guided by this result, the patient was re-challenged with an oxaliplatin-based regimen (capecitabine plus oxaliplatin, or CAPOX), which led to marked clinical improvement. This included normalization of liver enzyme levels, stabilization of tumor markers, and resolution of malignant ascites. However, due to the highly refractory nature of the disease and extensive metastatic burden, disease progression occurred six months later [63].

This case highlights the potential value of incorporating PDCO-guided drug sensitivity testing earlier in the treatment journey. Had such testing been available at diagnosis, it may have helped avoid ineffective therapies, delayed disease progression, and improved the patient's quality of life. These findings strongly support the need to accelerate the clinical translation of organoid-based technologies and underscore the importance of bridging the gap between PDCO research and real-world oncology practice.

Looking ahead, integrating polygenic risk modeling with PDCO-guided drug testing represents a promising “drug avatar” approach. This strategy enables clinicians to prospectively evaluate the efficacy of multiple therapeutic options for each patient, facilitating personalized

treatment selection while minimizing unnecessary toxicity, treatment delays, and financial burden.

Radiotherapy

Radiotherapy, one of the major approaches in cancer treatment, utilizes high-energy radiation—such as X-rays or particle beams—to induce DNA damage in tumor cells, leading to their elimination or the inhibition of their proliferation [64, 65]. While radiotherapy can be used alone, it is most often combined with chemotherapy to enhance therapeutic outcomes. Hsu et al. [66] explored the intrinsic radiosensitivity of CRC using PDCOs in conjunction with the single-hit multi-target (SHMT) algorithm. This model quantifies a tumor's inherent sensitivity to radiation by analyzing the D0 value—defined as the dose required to reduce the surviving fraction of cells to 37%. The higher the D0 value, the more radioresistant the tumor cells are. Interestingly, their study revealed that during the progression from colorectal adenoma to carcinoma, tumor cells acquire features that paradoxically increase their susceptibility to radiation-induced DNA damage. This counters the typical assumption that advanced tumors are more resistant to treatment [67]. The integration of PDCOs with the SHMT model provides a standardized in vitro platform to estimate the minimal effective radiation dose for individual patients. If validated in larger cohorts and across other cancer types, this approach could enable pre-treatment stratification and guide more personalized radiotherapy protocols, potentially improving outcomes while minimizing unnecessary toxicity.

Beyond radiosensitivity assessment, PDCOs are also proving useful in predicting responses to neoadjuvant chemoradiotherapy (nCRT). In a study by Yao et al. [68], organoid responses to chemoradiation in locally advanced rectal cancer (LARC) showed strong concordance with clinical responses, achieving 84.43% accuracy, 78.01% sensitivity, and 91.97% specificity. These results suggest that patients with PDCOs sensitive to nCRT may proceed with standard treatment, while those with resistant PDCOs could be spared ineffective therapy and associated toxicity. Following nCRT, LARC patients usually undergo surgery and adjuvant chemotherapy (AC) to reduce the risk of recurrence or metastasis [69]. For patients who respond poorly to nCRT, selecting the most effective AC regimen is critical. To address this, Xue et al. [70] developed a PDCO platform using tumor tissues from LARC patients who had poor responses to nCRT. These PDCOs were used to test multiple AC agents, enabling prediction of individual therapeutic benefits. By combining PDCO drug sensitivity data with tumor regression grade (TRG) and pathological nodal status, the researchers created a model that predicted 2- and 3-year disease-free survival (DFS) and overall survival (OS) with

high accuracy (AUCs of 0.902 and 0.885, respectively). Notably, the entire testing process was completed within 15–18 days—allowing timely post-surgical decision-making. Together, these studies highlight the growing clinical utility of organoid-based platforms in personalizing chemoradiotherapy regimens, stratifying patients based on expected response, and informing post-operative treatment choices.

Targeted therapy

Targeted therapy functions by selectively inhibiting molecular pathways that are abnormally activated in cancer, such as dysregulated kinases, epigenetic modifiers, and cell cycle regulators [57, 71]. Unlike conventional chemotherapy, which non-selectively kills rapidly dividing cells, targeted therapies aim to minimize harm to normal tissues by focusing on tumor-specific molecular alterations (e.g., HER2 amplification, ALK rearrangements). However, despite their precision, resistance to targeted therapy remains a major clinical hurdle [72, 73]. Tumor heterogeneity and adaptive evolution under therapeutic pressure can lead to resistance mechanisms, including loss of the drug target or activation of compensatory pathways.

In this context, PDCOs offer a valuable tool for personalized and translational oncology. They can be used for high-throughput drug screening, identification of novel drug targets, and rapid preclinical testing of new or repurposed compounds. For instance, Duan et al. [74] used PDCOs from pancreatic ductal adenocarcinoma (PDAC) to screen over 6,000 compounds, including FDA-approved drugs. Their study identified Perhexiline maleate as a selective inhibitor of KRAS-mutant PDAC organoids, with minimal toxicity to normal tissue-derived organoids. This is particularly significant given the limited clinical progress in targeting KRAS^{G12D/V} mutations—only MRTX1133 has entered clinical trials to date. Mechanistically, Perhexiline maleate suppresses SREBP2, a downstream effector in the PI3K/AKT/mTOR pathway, and interferes with metabolic reprogramming driven by mutant KRAS. For patients who develop resistance to first-line KRAS inhibitors, this drug—especially in combination with chemotherapy—offers a promising therapeutic alternative. Moreover, Yang et al. [75] systematically investigated lenvatinib resistance mechanisms using a multi-regional PDCO biobank derived from HCC. Their study identified c-Jun as a key mediator of resistance, operating through coordinated activation of JNK and β -catenin signaling pathways. Building on this mechanistic insight, the team developed a novel combinatorial therapeutic approach pairing lenvatinib with Veratramine (a c-Jun inhibitor), formulated as PKUF-01. This strategy demonstrated significant efficacy, increasing sensitivity in resistant organoids by 20%. This illustrates

a robust translational research model—from uncovering resistance mechanisms to rational drug design—enabled by organoid technology. Moreover, Vlachogiannis et al. [76] showed that PDCOs faithfully replicate patient tumors, enabling precise drug response predictions and advancing personalized medicine. For example, PDCOs identified differential sensitivity to TAS-102 in metastatic CRC, linking efficacy to thymidine kinase 1 (TK1) expression—a potential predictive biomarker. By modeling intra-tumor heterogeneity, PDCOs help tailor treatments to specific tumor subclones while avoiding ineffective therapies. Unlike genomic profiling alone, PDCOs functionally validate drug responses, improving clinical decision-making. Sequential PDCOs also track resistance evolution, allowing real-time therapy adjustments. Their integration into co-clinical trials accelerates precision oncology by matching treatments to individual patients, ensuring drugs like TAS-102 are used optimally

(Fig. 4). PDCOs thus bridge the gap between lab research and clinical practice, maximizing therapeutic success.

What's more, PDCOs also provide a critical platform for rare cancer types, where clinical trial data are often lacking due to low patient numbers. One such example is fibrolamellar carcinoma (FLC), a rare and aggressive liver cancer affecting adolescents and young adults, for which there are currently no approved therapies [77, 78]. A team led by Hans Clevers successfully generated FLC organoids and discovered that a fusion gene, BAP1-PRKAR2A, drives constitutive activation of PKA signaling and promotes tumor growth. They further showed that combined loss of BAP1 and PRKAR2A exerts synergistic oncogenic effects [79]. These findings highlight PKA signaling and epigenetic pathways as potential therapeutic targets in FLC. Although these strategies require further clinical validation, this work marks a critical step toward developing effective treatments for rare

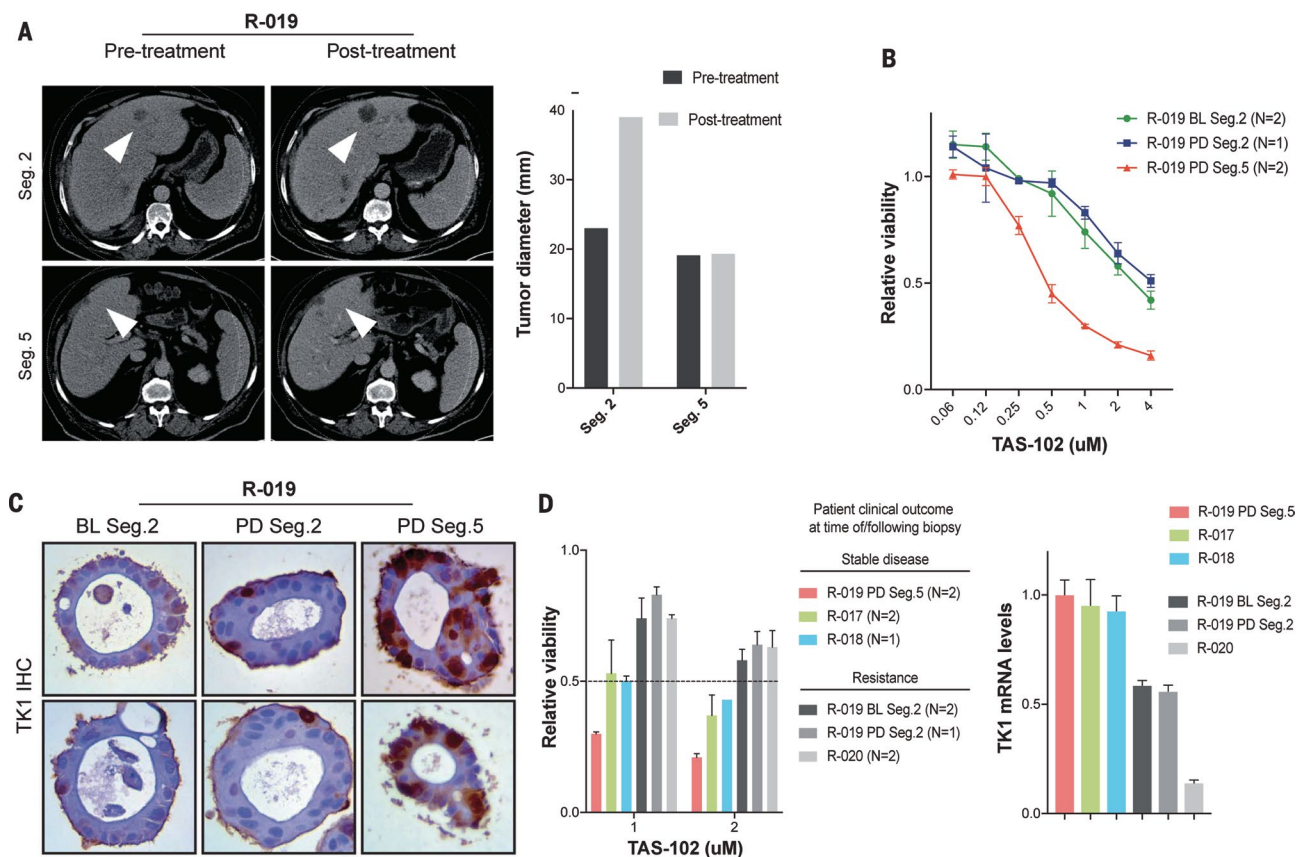


Fig. 4 Patient-derived organoids recapitulate intra- and inter-patient heterogeneity in response to TAS-102. **A** PDOs were established from a patient (R-019) with mixed response to TAS-102. While the segment 2 metastasis rapidly progressed, the segment 5 one remained stable upon TAS-102 treatment (white arrows in the CT-scan indicate metastases; bars indicate pre- and post-treatment measurement of the indicated metastases). **B** Ex vivo dose-response curves in baseline (BL) and post-treatment (PD) multi-region PDOs from patient R-019 (with mixed response to TAS-102). N= independent experiments; viability values are expressed as mean $\pm$ SEM. **C** TK1 immunohistochemistry (IHC) expression in TAS-102 refractory (segment 2) and sensitive (segment 5) PDOs. BL= baseline; PD= post-treatment/progressive disease. **D** Cell viability (left) and TK1 mRNA expression (right) in PDOs from TAS-102 responsive and refractory patients. BL= baseline; PD= post-treatment/progressive disease. N indicates independent experiments; viability values are expressed as mean $\pm$ SEM. CT: Computed tomography. Reproduced from Vlachogiannis et al., Science, DOI: <https://doi.org/10.1126/science.aao2774>, copyright 2018, AAAS.

liver tumors. In summary, PDCO platforms bridge the gap between laboratory research and clinical practice by enabling drug screening, elucidating resistance mechanisms, and supporting therapy development—especially in cases where conventional approaches fall short.

Immunotherapy

Over the past decade, immunotherapy has emerged as a cornerstone in cancer treatment, particularly with the introduction of immune checkpoint inhibitors (ICIs), adoptive cell therapies, and oncolytic viruses [59, 60, 80, 81]. These approaches either reactivate the host immune system to recognize and destroy cancer cells or directly lyse tumor cells using genetically engineered viruses. While immunotherapy has yielded promising results in some GI malignancies, a significant number of patients fail to respond [82]. This variability in response is largely due to tumor-intrinsic and microenvironmental factors that promote immune escape. Mechanisms such as tumor hypoxia [83], defective antigen presentation [84], abnormal angiogenesis [85], and metabolic reprogramming [86, 87] contribute to an immunosuppressive TME, undermining immunotherapeutic efficacy. However, PDCOs offer a promising platform to address these challenges. As *ex vivo* 3D cultures that retain the genetic and phenotypic characteristics of the original tumor, PDCOs can be co-cultured with autologous immune cells to model individualized immune responses [18]. This enables clinicians to evaluate a patient's likelihood of benefiting from specific immunotherapies, optimize treatment combinations, and avoid unnecessary toxicities and costs associated with ineffective interventions. In this way, PDCOs are accelerating the development of precision immunotherapy in digestive system cancers.

Currently, the most widely used ICIs target PD-1, PD-L1, and CTLA-4 [88]. Tumor cells can upregulate these checkpoints to evade immune surveillance, and in some cases, they also activate oncogenic signaling pathways. For example, in PDAC, PD-1 signaling has been shown to enhance tumor progression via the MAPK and PI3K/AKT pathways [89]. Gao et al. [89] found that PD-L1 exposure increased proliferation and MAPK activation in PDAC-derived PDCOs. Importantly, the combination of anti-PD-1 therapy with MEK1/2 inhibitors significantly enhanced cytotoxicity, suggesting that PD-1/PD-L1 expression may serve as a predictive biomarker to guide combination immunotherapy in PDAC. More advanced organoid models are also being developed to better simulate tumor–immune interactions. Zou et al. [90] created a microfluidic “organoid-on-a-chip” model by co-culturing HCC-PDCOs with CAFs, mesenchymal stromal cells (MSCs), and peripheral blood mononuclear cells (PBMCs). This system more accurately mimicked the TME and showed improved predictive sensitivity to

chemotherapy, targeted therapies, and ICIs compared to traditional PDCO cultures. Combination immunotherapies targeting multiple checkpoints are gaining momentum. Dual inhibition of PD-1 and CTLA-4 has been shown to improve response rates in metastatic melanoma [91], and similar approaches are being explored in digestive system cancers. Chalabi et al. [92] used a co-culture of early-stage CRC-PDCOs with PBMCs to evaluate neoadjuvant immunotherapy. The combination of PD-1 and CTLA-4 inhibitors yielded a robust pathological response in mismatch repair-deficient tumors, whereas only partial responses were observed in mismatch repair-proficient cases. These findings underscore the importance of patient stratification in immunotherapy, as a one-size-fits-all strategy remains inadequate.

In addition, PDCOs are being used to test and refine emerging immunotherapies such as chimeric antigen receptor T-cell (CAR-T) and chimeric antigen receptor natural killer cell (CAR-NK) therapies. Several studies have evaluated the cytotoxicity and safety of these therapies using digestive tumor PDCOs [93–95]. While CAR-T therapy alone shows limited efficacy, its combination with the apoptosis sensitizer birinapant significantly enhances PDCO killing through a tumor necrosis factor (TNF)-mediated bystander effect [93]. Notably, CAR-T cells can induce apoptosis in nearby tumor cells even without direct contact, supporting the integration of TNF-related biomarkers—such as serum TNF levels or TNF receptor expression—for treatment monitoring and adjustment. CAR-NK cell therapy has also demonstrated promise. Schnalzger et al. [95] showed that CAR-NK-92 cells selectively eradicated CRC-PDCOs while sparing normal tissue organoids, even in tumors with low antigen expression. These studies indicate that the use of patient-derived organoids allows for individualized evaluation of CAR therapies, facilitating the identification of tumor-specific antigens and predicting patient responses.

Oncolytic virotherapy is another novel immunotherapy being evaluated using PDCOs. In PDAC models, oncolytic adenoviruses selectively infected and destroyed tumor organoids without harming normal tissue [96]. These viruses also enhanced the efficacy of chemotherapy and helped overcome drug resistance by modulating the TME. Miao et al. [97] demonstrated that combining oncolytic viruses with bromodomain inhibitors (e.g., iBET-762, OTX-015) simultaneously eliminated tumor and stromal cells, outperforming standard therapies. Moreover, by engineering oncolytic viruses to express immune-stimulating payloads—such as PD-L1–targeting Fc fusion proteins—researchers have enhanced antitumor immunity while minimizing immune-related adverse events [98, 99]. Altogether, PDCO-based immunotherapy platforms enable the identification of effective treatment combinations and improve patient selection for tailored

interventions. These systems offer a path toward making neoadjuvant immunotherapy a standard of care in select digestive system cancers. However, robust validation through large-scale clinical trials remains essential.

Microbial-assisted therapy

The mammalian digestive tract plays a vital role in digestion and nutrient absorption and serves as a habitat for diverse microbial communities, including bacteria and viruses. While much of the previous research has centered on the pathogenic mechanisms of these microorganisms and their interactions with the host [100–102], the beneficial roles of certain microbiota—such as probiotics—have often been overlooked. Probiotics like *Lactobacillus* species and yeast have been widely reported to support gastrointestinal function and exert protective effects [103, 104]. However, their potential role in the treatment of digestive system cancers remains relatively underexplored.

Organoid technology offers a promising platform to investigate the therapeutic application of probiotics in cancer treatment [105–108]. Probiotics may inhibit tumor growth and proliferation in the digestive system, and their protective effects against tumorigenesis can be effectively captured using organoid models. Targeting these microorganisms may prove valuable in enhancing therapeutic efficacy. For instance, a study utilizing intestinal organoids co-cultured with lamina propria lymphocytes demonstrated that *Lactobacillus reuteri* D8 could protect organoids from treatment-induced toxicity, mitigate TNF- α -induced inflammation, and preserve organoid structure [105]. Another study identified a novel strain of *Lactococcus lactis*, HkyuLL 10, isolated from the feces of healthy individuals but depleted in CRC patients. This strain significantly induced PDCO death through two main mechanisms: restoration of intestinal microbiota homeostasis and secretion of the functional protein α -mannosidase. Since N-glycans promote tumor growth, oncogenic signaling, and metastasis [106], the α -mannosidase secreted by HkyuLL 10 exerts anti-tumor effects by catalyzing their degradation. Moreover, *Lactobacillus acidophilus* has demonstrated anticancer activity through the production of pentanoic acid, which was validated in HCC organoids associated with non-alcoholic fatty liver disease [107]. Exogenous lactate, by altering the NADH/NAD⁺ redox balance in tumors, has shown potential in inhibiting the growth and progression of Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC) PDCOs, while also modulating the inflammatory TME [108]. This opens the door for combining lactate with immunotherapy as a novel strategy for digestive system cancer treatment.

Oxaliplatin, a standard chemotherapy for digestive system cancers, exerts its antitumor activity partly through

interactions with the gut microbiome. Certain prebiotic microbiota can enhance myeloid cell infiltration and promote anti-tumor responses via reactive oxygen species (ROS) production, indicating that they may serve as adjuvants to improve therapeutic efficacy and mitigate resistance [109]. Clinically, probiotic-based oral supplements are sometimes used to prevent gastrointestinal side effects and may also support anticancer therapies. *Fusobacterium nucleatum* (Fn), known for its dual role, has previously been associated with tumor progression and chemoresistance [110]. However, in CRC-PDCO models, a high abundance of Fn was found to significantly enhance tumor sensitivity to PD-L1 blockade by promoting the infiltration of interferon- γ -producing CD8⁺ tumor-infiltrating lymphocytes (TILs), thereby improving the efficacy of ICIs [111].

Currently, microbial-assisted therapies are only practiced in a limited number of clinical settings. Nevertheless, with further validation of existing studies, the integration of microbiota-based strategies into mainstream cancer treatment is likely to accelerate and become an integral part of personalized oncology.

Photodynamic therapy

Photodynamic therapy (PDT) is a minimally invasive cancer treatment that has gained increasing attention for its therapeutic potential. PDT involves a two-step process: first, a photosensitizing agent is administered and accumulates in the tumor tissue; second, the tumor is exposed to a specific wavelength of light, which activates the photosensitizer in the presence of oxygen to generate reactive ROS. These ROS then induce tumor cell death or damage the tumor vasculature [112]. PDCOs, which closely mimic the histological and functional features of native human tissues, are emerging as a valuable tool to study PDT efficacy. Because organoids preserve tissue architecture and properties such as thickness and optical absorption, they allow for more accurate modeling of light penetration and ROS generation—factors that are critical for PDT success [113].

PDT can be used as a standalone treatment or in combination with conventional therapies. Its clinical relevance in digestive system cancers, such as CCA and pancreatic cancer, is being increasingly recognized [114–118]. Notably, PDT has already been incorporated into the National Comprehensive Cancer Network (NCCN) guidelines for palliation in patients with unresectable CCA [119], where it helps ablate tumors and relieve biliary obstruction. For example, Fujiwara et al. [114] demonstrated that CCA-derived PDCOs exhibited significantly higher photodynamic activity than organoids derived from non-cancerous bile ducts. This distinction suggests that PDCO-based PDT response could serve as a biomarker to identify patients who may benefit

from the therapy and potentially help classify indeterminate lesions. Beyond monotherapy, combining PDT with pharmacological agents shows strong therapeutic potential. Zheng et al. [115] found that combining PDT with sulfasalazine significantly increased apoptosis in CCA-PDCOs, achieving greater efficacy than either treatment alone. PDT-generated ROS can also induce ferroptosis, an iron-dependent form of programmed cell death [120]. In another study, Huang et al. [116] reported that combining sorafenib with PDT led to rapid ROS accumulation in CCA-PDCOs, allowing for dose reduction while maintaining strong tumoricidal effects.

In pancreatic cancer, the dense fibrotic stroma—rich in CAFs—presents a major barrier to effective treatment and contributes to therapeutic resistance [121]. Broekgaarden et al. [117] used PDCOs derived from metastatic pancreatic cancer to study the synergy between PDT and the chemotherapy drug oxaliplatin. They found that PDT enhanced both the immediate cytotoxic and long-term effects of chemotherapy, offering a potential strategy to overcome drug resistance. CAFs, which are particularly sensitive to PDT, also represent a promising therapeutic target. By disrupting these stromal cells, PDT may help reverse immunosuppression and improve immunotherapy outcomes [122, 123]. A particularly innovative approach was reported by Obaid et al. [118], who developed photothermal immuno-nanocomplexes (PINs) by combining photosensitizers with immune-targeting agents such as anti-EGFR monoclonal antibodies. When tested in co-cultures of pancreatic cancer PDCOs and CAFs, PINs—activated by near-infrared light—simultaneously eliminated tumor cells and CAFs. This disrupted the tumor-supportive microenvironment and significantly reduced PDCO viability, highlighting the potential of combining PDT with immunotherapy in stromal-rich tumors. Although PDT is currently used mainly for treating superficial malignancies like skin cancer, these emerging studies suggest that it could also be effective in deep-seated digestive system cancers, particularly when combined with targeted or immune-based therapies. The use of PDCOs has been instrumental in revealing these therapeutic opportunities, reinforcing their value in pre-clinical cancer research and personalized medicine.

Organoids in toxicity assessment

Conventional cancer treatments such as chemotherapy and radiotherapy remain essential for managing gastrointestinal (GI) tumors that cannot be surgically removed. However, these therapies often damage healthy tissues, leading to significant side effects. This not only adds to patients' physical and emotional burden but can also reduce their ability to tolerate and complete treatment. Unfortunately, many toxic effects only become apparent after drugs have entered clinical use. Therefore, thorough

preclinical toxicity testing is critical to minimize side effects, especially in the context of personalized oncology. Currently, animal models are indispensable for evaluating systemic toxicity, long-term safety, and fulfilling regulatory requirements, as they capture whole-body responses. At the same time, organoids—three-dimensional, lab-grown models of human organs—provide a valuable complementary platform. Organoids enable toxicity testing directly on human tissues and support faster, higher-throughput screening. Because they more accurately replicate human biology and help reduce reliance on animal testing, organoids are playing an increasingly important role in early drug development and personalized treatment planning.

Organoids for predicting drug-induced toxicity

Drug-induced toxicity, particularly within the GI tract—the primary site of drug absorption—is a common and often dose-limiting adverse effect during cancer treatment and other pharmacotherapies [124, 125]. While GI symptoms such as nausea, vomiting, and diarrhea are generally not life-threatening, they can severely compromise patient quality of life and treatment adherence. However, traditional preclinical models, especially animal studies, frequently fall short in predicting these toxicities with sufficient accuracy. For example, GI toxicity prediction rates remain suboptimal, reaching only 63% in non-rodent models and 43% in rodents [126]. To address these limitations, the U.S. FDA has endorsed the incorporation of advanced in vitro models, including human-derived organoid systems, into preclinical toxicity testing frameworks [127]. One particularly promising innovation is the air–liquid interface (ALI) intestinal organoid model developed by Peters et al. [128]. This system cultures human intestinal epithelial cells at the interface between air and culture medium, promoting differentiation and organization into a physiologically relevant epithelial barrier that closely mimics in vivo intestinal architecture.

This ALI-cultured organoid model was designed specifically to assess drug-induced diarrhea, a common but difficult-to-predict GI side effect. By exposing the apical surface of the organoid layer to test compounds while maintaining basolateral support, this setup enables accurate evaluation of epithelial integrity, fluid transport, and secretory responses. In validation studies, this model achieved 84% accuracy and 88% specificity in predicting diarrhea-inducing compounds—substantially outperforming conventional rodent models [128]. This approach not only enhances the physiological relevance of pre-clinical GI toxicity assays but also aligns with regulatory efforts to promote non-animal alternatives in safety assessment. As such, ALI intestinal organoids represent a valuable tool for improving the predictability and translational value of toxicity testing in drug development.

Beyond GI toxicity, organoids are now being used to evaluate liver and kidney toxicity—two other major concerns in systemic cancer therapy. Choi et al. [129] created liver organoids to study drug-induced liver injury (DILI), a leading cause of acute liver failure. This model successfully differentiated hepatotoxic drugs (e.g., ketoconazole, troglitazone, tolcapone) from non-toxic compounds (e.g., sucrose, ascorbic acid, biotin) by assessing cell viability and liver-specific functional markers like albumin secretion. Similarly, Digby et al. [130] used human-induced pluripotent stem cell (iPSC)-derived kidney organoids to model cisplatin-induced acute kidney injury (AKI). The organoids exhibited hallmark features of nephrotoxicity, such as DNA damage and tubular injury, when exposed to clinically relevant doses of cisplatin. These results demonstrate that PDOs can help predict individual susceptibility to nephrotoxicity, paving the way for personalized chemotherapy dosing. Additionally, such models offer valuable platforms for studying AKI mechanisms and evaluating nephroprotective interventions—aligned with the 3Rs principle (Replacement, Reduction, and Refinement) in preclinical research.

Multi-organ platforms and systemic toxicity

As organs function in a coordinated manner, toxicity assessment must extend beyond single-tissue models. Multi-organ organoid systems have emerged as advanced platforms to evaluate inter-organ responses to drug exposure. Skardal et al. [131] demonstrated that liver–cardiac organoid co-cultures better recapitulated toxicity profiles of FDA-recalled drugs, including troglitazone and rofecoxib, than 2D cultures or animal models. These organoids detected toxicities at clinically relevant doses that had previously gone unnoticed in preclinical testing. In addition, Skardal's team also developed a 3D organ-on-a-chip system that integrates liver, heart, and lung organoids into a circulating medium loop to simulate physiological organ interactions [132]. This setup enabled real-time monitoring of drug metabolism and downstream effects. For example, liver organoids accurately predicted acetaminophen toxicity and N-Acetylcysteine-mediated rescue, mirroring clinical outcomes. Moreover, this system uncovered previously unrecognized multi-organ toxicity from bleomycin, underscoring its ability to detect indirect or delayed toxic effects not evident in single-organ assays [132]. In summary, organoid-based toxicity platforms—ranging from single-tissue to multi-organ systems—offer enhanced predictive value and human relevance. Their adoption in preclinical drug development can improve safety assessments, support personalized medicine, and reduce reliance on animal testing.

Organoid models for radiation-related toxicity

Radiotherapy remains a cornerstone in the treatment of many digestive system cancers. While it is effective in killing tumor cells by inducing DNA damage, it inevitably causes collateral damage to surrounding healthy tissues. Escalating radiation doses to improve treatment outcomes has shown limited success and often leads to increased toxicity without substantial gains in efficacy [65, 133]. Currently, no FDA-approved therapies exist to prevent or treat radiation-induced injury. However, recent studies have explored several promising strategies to mitigate these adverse effects and improve tumor radiosensitivity [134, 135].

Nutritional and microbiota-based radioprotectants

Natural compounds, particularly plant polyphenols [136, 137], and dietary interventions—such as probiotics and prebiotics [138, 139]—have shown potential in reducing radiation-related GI toxicity. For example, studies using colon PDOs revealed that microbiota-derived tryptophan metabolites—especially indole-3-carboxaldehyde(I3A)—could protect intestinal tissue from radiation damage. I3A promotes the growth of beneficial gut bacteria like *Bifidobacterium* and *Lactobacillus*, while suppressing harmful species [134]. Since tryptophan is commonly found in foods such as chicken, soybeans, oats, and nuts, these findings suggest that dietary modulation may serve as an adjunct to reduce radiation-induced toxicity.

Gut-derived short-chain fatty acids as radiosensitizers

Improving tumor sensitivity to radiation may allow for effective treatment with lower, safer radiation doses. In a study using CRC-PDOs, Park et al. [135] tested the potential of short-chain fatty acids (SCFAs)—butyrate, propionate, and acetate—as radiosensitizers. Notably, butyrate significantly enhanced the effect of radiotherapy by activating FOXO3A, a transcription factor involved in stress response and cell cycle regulation. At the same time, it triggered cytoprotective responses in normal cells. SCFAs are natural products of fiber fermentation by gut microbes, highlighting the potential role of fiber-rich diets (e.g., grains, vegetables, fruits) in supporting treatment efficacy and reducing toxicity [140].

Together, these studies underscore the value of organoid-based systems in evaluating radiation toxicity and identifying potential radioprotective or radiosensitizing agents. Organoids offer a clinically relevant model for testing nutritional, microbial, and pharmacologic interventions, and may ultimately help tailor radiotherapy regimens that maximize efficacy while minimizing harm.

Clinical applications of PDCOs in digestive system malignancies

Organoid models, including PDCOs, have shown considerable promise in preclinical studies for drug screening, target validation, and patient stratification. Despite these encouraging findings, their direct clinical application remains under development and requires further validation. To assess the current landscape of PDCOs in clinical oncology, especially for digestive system cancers, we conducted a literature review through PubMed and ClinicalTrials.gov. This search yielded 46 peer-reviewed studies reporting PDCO establishment success rates, as well as 60 registered clinical trials focused on their clinical utility.

The success rate of establishing PDCOs varies significantly across cancer types (Table 2). For example, reported rates range from 26 to 91.3% for liver cancer, 57% for cholangiocarcinoma, 31–78% for esophageal cancer, 50–100% for gastric cancer, 59–100% for colorectal cancer, and 34.3–87.8% for pancreatic cancer. These PDCO models have primarily been used to study tumor heterogeneity, investigate drug resistance mechanisms, conduct drug screening, and identify novel therapeutic targets. Chemotherapy and targeted agents such as 5-FU, oxaliplatin, carboplatin, gemcitabine, paclitaxel, sorafenib, and lenvatinib have been tested across studies. Among the digestive cancers, colorectal and pancreatic cancers were most frequently studied, reflecting the clinical need for improved therapeutic strategies in these often treatment-refractory diseases.

One particularly impactful study by Boilève et al. [176] demonstrated the clinical relevance of PDCOs in guiding treatment for patients with advanced PDAC. The authors successfully generated organoids from 62% of 87 PDAC patients and found a high molecular concordance (91%) between the organoids and the original tumors. Drug testing performed on these organoids predicted treatment responses with a sensitivity of 83.3% and a specificity of 92.9%. Notably, patients treated with organoid-predicted drugs had significantly improved progression-free survival (PFS) (3.0 vs. 1.8 months) and OS (6.0 vs. 3.1 months). This study underscores the potential of PDCOs in guiding personalized treatment decisions and avoiding ineffective therapies. Furthermore, the study revealed a synergistic effect between anti-KRAS^{G12D} inhibitors (MRTX1133) and EGFR inhibitors, suggesting a novel combination strategy for KRAS-mutant PDAC. The result of this study showed that organoid-predicted therapies improved PFS and OS in advanced PDAC patients—yet this remains investigational. Leading in this approach enables personalized therapy and accelerates drug discovery. However, clinical implementation still faces challenges, as the median turnaround time varies across different cancer types, which also depends on the

source of samples and the type and scope of drug screening [75, 148, 151, 157, 174]. Moreover, most PDCO systems lack components of the immune microenvironment.

As of now (July 2025), no organoid-based personalized cancer medicine has received formal regulatory approval (e.g., by the FDA, EMA, or other major agencies) for routine clinical use. However, organoids are actively being used in translational and clinical research, especially in early-phase trials and precision oncology pilot programs. Clinical research involving PDCOs in digestive system cancers is rapidly advancing. We identified 60 clinical trials registered on ClinicalTrials.gov that incorporate PDCOs into digestive cancer research. Of these, five studies have been completed—though only one has published results—while 35 are ongoing and 20 remain in unknown status (Table 3). Notably, more than one-third of these trials aim to evaluate how well PDCO-based drug response predictions align with actual clinical outcomes in patients. This growing body of research highlights the increasing recognition of PDCOs as a promising tool in precision oncology. With continued optimization—particularly through the integration of TME components—PDCOs may soon offer a practical and reliable platform for guiding real-time, patient-tailored treatment decisions in clinical practice. Figure 5 illustrates the workflow of using PDCOs to assist in patient care. This workflow supports precision oncology by predicting therapeutic efficacy, minimizing ineffective treatments, and accelerating personalized therapy design. Future enhancements include integrating TME co-cultures and AI-driven automation.

Challenges and future perspectives

PDCOs hold great promise for transforming personalized treatment strategies in digestive system cancers. However, their clinical translation is currently limited by a range of biological, technical, and operational challenges that must be systematically addressed to realize their full potential. First, a major biological hurdle lies in the variability of PDCO establishment across tumor types. Success rates are inconsistent, largely influenced by tumor cellularity, stromal content, and biopsy quality [180, 181]. Mucinous, necrotic, or poorly cellular tumors often yield low organoid generation efficiency, hindering consistent model development [156, 182]. Second, conventional PDCOs do not fully recapitulate the TME, which is critical for evaluating therapies—particularly immunotherapies—that depend on TME interactions [183]. To overcome this, recent advances have introduced co-culture systems incorporating autologous immune and stromal components, including TILs, CAFs, dendritic cells, and peripheral immune cells [18, 184]. For instance, co-culturing PDCO with matched CD8⁺ T cells has enabled functional assessment of immune checkpoint

Table 2 Clinical application of patient-derived cancer organoids from pubmed database

Cancer Type	Patient Sample Size	PDCOs culture time	PDCOs success rate	Application	Drugs used	Year	References
Liver cancer	8	/	26%	Biobank Drug testing	Sorafenib	2018	[141]
Liver cancer	66	2–3 weeks	40.9%	Drug screening Coherence assessment Personalized treatment	Sorafenib, Regorafenib, Donafenib, Lenvatinib, Cabozantinib, Oxaliplatin, FOLFOX, XELOX ...	2024	[142]
Liver cancer	144	/	75.6%	Resistance prediction Biomarker development	Sorafenib, Lenvatinib, Regorafenib, Apatinib, Bevacizumab ...	2024	[75]
Liver cancer	77	≥ 8 weeks	91.3%	Mutation identification Drug screening Clinical relevance	Sorafenib, Lenvatinib, Regorafenib ...	2025	[143]
Intrahepatic Cholangiocarcinoma	6	> 1 year	50%	Mutation identification Drug screening Biomarker development	EGFR inhibitors, Kinase inhibitors, mTOR inhibitors, Proteasome inhibitors ...	2019	[144]
Cholangiocarcinoma	8	1–3 weeks	57%	Target testing Drug testing	Gemcitabine, Gemcitabine + Cisplatin, Cisplatin, Cabozantinib, JAK inhibitor ...	2025	[145]
Hepatobiliary cancer	/	/	> 60%	Gene-drug associations Drug screening	KEAP1-beta-Lapachone, NF1-afatinib ...	2024	[146]
Esophageal cancer	32	> 6 months	31%	Clonal dynamics Drug testing	ERK inhibitor/EGFR inhibitor ...	2018	[53]
Esophageal cancer	28	1 year	57.2%	Coherence assessment Drug testing	Cisplatin, Paclitaxel, 5-FU ...	2020	[147]
Esophageal cancer	9	/	78%	Drug testing	Cisplatin, Paclitaxel, γ-irradiation, FOLFOX	2021	[148]
Esophageal cancer	55	/	61.8%	Drug testing Coherence assessment	Cisplatin, Paclitaxel + Cisplatin, Vinorelbine + Cisplatin, 5-FU + Cisplatin	2024	[149]
Gastric Cancer	34	/	> 50%	Heterogeneity Exploration Drug screening	5-FU, Cisplatin, Oxaliplatin, Napabucasin, Abemaciclib ...	2018	[32]
Gastric Cancer	3	5–8 days	100%	Drug Validation	Paclitaxel	2020	[150]
Gastric Cancer	10	/	80%	Drug testing	Albu-PTX, Lipo-PTX	2022	[151]
Gastric Cancer	24	/	54%	Resistance prediction Drug testing	FLOT, FOLFOX	2023	[152]
Gastric Cancer	11	/	100%	Drug Validation	Luteolin	2023	[153]
Gastric Cancer	50	/	56%	Drug testing Combination therapy Target testing	5-FU, Oxaliplatin, Docetaxel, 5-FU + Veliparib	2024	[154]
Gastric Cancer	18	2 weeks	> 60%	Drug testing Drug screening	5-FU, Paclitaxel, Oxaliplatin, Irinotecan, Epirubicin, Afatinib ...	2024	[155]
Gastric Cancer	73	/	78%	Drug screening/TME Coherence assessment	5-FU, Oxaliplatin, Cisplatin, Paclitaxel, Doxorubicin, SN-38	2024	[33]
Colorectal Cancer	20	/	~ 90%	Biobank Drug screening	Cetuximab, Oxaliplatin, 5-FU ...	2015	[156]
Colorectal Cancer	61	/	63%	Drug testing Coherence assessment	Irinotecan, 5-FU + Irinotecan, 5-FU + Oxaliplatin	2019	[157]
Colorectal Cancer	28	/	68%	Drug testing	FOLFOX, FOLFIRI	2020	[158]
Colorectal Cancer	22	/	76%	Drug testing Resistance prediction	Ipatasertib, Linsitinib, Targeting EGFR drugs...	2020	[159]
Colorectal Cancer	54	/	75%	Drug testing Drug repurposing screening	5-FU, oxaliplatin, Cetuximab, SN38	2022	[160]
Colorectal Cancer	50	/	82%	Drug testing Biomarker development Resistance prediction	5-FU	2022	[161]

Table 2 (continued)

Cancer Type	Patient Sample Size	PDCOs culture time	PDCOs success rate	Application	Drugs used	Year	References
Colorectal Cancer	36	/	80.6%	Drug testing Coherence assessment	FOLFOX, FOLFIRI	2022	[162]
Colorectal Cancer	52	/	80.8%	Drug screening Coherence assessment	5-FU, Oxaliplatin, Irinotecan	2023	[163]
Colorectal Cancer	34	/	59%	Drug screening Coherence assessment	SN-38, Palbociclib, Trametinib, Alpelisib ...	2023	[164]
Colorectal Cancer	31/9	3 weeks	80%/60% ^{†1}	Drug testing	5-FU, Irinotecan, Oxaliplatin, Cetuximab, Cetuximab + Encorafenib ...	2023	[165]
Colorectal Cancer	12/5	/	83.3%/80% ^{†2}	Drug testing Coherence assessment Resistance prediction	5-FU, Oxaliplatin, Leucovorin, SN-38, Regorafenib	2024	[166]
Colorectal Cancer	8	> 3 months	100%	Drug repurposing Drug screening	Romidepsin, Trametinib, Bortezomib, 5-FU, Regorafenib ...	2024	[167]
Colorectal Cancer	16	/	93.7%	Target testing Drug testing	FOLFOX, FOLFIRI, Carboplatin, 5-FU ...	2025	[168]
Rectal Cancer	58	/	77%	Drug testing Radiation response Target validation	5-FU, FOLFOX, Cetuximab	2019	[169]
Rectal Cancer	112	/	85.7%	Drug testing Coherence assessment	NCRT	2020	[68]
Rectal Cancer	138	/	62.3%	Drug testing	5-FU, Oxaliplatin	2023	[70]
Pancreatic cancer	20	3–7 weeks	85%	Drug testing	Gemcitabine, G9a (A366), EZH2	2015	[170]
Pancreatic cancer	138	/	75%	Drug screening Coherence assessment	Gemcitabine, Nab-Paclitaxel, Irinotecan 5-FU, Oxaliplatin	2018	[171]
Pancreatic cancer	15	/	81%	Heterogeneity Exploration	/	2021	[172]
Pancreatic cancer	8	/	42%	Drug Discovery Drug screening Coherence assessment	Gemcitabine, Paclitaxel, Kinase inhibitor	2022	[173]
Pancreatic cancer	31	3 weeks	87.8%	CTCs-PDCOs Drug testing	5-FU, Oxaliplatin, Paclitaxel, Irinotecan	2022	[174]
Pancreatic cancer	48	/	71%	Drug testing Coherence assessment	ERK inhibitor + Chloroquine	2024	[175]
Pancreatic cancer	87	/	62%	Coherence assessment	Oxaliplatin, Carboplatin, kinase inhibitors, Epigenetic drugs ...	2024	[176]
Pancreatic cancer	16	/	52.2%	Heterogeneity Exploration	/	2025	[177]
Pancreatic cancer	67	/	34.3%	Drug screening Coherence assessment	FOLFIRINOX, Gemcitabine + Paclitaxel, Cisplatin + Olaparib ...	2025	[178]
Gastrointestinal cancers	71	/	70%	Drug testing Biomarker development	Paclitaxel, Cetuximab, Regorafenib, TAS-102	2018	[76]
Esophago-gastric cancer	120	/	61%	Drug testing Patient stratification	5-FU, Oxaliplatin, Docetaxel, 5-FU + Calcium folinate ...	2024	[179]

Note: Search strategy: The following keywords were searched in the PubMed database: (patient-derived cancer organoids[Title]) AND (digestive cancer[Title/Abstract]); (patient-derived tumor organoids[Title]) AND (digestive cancer[Title/Abstract]); (patient-derived organoids[Title]) AND (digestive cancer[Title/Abstract]). "Digestive cancer" can be replaced with liver cancer, cholangiocarcinoma, gastric cancer, colorectal cancer, intestinal cancer, esophageal cancer, and pancreatic cancer for comprehensive retrieval. After inclusion and exclusion based on relevance to this article, 46 experiments were included for analysis

FOL: Folinic acid. **5-FU:** 5-Fluorouracil. **SN38:** An active metabolite of Irinotecan. **FOLFOX:** 5-FU + Oxaliplatin. **XELOX:** Oxaliplatin + Capecitabine. **FLOT:** Docetaxel + Oxaliplatin. **FOLFIRI:** FOL + 5-FU + Irinotecan. **FOLFIRINOX:** 5-FU + Leucovorin + Irinotecan + Oxaliplatin. **mTOR inhibitor:** Mechanistic target of rapamycin inhibitor. **JAK inhibitor:** Janus Kinase inhibitor. **ERK inhibitor:** Extracellular Signal-Regulated Kinase inhibitor. **Albu-PTX:** Albumin-bound Paclitaxel. **Lipo-PTX:** Paclitaxel Liposome. **EGFR inhibitor:** Epidermal growth factor receptor inhibitor. **KEAP1:** Kelch-like ECH-associated protein 1. **NF1:** Neurofibromin 1. **NCRT:** Neoadjuvant chemoradiotherapy. **PDCOs:** Patient-derived cancer organoids. **CTCs:** Circulating tumor cells. ^{†1}: The PDCOs success rates were derived from two cohorts. ^{†2}: The two PDCO success rates were derived from the primary site (left) and liver metastasis (right), respectively

Table 3 Clinical application of patient-derived cancer organoids from clinicaltrials.gov

NCT Number	Status	Cancer Type	Stage of Cancer	Phase	Application	Outcome Measurement	Sample Size	Start date
NCT06196554	Recruiting	Gastric Cancer	All stage	NA	Drug screening	Sensitivity, Specificity, Consistency	40	2023-05-01
NCT06100003	Recruiting	Gastric Cancer	All stage	Phase 1	Drug testing	DFS, Sensitivity, Specificity	104	2023-10-18
NCT05442138	Unknown	Gastric Cancer	Early	NA	Drug testing	ORR, TRG	54	2022-06-28
NCT05351398	Unknown	Gastric Cancer	Advanced	NA	Treatment comparison	ORR, TRG, RR	54	2022-04-28
NCT05203549	Unknown	Gastric Cancer	All stage	NA	Treatment consistency observations	PDCOs success rate, Correlation	250	2021-05-01
NCT06907342	Recruiting	Colorectal Cancer	Metastasis	Phase 2	Drug testing	PDCOs success rate, ORR, DCR, PFS, OS, DOR	148	2025-05-23
NCT06787625	Recruiting	Colorectal Cancer	Primary/ Metastasis	NA	PDCOs Establishment	PDCOs success rate	10	2024-05-25
NCT06136949	Recruiting	Colorectal Cancer	All stage	NA	Drug testing	PFS, Correlation	150	2023-05-22
NCT06100016	Recruiting	Colorectal Cancer	All stage	Phase 1	Drug testing	PFS, Sensitivity, Specificity	105	2023-10-18
NCT05955196	Recruiting	Colorectal Cancer	All stage	NA	Drug testing	PDCOs success rate	115	2023-01-09
NCT05883683	Not yet recruiting	Colorectal Cancer	Advanced/ Recurrent	NA	Drug testing/ Target prediction	Viability of PDCOs, Correlation, Sensitivity	100	2024-10-01
NCT05832398	Recruiting	Colorectal Cancer	All stage	NA	Drug testing	Correlation, PFS, OS	186	2023-05-01
NCT05725200	Recruiting	Colorectal Cancer	Metastasis	Phase 2	Treatment consistency observations	ORR, PFS, DOR, OS, Safety and tolerability	40	2022-09-27
NCT05401318	Recruiting	Colorectal Cancer	All stage	NA	Drug testing	PDCOs success rate, Sensitivity	40	2022-03-28
NCT05352165	Not yet recruiting	Rectal Cancer	Advanced	NA	Treatment comparison	PCR, Complication, Recurrence, Metastasis	192	2023-01-01
NCT05304741	Recruiting	Colorectal Cancer	Advanced/ Recurrent/ Metastasis	NA	PDCOs Establishment/ Drug screening	PFS, OS, Sensitivity, Specificity	30	2020-01-01
NCT05183425	Unknown	Colorectal Cancer	Metastasis	NA	Drug testing	Correlation, PDCOs success rate	60	2022-01-01
NCT05038358	Recruiting	Colorectal Cancer	Primary	NA	Drug Resistance	PDCOs success rate	60	2022-12-12
NCT04996355	Unknown	Colorectal Cancer	All stage	NA	Drug screening	Accuracy, Specificity, Sensitivity	52	2021-05-31
NCT04906733	Unknown	Colon Cancer	All stage	NA	Treatment consistency observations	Sensitivity, Correlation	80	2021-04-15
NCT04755907	Unknown	Colorectal Cancer	All stage	NA	Model comparison	PDCOs sensitivity, Correlation, DFS	120	2021-03-01
NCT04371198	Completed	Rectal Cancer	All stage	NA	PDCOs Establishment	PDCOs success rate	20	2020-09-18

Table 3 (continued)

NCT Number	Status	Cancer Type	Stage of Cancer	Phase	Application	Outcome Measurement	Sample Size	Start date
NCT03577808	Unknown	Rectal Cancer	All stage	NA	Drug/Irradiation testing	Correlation, PDCOs success rate	80	2018-08-17
NCT07054086	Recruiting	Esophageal Cancer	All stage	NA	Drug testing	PDCOs success rate, Correlation	30	2025-03-01
NCT03283527	Recruiting	Esophageal Cancer	All stage	NA	Drug/Irradiation testing	PDCOs success rate, Sensitivity, PFS, OS	100	2017-12-01
NCT06929845	Recruiting	Liver Cancer	All stage	NA	PDCOs Establishment	PDCOs success rate, Correlation	150	2024-10-01
NCT06699524	Active	Liver Cancer	All stage	NA	Drug testing	PFS	122	2022-09-20
NCT06355700	Recruiting	Liver Cancer	All stage	NA	PDCOs Establishment	co-culture PDCOs success rate, Correlation	10	2023-06-15
NCT05932836	Active	Liver Cancer	All stage	NA	Drug testing	PDCOs success rate, Accuracy	165	2023-03-01
NCT05913141	Recruiting	Liver Cancer	All stage	NA	Drug testing	PDCOs/co-culture PDCOs success rate, ORR, PFS, RFS...	30	2023-06-22
NCT05644743	Not yet recruiting	Intrahepatic Cholangiocarcinoma	All stage	NA	Treatment consistency observations	Correlation, Drug resistance model	40	2022-12-09
NCT05634694	Unknown	Cholangiocarcinoma	All stage	NA	Treatment consistency observations	PFS, Sensitivity	40	2022-11-30
NCT04561453	Completed	Cholangiocarcinoma	All stage	NA	Drug testing	PDCOs success rate, Correlation	14	2020-07-08
NCT04072445	Completed	Cholangiocarcinoma	Advanced	Phase 2	Drug resistance	Correlation, Clinical Benefit	28	2019-10-18
NCT06813079	Not yet recruiting	Pancreatic Ductal Carcinoma	Advanced	Phase 2	Drug testing	ORR, DCR, DOR, PFS, OS, Side effects	25	2025-02-17
NCT06666803	Not yet recruiting	Pancreatic Ductal Adenocarcinoma	All stage	NA	PDCOs Establishment	PDCOs success rate, Correlation	60	2024-10-31
NCT06615830	Not yet recruiting	Pancreatic Cancer	Metastasis	Phase 2	Drug testing	PDCOs sensitivity, PFS, Correlation	185	2024-09-27
NCT05927298	Recruiting	Pancreatic Cancer	All stage	NA	PDCOs Establishment	PDCOs success rate, Correlation, AI platform establishment	200	2023-03-06
NCT05351983	Unknown	Pancreatic Cancer	All stage	NA	Drug screening	PDCOs success rate, Safety, Contamination rates	50	2022-09-22
NCT05196334	Recruiting	Pancreatic Cancer	All stage	NA	Drug testing	PDCOs response to therapy	88	2021-07-01
NCT04931394	Unknown	Pancreatic Cancer	All stage	Phase 3	Drug testing	DFT, OS, DFS PDCOs success rate, Correlation	200	2021-06-01
NCT04931381	Unknown	Pancreatic Cancer	Advanced	Phase 3	Drug testing	DCR, PFS, OS, PDCOs success rate, Correlation	100	2021-06-01
NCT04777604	Not yet recruiting	Pancreatic Cancer	All stage	NA	Drug testing/Prognosis	OS	300	2021-03-01

Table 3 (continued)

NCT Number	Status	Cancer Type	Stage of Cancer	Phase	Application	Outcome Measurement	Sample Size	Start date
NCT04736043	Recruiting	Pancreatic Cancer	All stage	NA	Drug testing/Prognosis	OS	300	2021-01-31
NCT04469556	Active	Pancreatic Cancer	Advanced/Metastasis	Phase 2	Treatment comparison	PFS, ORR, OS, Correlation	150	2020-10-14
NCT03990675	Unknown	Pancreatic Cancer	All stage	NA	PDCOs Establishment	PDCOs success rate	50	2018-12-01
NCT03544255	Unknown	Pancreatic Cancer	All stage	NA	Drug screening	PDCOs success rate, Correlation	50	2018-05-01
NCT03500068	Unknown	Pancreatic Cancer	Metastasis	NA	PDCOs Establishment	PDCOs success rate, Biomarkers	30	2017-09-04
NCT03140592	Unknown	Pancreatic Cancer	All stage	NA	PDCOs Establishment	PDCOs success rate	300	2015-01-14
NCT06519500	Recruiting	Gastroenteropancreatic Neuroendocrine Tumor	All stage	NA	PDCOs Establishment	PDCOs success rate	40	2023-03-03
NCT06246630	Recruiting	Pancreatic Neuroendocrine Tumor	Advanced	NA	Drug testing	PDCOs success rate, ORR, PFS, OS, Correlation	20	2024-04-03
NCT04927611	Unknown	Gastroenteropancreatic Neuroendocrine Tumor	All stage	NA	PDCOs Establishment	PDCOs success rate, TME	200	2021-06-06
NCT06332716	Recruiting	Gastrointestinal Tumors	All stage	Phase 3	Drug testing	PFS, OS	68	2022-08-26
NCT06077591	Recruiting	Liver Cancer/Colorectal Cancer	Advanced/Inoperable	Phase 3	Drug testing	Tumor response, ORR, PFS, OS, PDCOs success rate	40	2024-10-18
NCT05842187	Unknown	Pancreatic Cancer/Gastric Cancer	Metastasis	NA	Treatment consistency observations	PFS, DCR	20	2023-03-03
NCT05652348	Recruiting	Gastric Cancer/Colorectal Cancer	All stage	NA	Drug testing	Sensitivity, DNA/RNA sequence	48	2022-12-08
NCT05384184	Completed	Liver Cancer/Colorectal Cancer	Metastasis	NA	PDCOs Establishment	PDCOs success rate, Correlation	48	2019-06-06
NCT04611035	Unknown	Gastrointestinal Cancers	All stage	NA	Drug screening	PDCOs success rate, ORR	100	2020-01-20
NCT03429816	Completed	Gastric Cancer/Esophageal Cancer	All stage	NA	Drug testing	Correlation, Histological regression, PFS, OS	120	2018-04-15
NCT02436564	Unknown	Cholangiocarcinoma/Liver Cancer/Pancreatic Cancer	All stage	NA	PDCOs Establishment	PDCOs success rate	75	2015-05-07

Note: Search strategy: A combined search was performed on the ClinicalTrials.gov platform by selecting the disease: Digestive cancer/tumor; and the term: organoids. "Digestive cancer" can be replaced with liver cancer, cholangiocarcinoma, gastric cancer, colorectal cancer, intestinal cancer, esophageal cancer, and pancreatic cancer for comprehensive retrieval. After inclusion and exclusion based on relevance to this article, 60 clinical trials were included for analysis

PDCOs: Patient-derived cancer organoids. DFS: Disease-free survival. ORR: Objective response rate. TRG: Tumor regression grading. RR: Recurrence rate. DCR: Disease control rate. OS: Overall survival. DOR: Duration of Response. PFS: Progression-free survival. RFS: Recurrence-free survival. DFT: Disease free time. PCR: Pathologic complete response. TME: Tumor microenvironment

inhibitor responses, offering individualized insights into immune competence and therapeutic sensitivity [185, 186]. Third, the absence of vascularization and perfusion in static PDCO cultures restricts nutrient diffusion and compromises physiological relevance. Organ-on-a-chip technologies offer a promising solution by integrating

PDCOs into microfluidic systems that mimic mechanical forces, real-time drug exposure, and immune cell trafficking [187, 188]. These platforms allow for dynamic modeling of drug pharmacokinetics and anti-angiogenic responses in a patient-specific manner [189]. Notably, vascularized digestive tract tumor-on-chip systems

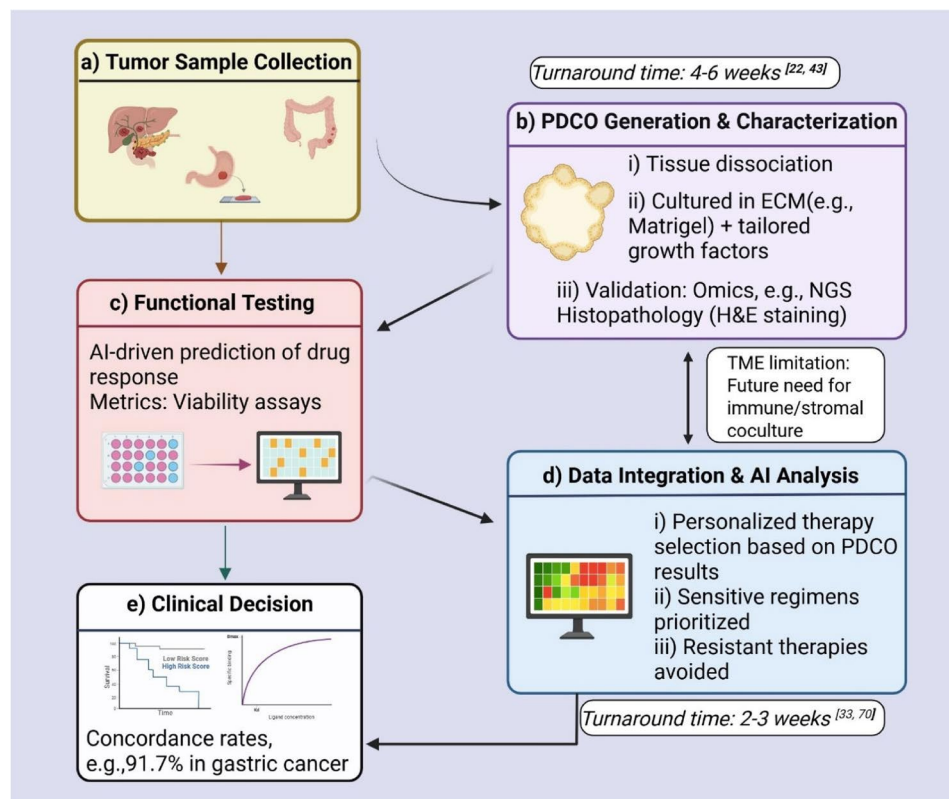


Fig. 5 The workflow of PDCOs-guided patient treatment. **a** Tumor Sample Collection: Biopsies or surgical samples are obtained from the patient. **b** PDCO Generation & Characterization: Tissue dissociation and culture in extracellular matrix (e.g., Matrigel) with tailored growth factors. Validation via omics (e.g., NGS) and histopathology (e.g., H&E staining). **c** Functional Testing: High-throughput drug screening (e.g., chemotherapy, targeted therapy). AI-driven prediction of drug response based on viability assays. **d** Data Integration & AI Analysis: Prioritize sensitive regimens and avoid resistant therapies. **e** Clinical Decision: Treatment selection informed by PDCO results (e.g., 91.7% concordance in gastric cancer). This figure was created with Biorender

have demonstrated superior predictive accuracy compared to conventional cultures and represent a scalable approach for functional precision oncology [157, 190]. Lastly, a significant clinical barrier is the long turnaround time—often several weeks—required to establish and test PDCOs. This delay can be critical for patients with advanced-stage disease who have a narrow therapeutic window [191, 192]. Addressing this issue requires a multifaceted approach, including biological optimization to accelerate organoid growth, engineering advances to enable high-throughput screening, and integration into clinical workflows such as the neoadjuvant setting, where treatment decisions may accommodate longer timelines [193]. For aggressive cancers, adaptive trial arms can use PDCO data to guide second-line therapy.

To fully unlock the translational potential of PDCOs, it is essential to overcome current technological and standardization challenges that limit their reproducibility, scalability, and predictive accuracy. A major hurdle is the continued reliance on undefined extracellular matrices such as Matrigel, which introduce batch-to-batch variability and compromise cross-laboratory reproducibility [194]. Recent advances in 3D bioprinting offer a

promising solution by enabling the creation of organoid models with defined architecture, spatial control, and consistent integration of tumor and stromal components. These engineered constructs better mimic the TME, including vascularization, thus improving the physiological relevance of drug screening [195, 196]. Moreover, bioprinting supports scalable, automated production, facilitating broader clinical and pharmaceutical application.

AI and machine learning are also reshaping organoid-based phenotyping and drug response prediction [197–199]. Deep learning algorithms—such as convolutional neural networks and variational autoencoders—can analyze high-content imaging data to classify organoid morphologies and quantify drug-induced changes with high sensitivity [199]. Multi-omic PDCO models that integrate genomic, transcriptomic, and pharmacologic data are being developed to forecast patient-specific therapeutic responses. Notably, AI-based stratification of colorectal PDCOs has already demonstrated predictive utility for response to FOLFOX and EGFR-targeted therapies [199]. Beyond prediction, AI-driven automation reduces inter-observer variability and enhances consistency in

therapeutic assessments, streamlining the integration of PDCOs into clinical workflows [197].

Gene editing technologies, particularly CRISPR/Cas9, add another dimension by enabling the functional interrogation of drug resistance mechanisms and therapeutic vulnerabilities [200, 201]. Although their direct clinical application remains limited, CRISPR can generate isogenic PDCO models for biomarker discovery, synthetic lethality screening, and elucidation of genotype-phenotype relationships—thereby enhancing the mechanistic depth of PDCO-based research.

Looking ahead, rigorous clinical validation remains paramount. While early studies have reported strong concordance between PDCO-based drug responses and patient outcomes, widespread adoption will require prospective, multi-institutional trials with harmonized protocols, clearly defined clinical endpoints, and regulatory oversight. The successful implementation of large-scale, multi-center trials—such as the Dutch TUMOROID study (Tumor Organoids: Feasibility to Predict Sensitivity to Treatment in Cancer Patients)—will be critical to demonstrating the clinical utility of PDCO-guided therapy. Positive outcomes from these studies could pave the way for formal endorsement by regulatory authorities such as the FDA and EMA. In parallel, robust frameworks for patient consent, ethical data governance, and secure biobanking must be established to ensure responsible and scalable integration into precision oncology pipelines.

In conclusion, the convergence of 3D bioprinting, AI, and gene editing—together with standardized workflows and large-scale clinical validation—offers a clear path toward making PDCOs a clinically actionable platform for personalized cancer therapy and drug development in digestive system oncology.

Abbreviations

PDCOs	Patient-derived cancer organoids
GI	Gastrointestinal
NGS	Next-generation sequencing
PDOs	Patient-derived organoids
3D	Three-dimensional
2D	Two-dimensional
PD-1	Programmed death-1
PD-L1	Programmed death-ligand 1
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
ALI	Air–liquid interface
PDXs	Patient-derived xenografts
GEMMs	Genetically engineered mouse models
AI	Artificial intelligence
HCC	Hepatocellular carcinoma
CCA	Cholangiocarcinoma
TME	Tumor microenvironment
GC	Gastric cancer
5-FU	5-Fluorouracil
CAFs	Cancer-associated fibroblasts
MSI-H	Microsatellite instability-high
CRC	Colorectal cancer
SHMT	Single-hit multi-target
nCRT	Neoadjuvant chemoradiotherapy
LARC	Locally advanced rectal cancer

AC	Adjuvant chemotherapy
TRG	Tumor regression grade
DFS	Disease-free survival
PFS	Progression-free survival
OS	Overall survival
PDAC	Pancreatic ductal adenocarcinoma
TK1	Thymidine kinase 1
FLC	Fibrolamellar carcinoma
ICIs	Immune checkpoint inhibitors
MSCs	Mesenchymal stromal cells
PBMCs	Peripheral blood mononuclear cells
CAR-T	Chimeric antigen receptor T-cell
CAR-NK	Chimeric antigen receptor natural killer cell
TNF	Tumor necrosis factor
BE	Barrett's esophagus
EAC	Esophageal adenocarcinoma
ROS	Reactive oxygen species
Fn	Fusobacterium nucleatum
TILs	Tumor-infiltrating lymphocytes
PDT	Photodynamic therapy
NCCN	National Comprehensive Cancer Network
PINs	Photothermal immuno-nanocomplexes
DILI	Drug-induced liver injury
iPSC	Induced pluripotent stem cell
AKI	Acute kidney injury
I3A	Indole-3-carboxaldehyde
SCFAs	Short-chain fatty acids

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12943-025-02429-0>.

Supplementary Material 1. Supplementary appendix. As the submitted manuscript is a review article, we are unable to provide full, uncropped images of gels and blots as supplementary material.

Authors' contributions

YW: Writing—original draft, Methodology, Data curation. LZ: Writing—original draft, Data curation. LZW: Methodology, Data curation, Revision. YC: Visualization, Revision. LH: Visualization, Revision. GS: Methodology, Revision. XC: Supervision, Conceptualization. LW: Writing—original draft, Supervision, Funding acquisition, Conceptualization. BCG: Supervision, Revision, Funding acquisition, Conceptualization. All authors contributed to the original preparation of respective sections and revisions of the whole manuscript. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have gone through the manuscript and agreed to publish in *Molecular Cancer*.

Competing interests

The authors declare no competing interests.

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Reference

- Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Cancer J Clin*. 2024;74(3):229–63.
- Murphy N, Jenab M, Gunter MJ. Adiposity and gastrointestinal cancers: epidemiology, mechanisms and future directions. *Nat Rev Gastroenterol Hepatol*. 2018;15(11):659–70.
- Meng C, Bai C, Brown TD, Hood LE, Tian Q. Human gut microbiota and gastrointestinal cancer. *Genom Proteom Bioinform*. 2018;16(1):33–49.
- Cheng WL, Feng PH, Lee KY, Chen KY, Sun WL, Van Hiep N, et al. The role of EREG/EGFR pathway in tumor progression. *Int J Mol Sci*. 2021. <https://doi.org/10.3390/ijms222312828>.
- Fernandes Q, Inchakalody VP, Bedhiafi T, Mestiri S, Taib N, Uddin S, et al. Chronic inflammation and cancer; the two sides of a coin. *Life Sci*. 2024;338:122390.
- Krzysiek-Maczka G, Brzozowski T, Ptak-Belowska A. *Helicobacter pylori*-activated fibroblasts as a silent partner in gastric cancer development. *Cancer Metastasis Rev*. 2023;42(4):1219–56.
- Arnold M, Abnet CC, Neale RE, Vignat J, Giovannucci EL, McGlynn KA, et al. Global burden of 5 major types of Gastrointestinal cancer. *Gastroenterology*. 2020;159(1):335–e4915.
- Lu L, Mullins CS, Schafmayer C, Zeißig S, Linnebacher M. A global assessment of recent trends in Gastrointestinal cancer and lifestyle-associated risk factors. *Cancer Commun (London England)*. 2021;41(11):1137–51.
- Wang F, Song M, Lu X, Zhu X, Deng J. Gut microbes in gastrointestinal cancers. *Semin Cancer Biol*. 2022;86(Pt 2):967–75.
- Ghosn M, Kourie HR, Tabchi S. Gastrointestinal cancers in the era of theranostics: updates and future perspectives. *World J Gastroenterol*. 2015;21(28):8473–7.
- Zhu G, Pei L, Xia H, Tang Q, Bi F. Role of oncogenic KRAS in the prognosis, diagnosis and treatment of colorectal cancer. *Mol Cancer*. 2021;20(1):143.
- Joshi SS, Badgwell BD. Current treatment and recent progress in gastric cancer. *CA Cancer J Clin*. 2021;71(3):264–79.
- Van Cutsem E, Sagaert X, Topal B, Haustermans K, Prenen H. Gastric cancer. *Lancet*. 2016;388(10060):2654–64.
- Kuboki Y, Yamashita S, Niwa T, Ushijima T, Nagatsuma A, Kuwata T, et al. Comprehensive analyses using next-generation sequencing and immunohistochemistry enable precise treatment in advanced gastric cancer. *Ann Oncol*. 2016;27(1):127–33.
- Xiang D, He A, Zhou R, Wang Y, Xiao X, Gong T, et al. Building consensus on the application of organoid-based drug sensitivity testing in cancer precision medicine and drug development. *Theranostics*. 2024;14(8):3300–16.
- Foo MA, You M, Chan SL, Sethi G, Bonney GK, Yong WP, et al. Clinical translation of patient-derived tumour organoids- bottlenecks and strategies. *Biomark Res*. 2022;10(1):10.
- Polak R, Zhang ET, Kuo CJ. Cancer organoids 2.0: modelling the complexity of the tumour immune microenvironment. *Nat Rev Cancer*. 2024;24(8):523–39.
- Neal JT, Li X, Zhu J, Giangarra V, Grzeskowiak CL, Ju J, et al. Organoid modeling of the tumor immune microenvironment. *Cell*. 2018;175(7):1972–e8816.
- Wan C, Keany MP, Dong H, Al-Alem LF, Pandya UM, Lazo S, et al. Enhanced efficacy of simultaneous PD-1 and PD-L1 immune checkpoint blockade in high-grade serous ovarian cancer. *Cancer Res*. 2021;81(1):158–73.
- Tang XY, Wu S, Wang D, Chu C, Hong Y, Tao M, et al. Human organoids in basic research and clinical applications. *Signal Transduct Target Ther*. 2022;7(1): 168.
- Clevers H. Modeling development and disease with organoids. *Cell*. 2016;165(7):1586–97.
- Tuveson D, Clevers H. Cancer modeling meets human organoid technology. *Science*. 2019;364(6444):952–5.
- Drost J, Clevers H. Organoids in cancer research. *Nat Rev Cancer*. 2018;18(7):407–18.
- Wilson HV. A new method by which sponges, may be artificially reared. *Science*. 1907;25(649):912–5.
- Smith E, Cochrane WJ. Cystic organoid teratoma; report of a case. *Can Med Assoc J*. 1946;55(2):151.
- Weiss P, Taylor AC. Reconstitution of complete organs from single-cell suspensions of chick, embryos in advanced stages of differentiation. *Proc Natl Acad Sci USA*. 1960;46(9):1177–85.
- Evans M. Origin of mouse embryonal carcinoma cells and the possibility of their direct isolation into tissue culture. *Reproduction*. 1981;62(2):625–31.
- Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126(4):663–76.
- Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998;282(5391):1145–7.
- Li ML, Aggeler J, Farson DA, Hatier C, Hassell J, Bissell MJ. Influence of a reconstituted basement membrane and its components on casein gene expression and secretion in mouse mammary epithelial cells. *Proc Natl Acad Sci U S A*. 1987;84(1):136–40.
- Shannon JM, Mason RJ, Jennings SD. Functional differentiation of alveolar type II epithelial cells in vitro: effects of cell shape, cell-matrix interactions and cell-cell interactions. *Biochim Biophys Acta*. 1987;931(2):143–56.
- Yan HHN, Siu HC, Law S, Ho SL, Yue SSK, Tsui WY, et al. A comprehensive human gastric cancer organoid biobank captures tumor subtype heterogeneity and enables therapeutic screening. *Cell Stem Cell*. 2018;23(6):882–e9711.
- Zhao Y, Li S, Zhu L, Huang M, Xie Y, Song X, et al. Personalized drug screening using patient-derived organoid and its clinical relevance in gastric cancer. *Cell Rep Med*. 2024;5(7): 101627.
- Sontheimer-Phelps A, Hassell BA, Ingber DE. Modelling cancer in microfluidic human organs-on-chips. *Nat Rev Cancer*. 2019;19(2):65–81.
- Shrestha S, Lekkala VKR, Acharya P, Kang SY, Vanga MG, Lee MY. Reproducible generation of human liver organoids (HLOs) on a pillar plate platform via microarray 3D bioprinting. *Lab Chip*. 2024;24(10):3918–28.
- Fujii M, Clevers H, Sato T. Modeling human digestive diseases with CRISPR-Cas9-modified organoids. *Gastroenterology*. 2019;156(3):562–76.
- Wan X, Xu C, Lin Y, Lu C, Li D, Sang J, et al. Uric acid regulates hepatic steatosis and insulin resistance through the NLRP3 inflammasome-dependent mechanism. *J Hepatol*. 2016;64(4):925–32.
- Weng W, Wei Q, Toden S, Yoshida K, Nagasaka T, Fujiwara T, et al. Circular RNA ciRS-7-a promising prognostic biomarker and a potential therapeutic target in colorectal cancer. *Clin Cancer Res*. 2017;23(14):3918–28.
- Li S, Cong X, Gao H, Lan X, Li Z, Wang W, et al. Tumor-associated neutrophils induce EMT by IL-17a to promote migration and invasion in gastric cancer cells. *J Exp Clin Cancer Res*. 2019;38(1): 6.
- Yoshida GJ. Applications of patient-derived tumor xenograft models and tumor organoids. *J Hematol Oncol*. 2020;13(1):4.
- Li QX, Feuer G, Ouyang X, An X. Experimental animal modeling for immunoncology. *Pharmacol Ther*. 2017;173:34–46.
- Politi K, Pao W. How genetically engineered mouse tumor models provide insights into human cancers. *J Clin Oncology: Official J Am Soc Clin Oncol*. 2011;29(16):2273–81.
- Xu H, Jiao D, Liu A, Wu K. Tumor organoids: applications in cancer modeling and potentials in precision medicine. *J Hematol Oncol*. 2022;15(1):58.
- Greener JG, Kandathil SM, Moffat L, Jones DT. A guide to machine learning for biologists. *Nat Rev Mol Cell Biol*. 2022;23(1):40–55.
- Wang H, Fu T, Du Y, Gao W, Huang K, Liu Z, et al. Scientific discovery in the age of artificial intelligence. *Nature*. 2023;620(7972):47–60.
- Liu B, Zhu Y, Yang Z, Yan HHN, Leung SY, Shi J. Deep learning-based 3D single-cell imaging analysis pipeline enables quantification of cell-cell interaction dynamics in the tumor microenvironment. *Cancer Res*. 2024;84(4):517–26.
- Deben C, De La Hoz EC, Compte ML, Van Schil P, Hendriks JMH, Lauwers P, et al. ORBITS: label-free and time-lapse monitoring of patient derived organoids for advanced drug screening. *Cell Oncol*. 2023;46(2):299–314.
- Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature*. 2009;459(7244):262–5.
- Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and barrett's epithelium. *Gastroenterology*. 2011;141(5):1762–72.
- Boj SF, Hwang CI, Baker LA, Chio IL, Engle DD, Corbo V, et al. Organoid models of human and mouse ductal pancreatic cancer. *Cell*. 2015;160(1–2):324–38.
- Broutier L, Mastrogianni G, Versteeg MM, Francies HE, Gavarró LM, Bradshaw CR, et al. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. *Nat Med*. 2017;23(12):1424–35.
- Nanki K, Toshimitsu K, Takano A, Fujii M, Shimokawa M, Ohta Y, et al. Divergent routes toward Wnt and R-spondin niche interdependency during human gastric carcinogenesis. *Cell*. 2018;174(4):856–e6917.

53. Li X, Francies HE, Secrier M, Perner J, Miremadi A, Galeano-Dalmau N, et al. Organoid cultures recapitulate esophageal adenocarcinoma heterogeneity providing a model for clonality studies and precision therapeutics. *Nat Commun*. 2018;9(1):2983.
54. Ajani JA, D'Amico TA, Bentrem DJ, Chao J, Cooke D, Corvera C, et al. Gastric cancer, version 2.2022, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw*. 2022;20(2):167–92.
55. Tempero MA, Malafa MP, Al-Hawary M, Behrman SW, Benson AB, Cardin DB, et al. Pancreatic adenocarcinoma, version 2.2021, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw*. 2021;19(4):439–57.
56. Van Cutsem E, Cervantes A, Adam R, Sobrero A, Van Krieken JH, Aderka D, et al. Esmo consensus guidelines for the management of patients with metastatic colorectal cancer. *Ann Oncol*. 2016;27(8):1386–422.
57. Min HY, Lee HY. Molecular targeted therapy for anticancer treatment. *Exp Mol Med*. 2022;54(10):1670–94.
58. Zhang XN, Gao Y, Zhang XY, Guo NJ, Hou WQ, Wang SW, et al. Detailed curriculum vitae of HER2-targeted therapy. *Pharmacol Ther*. 2023;245: 108417.
59. Riley RS, June CH, Langer R, Mitchell MJ. Delivery technologies for cancer immunotherapy. *Nat Rev Drug Discovery*. 2019;18(3):175–96.
60. Kennedy LB, Salama AKS. A review of cancer immunotherapy toxicity. *CA Cancer J Clin*. 2020;70(2):86–104.
61. Letai A, de The H. Conventional chemotherapy: millions of cures, unresolved therapeutic index. *Nat Rev Cancer*. 2025;25(3):209–18.
62. Gu Y, Yang R, Zhang Y, Guo M, Takehiro K, Zhan M, et al. Molecular mechanisms and therapeutic strategies in overcoming chemotherapy resistance in cancer. *Mol Biomed*. 2025;6(1):2.
63. Cartry J, Bedja S, Boileve A, Mathieu JRR, Gontran E, Annereau M, et al. Implementing patient derived organoids in functional precision medicine for patients with advanced colorectal cancer. *J Exp Clin Cancer Res*. 2023;42(1): 281.
64. Allen C, Her S, Jaffray DA. Radiotherapy for cancer: present and future. *Adv Drug Deliv Rev*. 2017;109:1–2.
65. Schae D, McBride WH. Opportunities and challenges of radiotherapy for treating cancer. *Nat Rev Clin Oncol*. 2015;12(9):527–40.
66. Hsu KS, Adileh M, Martin ML, Makarov V, Chen J, Wu C, et al. Colorectal cancer develops inherent radiosensitivity that can be predicted using patient-derived organoids. *Cancer Res*. 2022;82(12):2298–312.
67. Chiarella P, Bruzzo J, Meiss RP, Ruggiero RA. Concomitant tumor resistance. *Cancer Lett*. 2012;324(2):133–41.
68. Yao Y, Xu X, Yang L, Zhu J, Wan J, Shen L, et al. Patient-Derived organoids predict chemoradiation responses of locally advanced rectal cancer. *Cell Stem Cell*. 2020;26(1):17–e266.
69. Sauer R, Becker H, Hohenberger W, Rödel C, Wittekind C, Fietkau R, et al. Pre-operative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med*. 2004;351(17):1731–40.
70. Xue W, Wang T, Yao J, Wu W, Chen D, Yan B, et al. Use of patient-derived tumor organoid platform to predict the benefit of postoperative adjuvant chemotherapy for poor responders to neoadjuvant chemoradiotherapy in locally advanced rectal cancer. *Bioeng Transl Med*. 2023;8(6): e10586.
71. Soragni A, Knudsen ES, O'Connor TN, Tognon CE, Tyner JW, Gini B, et al. Acquired resistance in cancer: towards targeted therapeutic strategies. *Nat Rev Cancer*. 2025;25:613.
72. Marusyk A, Janiszewska M, Polyak K. Intratumor heterogeneity: the Rosetta stone of therapy resistance. *Cancer Cell*. 2020;37(4):471–84.
73. Montoya S, Soong D, Nguyen N, Affer M, Munamarty SP, Taylor J. Targeted therapies in cancer: to be or not to be, selective. *Biomedicines*. 2021. <https://doi.org/10.3390/biomedicines9111591>.
74. Duan X, Zhang T, Feng L, de Silva N, Greenspun B, Wang X, et al. A pancreatic cancer organoid platform identifies an inhibitor specific to mutant KRAS. *Cell Stem Cell*. 2024;31(1):71–e888.
75. Yang H, Cheng J, Zhuang H, Xu H, Wang Y, Zhang T, et al. Pharmacogenomic profiling of intra-tumor heterogeneity using a large organoid biobank of liver cancer. *Cancer Cell*. 2024;42(4):535–e518.
76. 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. Volume 359. New York, NY: Science; 2018. pp. 920–6. 6378.
77. Craig JR, Peters RL, Edmondson HA, Omata M. Fibrolamellar carcinoma of the liver: a tumor of adolescents and young adults with distinctive clinico-pathologic features. *Cancer*. 1980;46(2):372–9.
78. Honeyman JN, Simon EP, Robine N, Chiaroni-Clarke R, Darcy DG, Lim II, et al. Detection of a recurrent DNAJB1-PRKACA chimeric transcript in fibrolamellar hepatocellular carcinoma. *Science*. 2014;343(6174):1010–4.
79. Narayan NJC, Requena D, Lalazar G, Ramos-Espiritu L, Ng D, Levin S, et al. Human liver organoids for disease modeling of fibrolamellar carcinoma. *Stem Cell Reports*. 2022;17(8):1874–88.
80. Sharma P, Allison JP. The future of immune checkpoint therapy. *Sci (New York NY)*. 2015;348(6230):56–61.
81. Abken H. CAR T cell therapies in gastrointestinal cancers: current clinical trials and strategies to overcome challenges. *Nat Rev Gastroenterol Hepatol*. 2025;22(7):463–80.
82. Lu Z, Peng Z, Liu C, Wang Z, Wang Y, Jiao X, et al. Current status and future perspective of immunotherapy in gastrointestinal cancers. *Innovation (Camb)*. 2020;1(2): 100041.
83. Wu Q, You L, Nepovimova E, Heger Z, Wu W, Kuca K, et al. Hypoxia-inducible factors: master regulators of hypoxic tumor immune escape. *J Hematol Oncol*. 2022;15(1):77.
84. Jhunjunwala S, Hammer C, Delamarre L. Antigen presentation in cancer: insights into tumour immunogenicity and immune evasion. *Nat Rev Cancer*. 2021;21(5):298–312.
85. Do MH, Shi W, Ji L, Ladewig E, Zhang X, Srivastava RM, et al. Reprogramming tumor-associated macrophages to outcompete endovascular endothelial progenitor cells and suppress tumor neoangiogenesis. *Immunity*. 2023;56(11):2555–e695.
86. Sun L, Zhang H, Gao P. Metabolic reprogramming and epigenetic modifications on the path to cancer. *Protein Cell*. 2022;13(12):877–919.
87. Zhang H, Li S, Wang D, Liu S, Xiao T, Gu W, et al. Metabolic reprogramming and immune evasion: the interplay in the tumor microenvironment. *Biomark Res*. 2024;12(1):96.
88. Chong X, Madeti Y, Cai J, Li W, Cong L, Lu J, et al. Recent developments in immunotherapy for gastrointestinal tract cancers. *J Hematol Oncol*. 2024;17(1):65.
89. Gao M, Lin M, Moffitt RA, Salazar MA, Park J, Vacirca J, et al. Direct therapeutic targeting of immune checkpoint PD-1 in pancreatic cancer. *Br J Cancer*. 2019;120(1):88–96.
90. 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*. 2023;10(27): e2302640.
91. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med*. 2015;373(1):23–34.
92. Chalabi M, Fanchi LF, Dijkstra KK, Van den Berg JG, Aalbers AG, Sikorska K, et al. Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. *Nat Med*. 2020;26(4):566–76.
93. Michie J, Beavis PA, Freeman AJ, Vervoort SJ, Ramsbottom KM, Narasimhan V, et al. Antagonism of IAPs enhances CAR T-cell efficacy. *Cancer Immunol Res*. 2019;7(2):183–92.
94. Wang Y, Gao Y, Niu C, Wang B, Zhao S, Roex G, et al. Chimeric antigen receptor clustering via cysteines enhances t-cell efficacy against tumor. *Cancer Immunol Immunother*. 2022;71(11):2801–14.
95. Schnalzger TE, de Groot MH, Zhang C, Mosa MH, Michels BE, Röder J, et al. 3D model for CAR-mediated cytotoxicity using patient-derived colorectal cancer organoids. *EMBO J*. 2019. <https://doi.org/10.15252/embj.2018100928>.
96. Raimondi G, Mato-Berciano A, Pascual-Sabater S, Rovira-Rigau M, Cuatrecasas M, Fondevila C, et al. Patient-derived pancreatic tumour organoids identify therapeutic responses to oncolytic adenoviruses. *EBioMedicine*. 2020;56:102786.
97. Miao T, Symonds A, Hickman OJ, Wu D, Wang P, Lemoine N, et al. Inhibition of bromodomain proteins enhances oncolytic HAdV5 replication and efficacy in pancreatic ductal adenocarcinoma (PDAC) models. *Int J Mol Sci*. 2024. <http://doi.org/10.3390/ijms25021265>.
98. Dias JD, Hemminki O, Diaconu I, Hirvonen M, Bonetti A, Guse K, et al. Targeted cancer immunotherapy with oncolytic adenovirus coding for a fully human monoclonal antibody specific for CTLA-4. *Gene Ther*. 2012;19(10):988–98.
99. Hamdan F, Ylösmäki E, Chiaro J, Giannoula Y, Long M, Fucciello M, et al. Novel oncolytic adenovirus expressing enhanced cross-hybrid IgG4 Fc PD-L1 inhibitor activates multiple immune effector populations leading to enhanced tumor killing *in vitro*, *in vivo* and with patient-derived tumor organoids. *J Immunother Cancer*. 2021. <https://doi.org/10.1136/jitc-2021-003000>.

100. Rao S, Hossain T, Mahmoudi T. 3D human liver organoids: an in vitro platform to investigate HBV infection, replication and liver tumorigenesis. *Cancer Lett.* 2021;506:35–44.
101. Kakni P, Truckenmüller R, Habibović P, van Griensven M, Giselbrecht S. A microwell-based intestinal organoid-macrophage co-culture system to study intestinal inflammation. *Int J Mol Sci.* 2022. <https://doi.org/10.3390/ijms232315364>.
102. Liu B, Bukhari I, Li F, Ren F, Xia X, Hu B, et al. Enhanced LRP8 expression induced by *Helicobacter pylori* drives gastric cancer progression by facilitating β -catenin nuclear translocation. *J Adv Res.* 2025;69:299–312.
103. Shah AB, Baiseitova A, Zahoor M, Ahmad I, Ikram M, Bakhsh A, et al. Probiotic significance of *Lactobacillus* strains: a comprehensive review on health impacts, research gaps, and future prospects. *Gut Microbes.* 2024;16(1):2431643.
104. Shen S, Ren F, Qin H, Bukhari I, Yang J, Gao D, et al. *Lactobacillus acidophilus* NCFM and *Lactiplantibacillus plantarum* Lp-115 inhibit *Helicobacter pylori* colonization and gastric inflammation in a murine model. *Front Cell Infect Microbiol.* 2023;13:1196084.
105. Hou Q, Ye L, Liu H, Huang L, Yang Q, Turner JR, et al. *Lactobacillus* accelerates ISCs regeneration to protect the integrity of intestinal mucosa through activation of STAT3 signaling pathway induced by LPLs secretion of IL-22. *Cell Death Differ.* 2021;28(6):2025–7.
106. de-Souza-Ferreira M, Ferreira EE, de-Freitas-Junior JCM. Aberrant N-glycosylation in cancer: MGAT5 and β 1,6-GlcNAc branched N-glycans as critical regulators of tumor development and progression. *Cell Oncol.* 2023;46(3):481–501.
107. Lau HC, Zhang X, Ji F, Lin Y, Liang W, Li Q, et al. *Lactobacillus acidophilus* suppresses non-alcoholic fatty liver disease-associated hepatocellular carcinoma through producing valeric acid. *EBioMedicine.* 2024;100:104952.
108. Su SH, Mitani Y, Li T, Sachdeva U, Flashner S, Klein-Szanto A, et al. Lactate suppresses growth of esophageal adenocarcinoma patient-derived organoids through alterations in tumor NADH/NAD⁺ redox state. *Biomolecules.* 2024. <https://doi.org/10.3390/biom14091195>.
109. Zitvogel L, Galluzzi L, Vautour S, Vétizou M, Daillère R, Merad M, et al. Cancer and the gut microbiota: an unexpected link. *Sci Transl Med.* 2015;7(271):271ps1.
110. Liu Y, Baba Y, Ishimoto T, Tsutsuki H, Zhang T, Nomoto D, et al. *Fusobacterium nucleatum* confers chemoresistance by modulating autophagy in oesophageal squamous cell carcinoma. *Br J Cancer.* 2021;124(5):963–74.
111. Gao Y, Bi D, Xie R, Li M, Guo J, Liu H, et al. *Fusobacterium nucleatum* enhances the efficacy of PD-L1 blockade in colorectal cancer. *Signal Transduct Target Ther.* 2021;6(1):398.
112. Correia JH, Rodrigues JA, Pimenta S, Dong T, Yang Z. Photodynamic therapy review: principles, photosensitizers, applications, and future directions. *Pharmaceutics.* 2021. <https://doi.org/10.3390/pharmaceutics13091332>.
113. Yu R, Maswikiti EP, Yu Y, Gao L, Ma C, Ma H, et al. Advances in the application of preclinical models in photodynamic therapy for tumor: a narrative review. *Pharmaceutics.* 2023;15(1):197.
114. Fujiwara H, Takahara N, Tateishi K, Tanaka M, Kanai S, Kato H, et al. 5-aminolevulinic acid-mediated photodynamic activity in patient-derived cholangiocarcinoma organoids. *Surg Oncol.* 2020;35:484–90.
115. Zheng YW, Miao XY, Xiong L, Chen B, Kong FH, Zhou JJ, et al. Sulfasalazine sensitizes polyhematoporphyrin-mediated photodynamic therapy in cholangiocarcinoma by targeting xCT. *Front Pharmacol.* 2021;12:723488.
116. Huang YP, Wang YX, Zhou H, Liu ZT, Zhang ZJ, Xiong L, et al. Surufatinib combined with photodynamic therapy induces ferroptosis to inhibit cholangiocarcinoma in vitro and in tumor models. *Front Pharmacol.* 2024;15:1288255.
117. Broekgaarden M, Rizvi I, Bulin AL, Petrovic L, Goldschmidt R, Massodi I, et al. Neoadjuvant photodynamic therapy augments immediate and prolonged oxaliplatin efficacy in metastatic pancreatic cancer organoids. *Oncotarget.* 2018;9(16):13009–22.
118. Obaid G, Bano S, Mallidi S, Broekgaarden M, Kuriakose J, Silber Z, et al. Impacting pancreatic cancer therapy in heterotypic in vitro organoids and in vivo tumors with Specificity-Tuned, NIR-Activable photoimmunonanoparticles: towards conquering desmoplasia? *Nano Lett.* 2019;19(11):7573–87.
119. Benson AB, D'Angelica MI, Abrams T, Abbott DE, Ahmed A, Anaya DA, et al. NCCN guidelines® insights: biliary tract cancers, version 2.2023. *J Natl Compr Canc Netw.* 2023;21(7):694–704.
120. Meng X, Deng J, Liu F, Guo T, Liu M, Dai P, et al. Triggered all-active metal organic framework: ferroptosis machinery contributes to the apoptotic photodynamic antitumor therapy. *Nano Lett.* 2019;19(11):7866–76.
121. Procacci P, Moscheni C, Sartori P, Sommariva M, Gagliano N. Tumor-Stroma cross-talk in human pancreatic ductal adenocarcinoma: a focus on the effect of the extracellular matrix on tumor cell phenotype and invasive potential. *Cells.* 2018. <https://doi.org/10.3390/cells7100158>.
122. Zhou H, Zhang Y, Zhang R, Zhao M, Chen W, Liu Y, et al. A tumor-microenvironment-activatable molecular pro-theranostic agent for photodynamic and immunotherapy of cancer. *Adv Mater.* 2023;35(30):e2211485.
123. Zhou S, Zhen Z, Paschall AV, Xue L, Yang X, Bebin-Blackwell AG, et al. FAP-Targeted photodynamic therapy mediated by ferritin nanoparticles elicits an immune response against cancer cells and cancer associated fibroblasts. *Adv Funct Mater.* 2021;31(7):2007017.
124. Peters MF, Choy AL, Pin C, Leishman DJ, Moisan A, Ewart L, et al. Developing in vitro assays to transform gastrointestinal safety assessment: potential for microphysiological systems. *Lab Chip.* 2020;20(7):1177–90.
125. Patton K, Borshoff DC. Adverse drug reactions. *Anaesthesia.* 2018;73(Suppl 1):76–84.
126. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, et al. Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regul Toxicol Pharmacol.* 2000;32(1):56–67.
127. Wadman M. FDA no longer has to require animal testing for new drugs. *Science.* 2023;379(6628):127–8.
128. Peters MF, Landry T, Pin C, Maratea K, Dick C, Wagoner MP, et al. Human 3D gastrointestinal microtissue barrier function as a predictor of drug-induced diarrhea. *Toxicol Sci.* 2019;168(1):3–17.
129. Choi SY, Kim TH, Kim MJ, Mun SJ, Kim TS, Jung KK, et al. Validating well-functioning hepatic organoids for toxicity evaluation. *Toxics.* 2024. <https://doi.org/10.3390/toxics12050371>.
130. Digby JLM, Vanichapol T, Przepiowski A, Davidson AJ, Sander V. Evaluation of cisplatin-induced injury in human kidney organoids. *Am J Physiol-Renal Physiol.* 2020;318(4):F971–8.
131. Skardal A, Aleman J, Forsythe S, Rajan S, Murphy S, Devarasetty M, et al. Drug compound screening in single and integrated multi-organoid body-on-a-chip systems. *Biofabrication.* 2020;12(2):025017.
132. Skardal A, Murphy SV, Devarasetty M, Mead I, Kang HW, Seol YJ, et al. Multi-tissue interactions in an integrated three-tissue organ-on-a-chip platform. *Sci Rep.* 2017;7(1):8837.
133. Verginadis II, Citrin DE, Ky B, Feigenberg SJ, Georgakilas AG, Hill-Kayser CE, et al. Radiotherapy toxicities: mechanisms, management, and future directions. *Lancet.* 2025;405(10475):338–52.
134. Xie LW, Cai S, Lu HY, Tang FL, Zhu RQ, Tian Y, et al. Microbiota-derived I3A protects the intestine against radiation injury by activating AhR/IL-10/Wnt signaling and enhancing the abundance of probiotics. *Gut Microbes.* 2024;16(1):2347722.
135. Park M, Kwon J, Shin HJ, Moon SM, Kim SB, Shin US, et al. Butyrate enhances the efficacy of radiotherapy via FOXO3A in colorectal cancer patient-derived organoids. *Int J Oncol.* 2020;57(6):1307–18.
136. Xie LW, Cai S, Zhao TS, Li M, Tian Y. Green tea derivative (-)-epigallocatechin-3-gallate (EGCG) confers protection against ionizing radiation-induced intestinal epithelial cell death both in vitro and in vivo. *Free Radic Biol Med.* 2020;161:175–86.
137. Li M, Gu MM, Lang Y, Shi J, Chen BPC, Guan H, et al. The vanillin derivative VND3207 protects intestine against radiation injury by modulating p53/NOXA signaling pathway and restoring the balance of gut microbiota. *Free Radic Biol Med.* 2019;145:223–36.
138. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: the international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol.* 2017;14(8):491–502.
139. Delia P, Sansotta G, Donato V, Frosina P, Messina G, De Renzis C, et al. Use of probiotics for prevention of radiation-induced diarrhea. *World J Gastroenterol.* 2007;13(6):912–5.
140. Rauf A, Khalil AA, Rahman U-u, Khalid A, Naz S, Shariati MA, et al. Recent advances in the therapeutic application of short-chain fatty acids (SCFAs): an updated review. *Crit Rev Food Sci Nutr.* 2022;62(22):6034–54.
141. Nuciforo S, Fofana I, Matter MS, Blumer T, Calabrese D, Boldanova T, et al. Organoid models of human liver cancers derived from tumor needle biopsies. *Cell Rep.* 2018;24(5):1363–76.
142. Rao J, Song C, Hao Y, Chen Z, Feng S, Xu S, et al. Leveraging patient-derived organoids for personalized liver cancer treatment. *Int J Biol Sci.* 2024;20(13):5363–74.
143. Wong AM, Huang H, Wong AM, Leung HHW, Cheung WWY, Lam YK, et al. Patient-derived organoids inform pharmacogenomic vulnerabilities in liver cancer. *JHEP Rep.* 2025;7(7): 101426.

144. Saito Y, Muramatsu T, Kanai Y, Ojima H, Sukeda A, Hiraoka N, et al. Establishment of Patient-Derived organoids and drug screening for biliary tract carcinoma. *Cell Rep*. 2019;27(4):1265–e764.
145. Boden C, Esser LK, Dold L, Langhans B, Zhou T, Kaczmarek DJ, et al. The IL-6/JAK/STAT3 axis in cholangiocarcinoma and primary sclerosing cholangitis: unlocking therapeutic strategies through patient-derived organoids. *Bio-medicines*. 2025. <https://doi.org/10.3390/biomedicines13051083>.
146. Zhu Y, Tang S, Yuan Q, Fu J, He J, Liu Z, et al. Integrated characterization of hepatobiliary tumor organoids provides a potential landscape of pharmacogenomic interactions. *Cell Rep Med*. 2024;5(2): 101375.
147. Derouet MF, Allen J, Wilson GW, Ng C, Radulovich N, Kalimuthu S, et al. Towards personalized induction therapy for esophageal adenocarcinoma: organoids derived from endoscopic biopsy recapitulate the pre-treatment tumor. *Sci Rep*. 2020;10(1):14514.
148. Karakasheva TA, Gabre JT, Sachdeva UM, Cruz-Acuña R, Lin EW, DeMarshall M, et al. Patient-derived organoids as a platform for modeling a patient's response to chemoradiotherapy in esophageal cancer. *Sci Rep*. 2021;11(1):21304. <https://doi.org/10.1038/s41598-021-00706-8>.
149. Shen S, Liu B, Guan W, Liu Z, Han Y, Hu Y, et al. Advancing precision medicine in esophageal squamous cell carcinoma using patient-derived organoids. *J Transl Med*. 2024;22(1): 1168.
150. Xiao X, Chen W, Wei ZW, Chu WW, Lu XF, Li B, et al. The Anti-Tumor effect of Nab-Paclitaxel proven by Patient-Derived organoids. *OncoTargets Therapy*. 2020;13:6017–25.
151. Zou J, Wang S, Chai N, Yue H, Ye P, Guo P, et al. Construction of gastric cancer patient-derived organoids and their utilization in a comparative study of clinically used paclitaxel nanoformulations. *J Nanobiotechnology*. 2022;20(1):233. <https://doi.org/10.1186/s12951-022-01431-8>.
152. Yoon C, Lu J, Kim BJ, Cho SJ, Kim JH, Moy RH, et al. Patient-derived organoids from locally advanced gastric adenocarcinomas can predict resistance to neoadjuvant chemotherapy. *J Gastrointest Surg*. 2023;27(4):666–76.
153. Hao X, Zu M, Ning J, Zhou X, Gong Y, Han X, et al. Antitumor effect of luteolin proven by patient-derived organoids of gastric cancer. *Phytother Res*. 2023;37(11):5315–27.
154. Chen G, Han R, Wang L, Ma W, Zhang W, Lu Z, et al. Establishment of patient-derived organoids and a characterization based drug discovery platform for treatment of gastric cancer. *Cancer Cell Int*. 2024;24(1):288.
155. Xu J, Gong J, Li M, Kang Y, Ma J, Wang X, et al. Gastric cancer patient-derived organoids model for the therapeutic drug screening. *Biochimica et Biophysica Acta (BBA)*. 2024;1868(4): 130566.
156. van de Wetering M, Francies HE, Francis JM, Bounova G, Iorio F, Pronk A, et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell*. 2015;161(4):933–45.
157. Ooft SN, Weeber F, Dijkstra KK, McLean CM, Kaing S, van Werkhoven E, et al. Patient-derived organoids can predict response to chemotherapy in metastatic colorectal cancer patients. *Sci Transl Med*. 2019;11(513).
158. Narasimhan V, Wright JA, Churchill M, Wang T, Rosati R, Lannagan TRM, et al. Medium-throughput drug screening of patient-derived organoids from colorectal peritoneal metastases to direct personalized therapy. *Clin Cancer Res*. 2020;26(14):3662–70.
159. 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(15):4107–19.
160. Cho YW, Min DW, Kim HP, An Y, Kim S, Youk J, et al. Patient-derived organoids as a preclinical platform for precision medicine in colorectal cancer. *Mol Oncol*. 2022;16(12):2396–412.
161. Chen L, Tian B, Liu W, Liang H, You Y, Liu W. Molecular biomarker of drug resistance developed from patient-derived organoids predicts survival of colorectal cancer patients. *Front Oncol*. 2022;12: 855674.
162. Mo S, Tang P, Luo W, Zhang L, Li Y, Hu X, et al. Patient-derived organoids from colorectal cancer with paired liver metastasis reveal tumor heterogeneity and predict response to chemotherapy. *Adv Sci (Weinheim, Baden-Wurttemberg, Germany)*. 2022;9(31):e2204097.
163. He X, Jiang Y, Zhang L, Li Y, Hu X, Hua G, et al. Patient-derived organoids as a platform for drug screening in metastatic colorectal cancer. *Front Bioeng Biotechnol*. 2023;11:1190637.
164. Papaccio F, García-Mico B, Gimeno-Valiente F, Cabeza-Segura M, Gambardella V, Gutiérrez-Bravo MF, et al. Proteotranscriptomic analysis of advanced colorectal cancer patient derived organoids for drug sensitivity prediction. *J Exp Clin Cancer Res*. 2023;42(1): 8.
165. Martini G, Belli V, Napolitano S, Ciaramella V, Ciardiello D, Belli A, et al. Establishment of patient-derived tumor organoids to functionally inform treatment decisions in metastatic colorectal cancer. *ESMO Open*. 2023;8(3):101198.
166. Thng DKH, Hooi L, Siew BE, Lee KY, Tan IJ, Lieske B, et al. A functional personalised oncology approach against metastatic colorectal cancer in matched patient derived organoids. *NPJ Precis Oncol*. 2024;8(1): 52.
167. 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(4):285–304.
168. Liu J, Li D, Li J, Fu W, Jia Q, Bai Y, et al. Patient-derived organoids reveal marked heterogeneity in chemosensitivity profiles of colorectal cancer and a potential association with HER2 status. *Front Pharmacol*. 2025;16:1620764.
169. Ganesh K, Wu C, O'Rourke KP, Szelegin BC, Zheng Y, Sauvè CG, et al. A rectal cancer organoid platform to study individual responses to chemoradiation. *Nat Med*. 2019;25(10):1607–14.
170. Huang L, Holtzinger A, Jagan I, BeGora M, Lohse I, Ngai N, et al. Ductal pancreatic cancer modeling and drug screening using human pluripotent stem cell- and patient-derived tumor organoids. *Nat Med*. 2015;21(11):1364–71.
171. Tiriach H, Belleau P, Engle DD, Plenker D, Deschênes A, Somerville TDD, et al. Organoid profiling identifies common responders to chemotherapy in pancreatic cancer. *Cancer Discov*. 2018;8(9):1112–29.
172. Beato F, Reverón D, Dezi KB, Ortiz A, Johnson JO, Chen DT, et al. Establishing a living biobank of patient-derived organoids of intraductal papillary mucinous neoplasms of the pancreas. *Lab Invest*. 2021;101(2):204–17.
173. Watanabe S, Yogo A, Otsubo T, Umehara H, Oishi J, Kodo T, et al. Establishment of patient-derived organoids and a characterization-based drug discovery platform for treatment of pancreatic cancer. *BMC Cancer*. 2022;22(1):489.
174. Wu YH, Hung YP, Chiu NC, Lee RC, Li CP, Chao Y, et al. Correlation between drug sensitivity profiles of circulating tumour cell-derived organoids and clinical treatment response in patients with pancreatic ductal adenocarcinoma. *Eur J Cancer*. 2022;166:208–18.
175. Matsumoto K, Fujimori N, Ichihara K, Takeno A, Murakami M, Ohno A, et al. Patient-derived organoids of pancreatic ductal adenocarcinoma for subtype determination and clinical outcome prediction. *J Gastroenterol*. 2024;59(7):629–40.
176. 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(5):961–e7613.
177. Roalsø MTT, Alexeeva M, Oanæs C, Watson M, Lea D, Zaharia C, et al. Patient-derived organoids from pancreatic cancer after pancreatectomy: feasibility and organoid take rate in treatment-naïve periampullary tumors. *Pancreatol-ogy*. 2025;25(3):367–76.
178. Nitschke C, Phan C, Souto Y, Walter P, Goetz M, Simkus G, et al. Patient-derived tumor organoids highlight the potential of precision medicine in managing pancreatic ductal adenocarcinoma. *Int J Cancer*. 2025;157(4):760–72.
179. Schmäche T, Fohgrub J, Klimova A, Laaber K, Drukewitz S, Merboth F, et al. Stratifying esophago-gastric cancer treatment using a patient-derived organoid-based threshold. *Mol Cancer*. 2024;23(1):10.
180. Qu J, Kalyani FS, Liu L, Cheng T, Chen L. Tumor organoids: synergistic applications, current challenges, and future prospects in cancer therapy. *Cancer Commun (London England)*. 2021;41(12):1331–53.
181. Veninga V, Voest EE. Tumor organoids: opportunities and challenges to guide precision medicine. *Cancer Cell*. 2021;39(9):1190–201.
182. Fujii M, Matano M, Toshimitsu K, Takano A, Mikami Y, Nishikori S, et al. Human intestinal organoids maintain Self-Renewal capacity and cellular diversity in Niche-Inspired culture condition. *Cell Stem Cell*. 2018;23(6):787–e936.
183. Weeber F, Ooft SN, Dijkstra KK, Voest EE. Tumor organoids as a pre-clinical cancer model for drug discovery. *Cell Chem Biol*. 2017;24(9):1092–100.
184. Sachs N, Pappaspyropoulos A, Zomer-van Ommen DD, Heo I, Böttlinger L, Klay D, et al. Long-term expanding human airway organoids for disease modeling. *EMBO J*. 2019;38(4).
185. Dijkstra KK, Cattaneo CM, Weeber F, Chalabi M, van de Haar J, Fanchi LF, et al. Generation of tumor-Reactive T cells by Co-culture of peripheral blood lymphocytes and tumor organoids. *Cell*. 2018;174(6):1586–e9812.
186. Yuki K, Cheng N, Nakano M, Kuo CJ. Organoid models of tumor immunology. *Trends Immunol*. 2020;41(8):652–64.
187. Hassell BA, Goyal G, Lee E, Sontheimer-Phelps A, Levy O, Chen CS, et al. Human organ chip models recapitulate orthotopic lung cancer growth, therapeutic responses, and tumor dormancy in vitro. *Cell Rep*. 2017;21(2):508–16.

188. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE. Reconstituting organ-level lung functions on a chip. Volume 328. New York, NY: Science; 2010. pp. 1662–8. 5986.
189. Skardal A, Shupe T, Atala A. Organoid-on-a-chip and body-on-a-chip systems for drug screening and disease modeling. *Drug discovery today*. 2016;21(9):1399–411.
190. Kim J, Koo BK, Knoblich JA. Human organoids: model systems for human biology and medicine. *Nat Rev Mol Cell Biol*. 2020;21(10):571–84.
191. Gjorevski N, Sachs N, Manfrin A, Giger S, Bragina ME, Ordóñez-Morán P, et al. Designer matrices for intestinal stem cell and organoid culture. *Nature*. 2016;539(7630):560–4.
192. Skylar-Scott MA, Uzel SGM, Nam LL, Ahrens JH, Truby RL, Damaraju S, et al. Biomanufacturing of organ-specific tissues with high cellular density and embedded vascular channels. *Sci Adv*. 2019;5(9): eaaw2459.
193. Dekkers JF, Berkens G, Kruisselbrink E, Vonk A, de Jonge HR, Janssens HM, et al. Characterizing responses to CFTR-modulating drugs using rectal organoids derived from subjects with cystic fibrosis. *Sci Transl Med*. 2016;8(344): 344ra84.
194. 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(1): 30.
195. Ren Y, Yuan C, Liang Q, Ba Y, Xu H, Weng S, et al. 3D Bioprinting for engineering organoids and Organ-on-a-Chip: developments and applications. *Medicinal research reviews*; 2025.
196. Hu Y, Zhu T, Cui H, Cui H. Integrating 3D bioprinting and organoids to better recapitulate the complexity of cellular microenvironments for tissue engineering. *Adv Healthc Mater*. 2025;14(3): e2403762.
197. 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.
198. Lin H, Cheng J, Zhu C, Yang Z, Shen Q, Zou Y, et al. Artificial intelligence-enabled quantitative assessment and intervention for heart inflammation model organoids. *Angew Chem Int Ed Engl*. 2025;64(25): e202503252.
199. Huang K, Li M, Li Q, Chen Z, Zhang Y, Gu Z. Image-based profiling and deep learning reveal morphological heterogeneity of colorectal cancer organoids. *Comput Biol Med*. 2024;173: 108322.
200. Zhu Z, Shen J, Ho PC, Hu Y, Ma Z, Wang L. Transforming cancer treatment: integrating patient-derived organoids and CRISPR screening for precision medicine. *Front Pharmacol*. 2025;16: 1563198.
201. Hosseini SA, Salehifard Jouneghani A, Ghatrehsamani M, Yaghoobi H, Elahian F, Mirzaei SA. CRISPR/Cas9 as precision and high-throughput genetic engineering tools in gastrointestinal cancer research and therapy. *Int J Biol Macromol*. 2022;223(Pt A):732–54.

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