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Review

Patient-derived organoids in precision cancer medicine

Le Tong,[1,*] Weiyingqi Cui,[2] Boya Zhang,[3] Pedro Fonseca,[1] Qian Zhao,[3] Ping Zhang,[3] Beibei Xu,[4] Qisi Zhang,[4] Zhen Li,[4] Brinton Seashore-Ludlow,[1] Ying Yang,[1,5] Longlong Si,[4,*] and Andreas Lundqvist[1,*]

[1]Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden
[2]Chemical Biology Consortium Sweden, Science for Life Laboratory, Karolinska Institutet, Stockholm, Sweden
[3]Organcare (Shenzhen) Biotechnology Company, Shenzhen, China
[4]Key Laboratory of Quantitative Synthetic Biology, Shenzhen Institute of Synthetic Biology, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China
[5]Department of Respiratory Medicine, The Fourth Affiliated Hospital, International Institutes of Medicine, Zhejiang University School of Medicine, Zhejiang, China
*Correspondence: le.tong@ki.se (L.T.), ll.si@siat.ac.cn (L.S.), andreas.lundqvist@ki.se (A.L.)
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SUMMARY

Organoids are three-dimensional (3D) cultures, normally derived from stem cells, that replicate the complex structure and function of human tissues. They offer a physiologically relevant model to address important questions in cancer research. The generation of patient-derived organoids (PDOs) from various human cancers allows for deeper insights into tumor heterogeneity and spatial organization. Additionally, interrogating non-tumor stromal cells increases the relevance in studying the tumor microenvironment, thereby enhancing the relevance of PDOs in personalized medicine. PDOs mark a significant advancement in cancer research and patient care, signifying a shift toward more innovative and patient-centric approaches. This review covers aspects of PDO cultures to address the modeling of the tumor microenvironment, including extracellular matrices, air-liquid interface and microfluidic cultures, and organ-on-chip. Specifically, the role of PDOs as preclinical models in gene editing, molecular profiling, drug testing, and biomarker discovery and their potential for guiding personalized treatment in clinical practice are discussed.

INTRODUCTION

The advent of organoids, a groundbreaking development in the field of stem cell research over the past decade, has revolutionized various domains, including regenerative medicine, drug development, and precision medicine.[1] Organoids, essentially mini-organs, are derived from adult stem cells (ASCs) or pluripotent stem cells (PSCs) cultured in three-dimensional (3D) environments. These tissue analogs not only possess a complex spatial structure but also exhibit histological features and physiological functions highly similar to their corresponding human tissues.[2]

In cancer research, the evolution of tumor models has played a pivotal role in deepening our understanding of cancer biology and in the development of novel therapeutic approaches. Despite being widely used and offering insights into tumor progression, dissemination, and drug responses, murine models come with limitations, including high costs, time constraints, ethical considerations, and, most notably, significant biological differences from humans. Similarly, traditional two-dimensional (2D) tumor models, predominantly used for high-throughput drug screening, are limited in their ability to accurately replicate the 3D microenvironment of actual tumors, often leading to discrepancies in drug-response data.[3]

To overcome these challenges, the development of patient-derived organoid (PDO) cultures has emerged as a critical advancement. PDOs effectively mimic the 3D structure and function of human tissues, signifying an important advancement in cancer research.[3–5] This approach circumvents many of the ethical and biological issues inherent to *in vivo* models and potentially more accurately represents the physical and biological attributes of human tumors. Thus, PDOs serve as valuable tools to study several aspects of tumor biology and can enhance the predictive accuracy of drug efficacy assessments. The methodologies for establishing such cultures, including the culture of epithelial organoids in extracellular matrices, air-liquid interface (ALI) cultures of minced tumor fragments, microfluidic cultures, and organ-on-chip (OoC), have been well summarized and described.[6–9]

A significant milestone in the recognition of organoids as valuable research tools was the approval by the US Food and Drug Administration (FDA) approval of these models as New Alternative Methods for drug development, as per the FDA Modernization Act 2.0 of 2022.[10] This recognition not only highlights the potential use of organoids as alternatives or supplements to animal

studies but it also signals a shift toward more ethical and scientifically robust platforms in cancer research.

Numerous studies have successfully generated PDOs from a variety of human cancers, including but not limited to breast, colon, kidney, ovarian, pancreatic, and liver cancers.[11] These PDO models are pivotal in capturing the cellular heterogeneity and spatial organization found within tumors,[12,13] thereby providing valuable models for studying cancer development and progression. Furthermore, the culture of cancer organoids together with non-tumor stromal cells has opened new avenues for exploring the tumor microenvironment (TME). As innovative tools, PDOs have demonstrated predictive values, thus paving the way for personalized medicine and clinical decision making.[6,7,14]

This review focuses on the rapid advancements in the field of PDOs. It discusses various methods for establishing PDOs to more effectively replicate the TME. The multifaceted applications of PDOs are extensively examined in Table 1, including their use as pre-clinical models in genomic editing, molecular and biochemical profiling, drug testing, and biomarker discovery. Additionally, the current state of PDO biobanks and the integration of these models into clinical practice, ultimately aiming to facilitate personalized treatment strategies, are discussed (Figure 1).

GENERATION OF 3D ORGANOID CULTURES: MODELING THE TME

Tumors are specialized tissues, characterized by the infiltration of diverse stromal cells, such as fibroblasts, endothelial cells (ECs), and immune cells.[84,85] Tumor development and progression are contingent upon intricate interactions among various cell types within the TME.[86,87] Furthermore, the TME is recognized as playing a pivotal role in tumor metastasis, immunosuppression, and pharmacological responsiveness.[88,89] Consequently, elucidating the interactions between tumor cells, tumor-associated fibroblasts, ECs, immune cells, and other stromal cells within the TME is imperative for an in-depth understanding of the TME; this is crucial for discovering novel cancer therapies.[7,8,90,91] Currently, there are two main concepts for modeling the TME *in vitro*.[9]

The first concept involves the culture of cancer organoids within an extracellular matrix (ECM) to reconstitute the TME. Traditional models of this kind are typically composed of tumor epithelial cells, and they can also be supplemented with stromal cells to more accurately reconstruct the TME. The second approach focuses on preserving the intrinsic TME of the tumor by culturing tumor fragments using ALI or microfluidics to culture single-cell suspension-derived mini-tumor spheroids (Figure 2).

The *ex vivo* modulation of the TME through the co-culture of cancer cells with different stromal cells in 3-D matrices provides the groundwork for the different applications of PDOs discussed in the following sections.

Culture with immune cells

The burgeoning field of cancer research is increasingly utilizing organoid co-culture models to study complex interactions between cancer cells and the immune system. These models incorporate various immune cells such as lymphocytes,[22,58] cytotoxic T lymphocytes (CTLs),[59,60,75] dendritic cells (DCs),[23,59,60,75] natural killer (NK) cells,[21] and macrophages.[61]

In pancreatic ductal adenocarcinoma (PDAC), cultures of primary human PDAC organoids with matched peripheral blood mononuclear cells (PBMCs) have shown promise. Flow cytometry analysis of such cultures indicates significant changes in T cell subtypes, correlating with improved patient outcomes. This model is particularly effective for exploring personalized therapeutic strategies in PDAC.[58] For epithelial cancers, autologous tumor organoids cultured with lymphocytes can enrich for tumor-reactive T cells that attack the PDOs but not organoids derived from normal tissue. This method is vital for assessing the responsiveness of tumor cells to T cell-mediated reactivity, especially in mismatch repair-deficient colorectal cancer (CRC) and non-small cell lung cancer.[22]

The culture of gastric cancer organoids with autologous bone marrow-derived DCs and spleen-derived CTL cells sheds light on programmed cell death ligand 1(PD-L1)/programmed cell death protein 1 (PD-1) interactions in gastric cancer.[75] In human PDAC and gastric cancer, co-cultures with DCs and CTLs, optionally with myeloid-derived suppressor cells, offer insights into enhancing CTL effector function and targeting PD-L1-expressing cancer cells.[59,60]

Metastasis, particularly in breast and CRC, is another critical area of investigation. Novel 3D models using NK cells and tumor organoids have been developed to mimic the interactions between these cells, providing insights into metastatic biology and potential therapeutic targets. This approach is particularly useful for studying NK cell cytotoxicity against metastatic breast cancer cells.[21] In CRC, the interaction between DCs and metastatic tumor cells in a 3D co-culture system has been pivotal in understanding the CRC TME. This model has revealed how CRC organoids influence the behavior, phenotype, and function of monocyte-derived DCs, thereby offering new perspectives on CRC-driven DC dysfunction and potential therapeutic interventions.[23]

A recent study in pancreatic adenocarcinoma (PAAD) has leveraged PDOs cultured with macrophages to unravel the mechanisms behind gemcitabine resistance. This research highlights the potential of targeting the macrophage-CCL5-Sp1-AREG feedback loop, enhancing the efficacy of treatments for PAAD.[61]

Another study explored the feasibility of creating combined lymph node/melanoma organoids for personalized immunotherapy screening. Using primary melanoma and lymph node biopsies from the same patient, Votanopoulos and colleagues developed 3D "immune-enhanced" tumor organoids, maintaining tumor heterogeneity and immune components. The organoids, tested with various immunotherapies, demonstrated a high success rate in mimicking clinical responses and were used to activate patient-matched peripheral blood T cells for effective tumor cell killing, highlighting their potential in studying personalized immunotherapy responses.[53]

A bioprinting model featuring gastric cancer PDOs and tumor-infiltrating lymphocytes (TILs) was reported to investigate the dynamics of the immune response against tumors. The model, using a mix of alginate, gelatin, and basal membrane, enabled the investigation of TIL migratory patterns and their interactions with PDOs,

Table 1. PDOs and their applications

Cancer type	Source	Biological analysis	Applications	Reference
Bladder	resected tumor	IHC, RNA-seq, whole-exome sequencing, mutation and phylogeny analysis	xenograft, drug response assay	Lee et al.[15]
	mouse and human tumor tissues	IF, RNA-seq, mutation analysis	xenograft, drug-response assay	Mullenders et al.[16]
Brain	resected tumor	IHC, mutation analysis, RNA-seq, methylation assay, hypoxia detection	biobanking, drug testing, xenografts, CAR-T efficiency testing	Jacob et al.[17]
	resected tumor	IF, IHC	xenograft	Hubert et al.[18]
Breast	resected tumor	RNA-seq, somatic mutation, IF, IHC	xenotransplantation, drug screening	Hubert et al.[19]
	resected tumor	IHC, RNA-seq, whole-exome sequencing, WB	clinical outcome evaluation, drug response	Chen et al.[20]
Breast cancer (metastatic)	musculus with mouse mammary tumors and human breast tumors	cytotoxicity study	NK-organoid co-culture, biological research	Chan and Ewald[21]
Colon	surgically resected or needle biopsy-obtained samples	FC, IHC, WGS	organoid-lymphocyte co-culture to generate functional T cells	Dijkstra et al.[22]
	resected tumor	IF, FC	dendritic cell-organoid co-culture for tumor modeling	Subtil et al.[23]
	fresh tumor biopsy specimens	cytotoxicity, IHC	biological research, chemoradiotherapy treatment	Kong et al.[24]
	human normal and tumor tissues	killing assay, IHC	organoid-PBMC co-culture	Harter et al.s[25]
	resected tumor	gene expression microarray, WGS	xenotransplantation	Fujii et al.[26]
	resected tumor	DNA-seq	biological research	Weeber et al.[27]
	Resected tumor	whole-exome analysis, RNA-seq, IF	organoid-stroma biobanking, xenotransplantation, co-culture, drug testing and screening	Farin et al.[28]
	Biopsy samples	RNA-seq, IF, IHC	biobanking, drug screening	Luo et al.[29]
	resected tumor	viability assay, mutational profile, IHC, migration assay, RNA-seq	fibroblast-organoid co-culture, drug treatment	Atanasova et al.[30]

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Table 1. *Continued*

Cancer type	Source	Biological analysis	Applications	Reference
	resected tumor	targeted sequencing, viability	fibroblast-organoid co-culture	Naruse et al.[31]
	resected tumor	viability	drug treatment	Pinho et al.[32]
	resected tumor	FC	co-culture, drug treatment	Chen et al.[33]
	resected tumor	mass spectrometry	assessing patient-specific organoid proteome profile	Cristobal et al.[34]
	resected tumor	DNA-seq	clinical outcome evaluation, drug screening	Ooft et al.[35]
	resected tumor	viability assay, live imaging, FC	biomarker discovery, drug screening, xenograft	Verissimo et al.[36]
CRC liver metastasis	resected tumor	IHC, RNA-seq, and whole-exome and single-cell sequencing	drug testing	Mo et al.[13]
Head and neck	resected tumor	whole-exosome sequencing, IF, IHC	biobanking, drug and radiotherapy evolution, clinical correlation	Milen et al.[37]
	resected tumor	RNA-seq, HSV infection and quantification, NGS, IF, IHC	drug screening, radiation and chemoradiation treatment, xenotransplantation	Driehuis et al.[38]
	resected tumor	cell viability	assay development for drug screening	Driehuis et al.[39]
	resected tumor	IF, IHC	co-culture, biological research	Zhao et al.[40]
Kidney	resected tumor	bulk RNA-seq, whole-genome and single-cell sequencing, FC, IHC	drug screening	Calandrini et al.[41]
	resected tumor	RNA-seq, DNA mutation analysis, statistical analysis, and short tandem repeat evaluation, IF, IHC	biological research, personalized therapy	Grassi et al.[42]
	resected tumor	RNA-seq, IHC, IF	drug treatment	Esser et al.[43]
Liver	human normal and tumor tissues	whole-genome sequencing, RNA-seq, IHC and ISH, WB	xenotransplantation, drug screening	Broutier et al.[44]
	PDX	live imaging, RNA-seq	co-culture for tumor modeling	Lim et al.[45]
	resected tumor	IHC, RNA-seq, whole-genome and single-cell sequencing, HLA typing, co-IP and mass spectrometry, killing assay	immunogenic peptide incubation	Wang et al.[46]

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Table 1. ***Continued***

Cancer type	Source	Biological analysis	Applications	Reference
	resected tumor	single-cell sequencing, IHC, whole-genome sequencing, somatic mutation calling	biomarker discovery, drug screening	Zhao et al.[47]
Lung	resected tumor	whole-exome and TCR sequencing, FC, IHC	co-culture, neoadjuvant immunotherapy testing	Chalabi et al.[48]
	human normal and tumor tissues	RNA-seq, whole genome and hotspot sequencing, IHC, IF, functional organoid swelling assay, multiplex immunoassays	xenograft, drug screening, ALI cultures, neutrophil co-culture	Sachs et al.[49]
	human normal and tumor tissues	whole-genome sequencing, RNA-seq, ATAC-seq, IHC and ISH, capillary-based immunoassay	xenotransplantation, drug testing	Ebisudani et al.[50]
	biopsy samples	cell death	drug testing	Shin et al.[51]
	resected tumor from patients and mice	FC, IF, time-lapse imaging, RNA-seq, IHC	immunotherapy testing	Jenkins et al.[52]
Melanoma	tissue biospecimens	IF, IHC, viability	drug studies, adaptive immunity pilot study	Votanopoulos et al.[53]
Nasopharyngeal	resected tumor	IF, IHC, ISH	biobanking, biological research	Wang et al.[54]
Neuroendocrine	resected tumor	IHC, microarray, ATAC-seq, RNA-seq, whole-genome and whole-exome sequencing, mutation analysis, capillary-based immunoassay	xenograft, drug testing	Kawasaki et al.[55]
Ovary	resected tumor	IHC, RNA-seq, methylation and WGS	dose screening, xenograft	Kopper et al.[56]
	resected tumor	IHC, whole-exome sequencing	dose-response curves	Hill et al.[57]
Pancreas	resected tumor	FC	PBMC-organoid co-culture, biological research	Knoblauch et al.[58]
	resected tumor from patients and mice	IHC, IF, FC	immune cell-organoid co-culture, biological research	Holokai et al.[59]
	biopsy sample	IF, FC	immune cell-organoid co-culture	Chakrabarti et al.[60]
	resected tumor	FC, WB, IF cell viability, ELISA, single-cell RNA-seq, cytokine antibody array	macrophage-organoid co-culture	Jiang et al.[61]

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Table 1. *Continued*

Cancer type	Source	Biological analysis	Applications	Reference
	mouse tumor and human pancreatic cancer tissue sample	FC, IHC, IF, hypoxia assay, RNA-seq, RT-qPCR, T cell cytotoxicity assay	T cell-organoid co-culture, immunotherapy testing	Zhou et al.[62]
	human normal and tumor tissues	WGS and pharmacotranscriptomic analysis	biomarker discovery, biological research, drug testing	Tiriac et al.[63]
	human and murine surgical or biopsy-obtained sample	RNA-seq, proteomic analysis	transplantation, tumor modeling, biological research	Boj et al.[64]
	resected tumor	IHC, ISH, whole-exome sequencing, methylation, microarray analysis	CAF-organoid co-culture, xenotransplantation, biological research	Seino et al.[65]
	fresh resected and cryopreserved tumor	DNA fingerprinting, IHC, targeted DNA-seq	biobanking, biological research	Beato et al.[66]
	resected tumor	IHC, single-cell sequencing, FC	fibroblast-organoid co-culture, drug treatment	Kinny-Köster et al.[67]
	resected tumor	IF, single-cell sequencing	fibroblast-organoid co-culture, drug screening	Schuth et al.[68]
	resected tumor	IHC	fibroblast-organoid co-culture, tumor modeling	Tsai et al.[69]
	resected tumor and PDX	IHC, whole exome sequencing, RNA-seq, mutation signature, WB	PDX, drug screening	Hirt et al.[70]
	resected tumor	whole-exome sequencing	biomarker discovery, pharmacotyping	Seppälä et al.[71]
Prostate	resected tumor	IHC, RNA-seq, whole-exome sequencing, methylation, viability and proliferation	tumor modeling, biological research, xenotransplantation	Gao et al.[72]
	PDX and patient-derived samples	IF, RNA-seq, and whole-exome sequencing	biobanking, dose-response assays	Beshiri et al.[73]
Rectum	biopsy samples	whole-exome sequencing, IF, IHC	irradiation and drug testing	Yao et al.[74]
Stomach	mouse tumor biopsy tissue	FC, live imaging, tunnel assay	organoid-immune cell co-culture, biological research	Chakrabarti et al.[75]
	resected tumor	whole-genome sequencing, RNA-seq, DNA mutation analysis, IHC, ISH, and viability	biobanking, biological research, drug screening	Yan et al.[76]
	resected tumor	whole-genome sequencing, RNA-seq, DNA mutation analysis, IHC, ISH, FRET, capillary-based immunoassay, FC	biological research, xenograft	Nanki et al.[77]

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Table 1. *Continued*

Cancer type	Source	Biological analysis	Applications	Reference
Upper tract urothelial carcinoma	resected tumor	IHC, RNA-seq, and whole-exome and single-cell sequencing	drug screening	Li et al.[78]
Mixed (colon and rectum)	resected tumor	WGS, viability assays	biobanking, drug screening	Van de Wetering et al.[79]
Mixed (colon and duodenum)	human biopsies and animal necropsies	mass spectrometry, drug metabolizing enzyme activity and bidirectional transport studies	toxicity screening	Kourula et al.[80]
Mixed (colon and pancreas)	needle core biopsy	bulk RNA-seq, IF	drug treatment	Choi et al.[81]
Mixed (colon and esophagus)	mouse and human intestinal fragments, surgically resected intestinal tissues or endoscopic biopsies	IF, IHC	biological research	Sato et al.[82]
Mixed (lung, melanoma, kidney, bladder)	resected tumor from patients and mice	targeted panel and exome sequencing, mutation analysis, IHC, IF, cytotoxicity, single-cell sequencing	xenotransplantation, drug treatment	Neal et al.[83]

CAR-T, chimeric antigen receptor T cell; co-IP, co-immunoprecipitation; FC, flow cytometry; FRET, fluorescence resonance energy transfer, HSV, herpes simplex virus; IF, immunofluorescence; IHC, immunohistochemistry; ISH, *in situ* hybridization; PDX, patient-derived xenograft; WB, western blot.

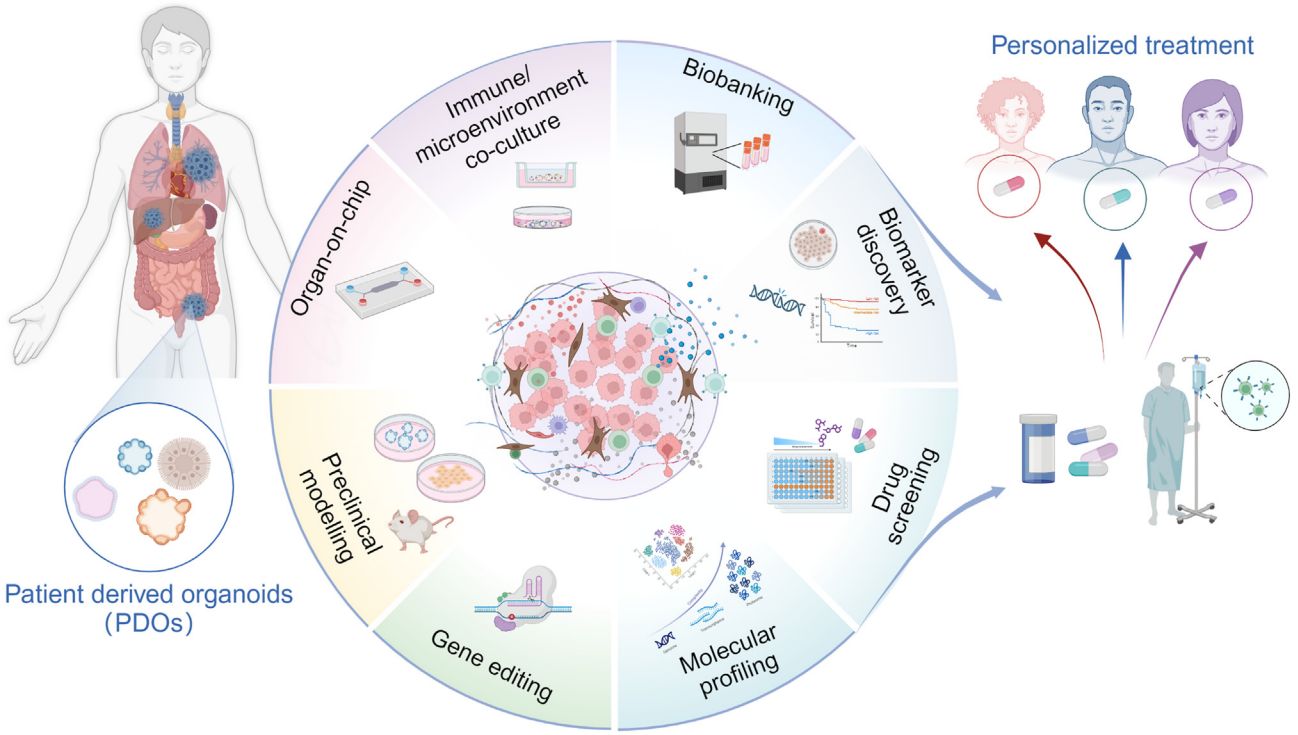

Figure 1. From PDOs to personalized treatment

An overview of PDOs in tailoring personalized cancer research. Topics covered include microenvironment modeling, OoC, gene editing, molecular profiling, *ex vivo* preclinical modeling, drug screening, biomarker discovery, and biobanking. Created with BioRender.com.

offering new insights into TIL activation, degranulation, and proteolytic activity, as well as to uncover specific stimuli to differentiate between passive and active cell migration mechanisms.[92]

Upon culturing rectal cancer PDOs with patient-matched TILs, checkpoint receptor blockade with anti-PD-1 antibody was evaluated by measuring cytotoxicity. With exposure to anti-PD-1 antibody, a partial restoration of TIL cytotoxicity was observed.[24]

Harter et al. designed a model of PDOs from intestinal tumor cells cultured together with immune cells to investigate on-target off-tumor toxicities using T cell-engaging bispecific antibodies. They observed that antibodies targeting epithelial cell adhesion molecules resulted in apoptosis in healthy organoids, whereas tumor organoids were more resistant to apoptosis.[25] Zhou et al. developed organoids containing a mix of tumor epithelial cells, ECs, fibroblasts, and macrophages to study T cell reactivity. They observed that treatment with the histone deacetylase inhibitor ITF2357 and the BET bromodomain inhibitor I-BET151, together with anti-PD-1 antibodies, led to an upregulation of major histocompatibility complex class I (MHC class I)-related antigen presentation in tumor cells, thereby improving T cell cytotoxicity.[62] Neo et al. reported that the presence of NK cells can influence the migration of uveal melanoma cells into liver organoids.[93] Immune cells such as microglia can represent a large proportion of the tumor tissue and are known to play a major role in the progression of glioblastoma.[94]

These examples highlight that organoid 3D co-cultures provide an avenue to study the crosstalk between immune cells and cancer cells. These models might be crucial to develop new immune checkpoint inhibitor and adaptive cell therapy modalities for cancer treatment. The challenge will be to accurately mimic the conditions immune cells face in the TME—for example, the hypoxic conditions present in many solid tumors that might hinder an immune response—but new technological developments discussed in this review might prove valuable in this endeavor.

In summary, the use of cancer organoid co-culture models marks a significant advancement in cancer research. By closely replicating the TME and facilitating the study of intricate cellular interactions, these models are instrumental in developing immune-related, targeted, patient-specific cancer therapies and enhancing our understanding of cancer biology.

Culture with cancer-associated fibroblasts

Solid tumors are infiltrated by a diverse and adaptable population of cancer-associated fibroblasts (CAFs), which have been shown to accelerate tumor progression and treatment resistance through various mechanisms. These mechanisms include promoting neoangiogenesis, creating dense stromal reactions that interfere with drug and immune cell infiltration, and directly inhibiting immune effector cells.[95] Recent studies have significantly advanced our understanding of the interactions between CAFs and PDOs, revealing their crucial role in modulating the behavior of PDOs across various cancers. CAFs achieve this, for instance, by facilitating tumor stem cell formation within PDOs,[40] by

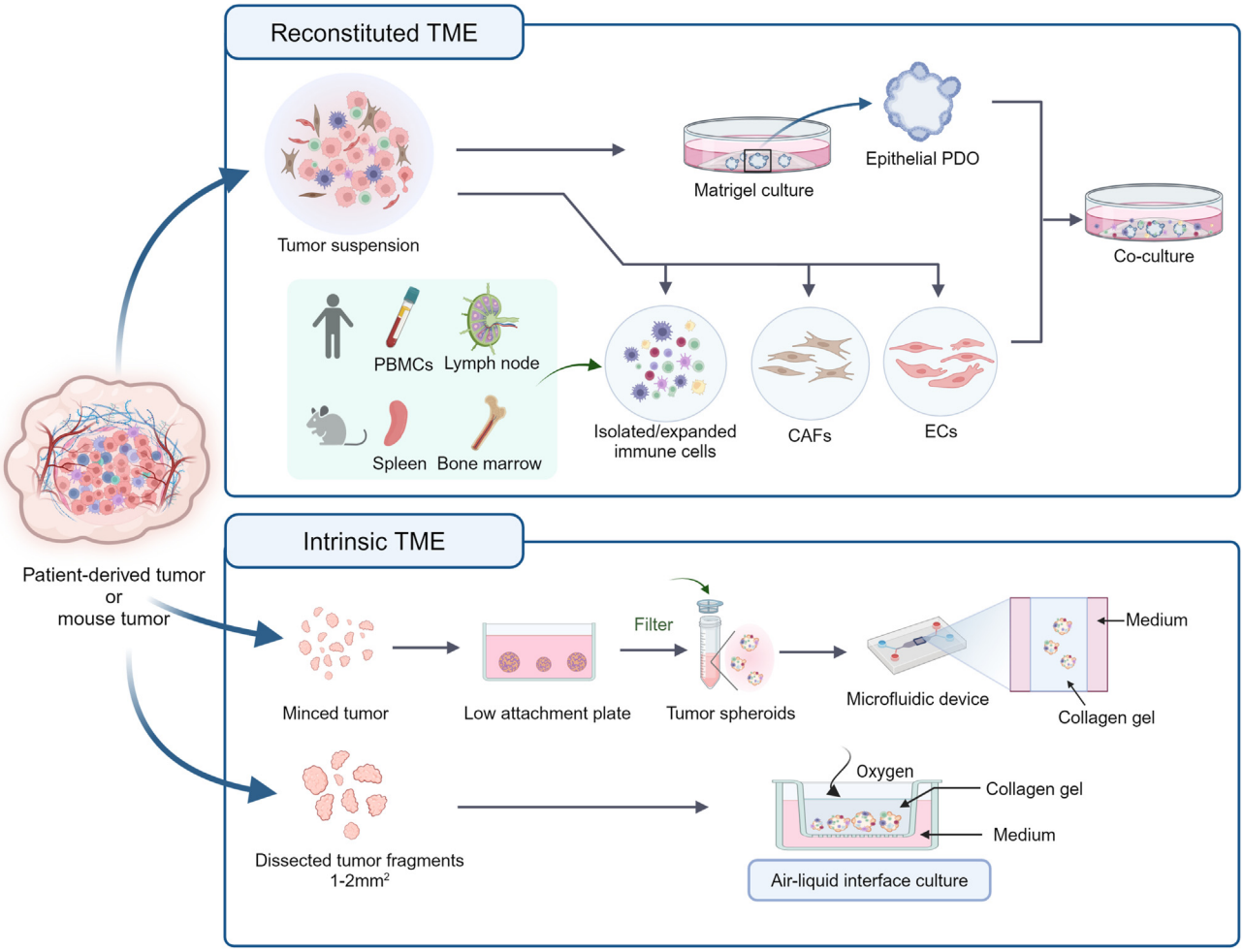

Figure 2. Three-dimensional modeling of the TME in PDO cultures

Illustration of two methodologies for *in vitro* modeling of the TME. (Top) Reconstituting TME that involves cultures of tumor cell suspensions in an ECM (e.g., Matrigel) to establish PDOs, typically tumor epithelial PDOs. Immune cells, CAFs, and ECs from various sources are isolated and can be used in cultures with PDOs. (Bottom) Maintaining intrinsic TME employs microfluidic cultures of tumor spheroids and ALI culture of tumor fragments to mimic the TME. Created with BioRender.com.

stimulating PDO proliferation,[30,31,68] by stabilizing the expression of tumor genes in PDOs,[31] by inducing inflammation,[67] and by potentially increasing the drug resistance of PDOs.[68,96]

In oral squamous cell carcinoma, culture with CAFs enhances the organoid-forming ability of cancer stem cells, indicating a pro-tumoral activity of CAFs.[40] Similarly, in CRC, culture of primary fibroblasts with tumor cells revealed that both cancer-associated and normal fibroblasts support cancer cell proliferation and increases cellular heterogeneity in 3D organoids, closely mimicking the *in vivo* tumor morphology. This study also highlighted the mutual crosstalk between tumor cells and fibroblasts, with significant deregulation in pathways linked to cell-cell communication and ECM remodeling, and identified Thrombospondin-1 as a key factor in fibroblast invasiveness.[30] CAFs can also restore the expression of certain genes downregulated in CRC organoids, particularly those related to immune response and external stimuli, such as the REG family and dual oxidases. These genes are known to contribute to malignant functions, leading to proliferative, anti-apoptotic, and drug-resistant phenotypes in tumor cells, indicating that culture systems based on PDOs together with CAFs can effectively mimic aspects of the TME.[31]

In PDAC, PDO-CAF cultures revealed an enhanced epithelial inflammatory response and expression of MHC class II genes.[67] Additionally, these co-cultures demonstrated the ability of matrix-activated CAFs to re-engineer the stiffness of the fibrotic environment through lysyl-oxidase-dependent crosslinking. This model also revealed that CAF cultures increase exosome production, which contributes to chemoresistance, and showed that inhibiting exosome hypersecretion can reduce chemoresistance, emphasizing the potential of the model in developing therapies targeting the biophysical aspects of tumor growth and chemoresistance.[96] Moreover, these co-cultures have been instrumental in revealing an increased PDAC organoid

proliferation, the induction of a pro-inflammatory phenotype in CAFs, and the upregulation of epithelial-to-mesenchymal transition genes, thereby further emphasizing the crucial role of CAFs in PDAC progression and chemoresistance.[68]

Another study successfully created complex organotypic models integrating tumor, stromal, and immune components; providing valuable insights into tumor-stroma and tumor-immune interactions; and assessing immunotherapeutics.[69] These findings collectively emphasize the critical role of CAFs in the TME across various cancers and highlight the potential of PDO-CAF co-culture systems as powerful tools for understanding cancer biology and developing effective treatments.

Culture with ECs

Tumor cells recruit ECs by secreting angiogenic factors, forming numerous irregular and fragile new blood vessels that supply oxygen and nutrients essential for tumor growth.[97–99] Targeting angiogenesis is a crucial aspect of cancer therapy.[100] Consequently, incorporating ECs into epithelial-immune cell co-cultures aids in the study of angiogenesis signaling, with the objective of developing novel angiogenesis-related treatments. Currently, there are few reports on tumor organoids and ECs, indicating a need for further research in this area. One study established co-culture models using a hydrogel system to mimic and study the angiocrine interactions between hepatocellular carcinoma organoids and ECs. These models showed that culture of patient-derived xenograft (PDX) organoids with ECs led to an inflammatory microenvironment, characterized by the upregulation of MCP-1, IL-8, and CXCL16. Furthermore, macrophages have been integrated into this co-culture system, with these cells showing polarization toward a pro-inflammatory and pro-angiogenic phenotype.

These findings indicate that co-culture models involving ECs and organoids may be pivotal in understanding and targeting the complex interplay between angiogenesis and the immune environment.[45] However, a major challenge is to mimic proper vascularized systems in *ex vivo* organoid cultures, but coupling new 3D culturing technologies such as ALI, microfluidics, OoC, and bioprinting techniques might circumvent such limitations.

ALI cultures

The ALI method involves embedding primary tissue fragments containing tumor and immune components in the ECM and then placing them into ECM-coated transwell culture dishes. This technique not only supports cells with a 3D matrix but it also ensures adequate oxygen supply due to the top exposure to air of the matrix. PDOs from various cancers, including colon, lung, and renal cancers, have been shown to maintain immune cells and fibroblasts within the tumor during 1–2 months of ALI *in vitro* culture.[33,43,83] ALI PDOs derived from non-small cell lung cancer, melanoma, renal cell carcinoma (RCC), and bladder cancer have been found to contain functional TILs. These TILs preserved the original tumor T cell receptor (TCR) repertoire and demonstrated the capacity to induce tumor cytotoxicity. Furthermore, they responded effectively to immune checkpoint blockade (ICB) therapies specifically targeting PD-1 or PD-L1 pathways.[83] By preserving the TME and immune cell interactions, potential future directions for translational applications of ALI PDOs include precision medicine approaches to optimize ICB therapies for various cancer types.

Microfluidic cultures of patient-derived organotypic tumor spheroids

The microfluidic culture of patient-derived organotypic tumor spheroids (PDOTs) involves culturing minced tumor tissues in a low-attachment plate to form spheroids, which are then cultured in a microfluidic device filled with collagen gel and medium. The device precisely controls the flow and concentration of the gel and medium through microchannels, ensuring a stable and dynamic environment for the spheroids. PDOs cultured in microfluidic devices successfully retain tumor cells along with endogenous immune cells, such as lymphocytes and myeloid cells. This technique has been used for the culture of melanoma, Merkel cell, head and neck, thyroid, lung, colon, and pancreatic cancer PDOs.[32,52,101] Jenkins et al. identified various lymphoid and myeloid populations in PDOTs that were derived from various cancer types. They found immune cells among epithelial cell adhesion molecule-positive tumor cells and observed dynamic cellular interactions.[52] Microfluidic cultures aid in developing more effective and personalized immunotherapies, enhancing the precision of high-throughput drug screening platforms, and advancing the understanding of tumor-stroma cell interactions to improve treatment strategies for various cancers.

OoC

OoC systems integrate multiple scientific disciplines to create sophisticated simulations of human physiology, and they represent an advanced approach compared to simpler tumor organoid/spheroid microfluidic cultures. The development of OoC technologies integrates cell biology, microfluidics, tissue engineering, biomaterial research, and microfabrication, thereby providing an ideal platform for simulating tumor physiology.[102] In comparison to traditional disease models, OoC platforms offer several significant advantages, the paramount being their ability to manipulate cellular and tissue environments, biomechanical, and biochemical forces to simulate human physiological responses. Additionally, vascularization and tissue perfusion offer the ability to supply nutrients and fluid flow. Ultimately, real-time sensors can be integrated to monitor the conditions and activities of cells.[103–105]

Leveraging OoC platform technologies facilitates the establishment of physiologically accurate *in vitro* 3D disease models, enabling the precise replication of the intricate pathological processes within the human body. Various tumor organ models, encompassing multiple cell types and structures resembling primary tumors, have been established, including glioblastoma, breast, lung, colorectal and pancreatic cancers.[106–112] For neuroblastoma studies, multiple cell types were combined with gelatin-methacrylate/fibrin to simulate the TME. Human neuroblastoma spheroids and human umbilical vein ECs were cultured with gelatin-methacrylate, allowing close contact between the two cell types. This setup directly reflected the interaction between tumor cells and blood vessels, successfully constructing a microvascular neuroblastoma tumor environment chip model.[113] In breast cancer research, various organ chips have

been developed. For instance, one chip contains two cell compartments, with breast cancer microtissue or normal matrix microtissue inoculated in each compartment, reshaping the extracellular tumor matrix to develop a breast cancer model. Another example is a 3D-based tubular chip model used to study the process of breast cancer cells transferring to bone. This model consists of gel channels containing bone differentiation, with human bone marrow-derived mesenchymal stem cells seeded in the matrix gel to form bone channels and ECs seeded in the central medium channel. Breast cancer cells are then introduced into this central channel. Since many anti-breast cancer drugs are metabolized through the liver, investigators developed a microarray chip to co-culture liver microtissues and breast tumor cells. Hepatocytes and ECs were seeded in the wells of the liver chip. The breast cancer cell chip was designed with a cross-shaped protruding structure, allowing breast cancer cells to directly contact cells in the liver chip. This chip provides a platform for *in vitro* drug screening, enabling researchers to study the interactions between liver metabolism and breast cancer drug efficacy.[106]

OoC has applications across diverse realms of oncological research, elucidating phenomena such as cancer cell migration and invasion, extracellular signal transduction, biophysical factors within the TME, mechanisms of resistance to chemotherapy and immunotherapy, and tumor heterogeneity. In addition to unveiling potential biological signals and interactions, OoC platforms are instrumental in investigating the contributions of biomechanical factors to tumor progression and therapeutic resistance, such as mechanical forces during respiration, interstitial flow, oxygen gradients, and shear stress during cancer cell invasion.[114–116] PDAC develops rapidly during its asymptomatic stage and creates an immunosuppressive TME, making immunotherapy impractical. To understand the key cellular interplay contributing to PDAC immunosuppressive TME, a 3D tumor model of PDAC was constructed, composed primarily of pancreatic stellate cells, endothelial ducts, and PDAC organoids.[107] To recapitulate cancer growth patterns and treatment responses at the organ level in lung cancer patients, a study injected human non-small cell lung cancer cell lines into primary alveolar and small airway organ chips. This model successfully mimicked the unique behavior of non-small cell lung cancer within its microenvironment.[108] Similarly, a colonic intestinal chip has been developed as a physiological model to mimic the human colonic epithelial-endothelial interface. Colon epithelial cells from patients are cultured in the top channel of this chip, with microvascular ECs cultured in the bottom channel. This chip serves as a valuable tool for analyzing the role of the colonic mucous layer in cancer.[109]

Due to the complexity and heterogeneity of tumors, there is a significant inter-patient variability when it comes to drug responses, requiring accurate assessment of individual patient treatment outcomes and the formulation of tailored anticancer therapeutic strategies.[117] OoC technologies address this potential shortcoming by integrating primary cells or organoids sourced from both healthy donors and patients, enabling the reconstruction of genetic and histological characteristics of the original tumor. For example, a microphysiological system was developed that combined self-assembled perfusion microvessels with 3D tumor spheroids. Patient-derived lung adenocarcinoma cells (A549) and ECs (human umbilical vein ECs) were assembled into multicellular spheroids to mimic solid lung tumors. These composite cancer spheroids were then injected into a microphysiological system embedded within an ECM hydrogel scaffold containing ECs and lung fibroblasts to successfully develop a 3D organotypic model of vascularized human lung adenocarcinoma. This model serves as a valuable platform for drug screening, enabling the evaluation of anticancer drug delivery in blood vessels, assessment of tumor-killing effects, and examination of vascular toxicity.[118] In a study of RCC, researchers developed a 3D human RCC chip. This innovative model integrates primary clear cell RCC (ccRCC) cells with human ECs, forming a ccRCC-on-chip system. Over time, the model demonstrated significant tumor angiogenesis characteristics, providing a promising platform for personalized drug selection.[119] This facilitates the assessment of patient-specific drug responses within organotypic human pathological environments.

The high-throughput characteristics of microfluidic chip organ models provide opportunities for large-scale drug screening, enabling the rapid, cost-effective identification of suitable drug combination regimens and the development of more personalized treatment modalities.[118,120,121] Using 3D bioprinting technology, researchers have developed patient-specific glioblastoma chips to replicate the *in vivo* structure of glioblastoma. This method involves printing tumor cells from cancer patients, along with vascular ECs and ECM from porcine brain tissue, to form a concentric-ring structure of cancer-stroma cells. The resulting glioblastoma chip reproduced key characteristics of the original glioblastoma microenvironment. Subsequently, the chip was used to simulate differential clinical treatment responses among patients with variations in drug resistance. Drug combinations were tested on the chip to evaluate their effects on specific patients, thereby determining optimal treatment plans based on drug efficacy assessments.[110] To achieve rapid anti-cancer drug susceptibility testing, another group combined a microfluidic chip with tumor organoids to develop an integrated superhydrophobic microwell array chip (InSMAR-chip). The researchers improved the processing method for tumor samples, using mechanical processing to extract a large number of lung cancer organoids from tumor tissue. These organoids were then integrated into the InSMAR-chip, enabling the completion of drug response tests within 1 week. This innovation significantly enhances the efficiency and speed of predicting personalized anti-cancer drug efficacy for patients with cancer.[120] Furthermore, OoC platforms can be applied to evaluate drug absorption, distribution, metabolism, and excretion, as well as the toxicity of chemotherapy, immunotherapeutic agents, or radiation therapy.[122,123] Consequently, the application of the OoC technology in the field of oncology opens new frontiers in cancer research and treatment, offering robust support for the realization of personalized medicine.

EX VIVO PRECLINICAL MODELING

Pre-clinical models include 2D tumor cell cultures encompassing both primary patient-derived cells (PDCs) and immortalized cell

lines, mouse models that cover genetically engineered mouse models and PDXs, and organoid models. The pros and cons of these models have been comprehensively summarized in various reviews.[14,124]

In simple terms, tumor cell monolayers or single-cell suspensions extensively utilized in cancer research are cultured in appropriate growth media. However, they inadequately represent primary tumors, fail to exhibit the cellular heterogeneity within tumors, and lack a complex TME. Animal models play a crucial role in pre-clinical cancer research. Tumors in mice can be induced through genetic manipulation such as gene knockouts or by transplanting patient-derived tumors into immunodeficient mice for culture. These processes require significant time and resources, and most discoveries fail to translate in human clinical trials.[125]

To overcome these hurdles, organoid models can serve as valuable tools by offering potent and scalable capabilities that can be used in a high-throughput format in basic and translational research, thereby accelerating the processes of disease treatment and new drug development. As an example, Kim et al. conducted RNA sequencing (RNA-seq) on PDCs and PDOs, extracted from various regions of a single CRC tumor, revealing that although 2D cultured PDCs maintained a moderate subregional heterogeneity, the 3D cultured PDOs more precisely mirrored the original consensus molecular subtype of the tumor.[12]

Typically, there are two approaches to constructing preclinical organoid models. One method, applicable to gene-related diseases, involves gene editing or creating organoid models that carry specific gene mutations, which is discussed in the next section. The other method entails inducing disease models through external stimuli, such as exposing organoid models to drugs, toxins, or disease-related proteins, to mimic clinical disease states. Organoid models can be constructed in response to various stimuli, such as inducing fatty liver disease with high-fat diets[126,127]; colitis with dextran sodium sulfate[128]; and creating models for respiratory, intestinal, vascular, and brain infections with COVID-19.[129–131] These models play a crucial role in the study of disease progression and treatment.

Organoid models usually contain only a subset of cell types and struggle to fully replicate the actual physiological environment found *in vivo*. Vascularization, multi-organ collaboration, and co-culture with other cells, particularly immune cells, are areas of ongoing exploration and research in the development of organoid models. Currently, efforts to better simulate the microenvironment of physiological states include the continuous optimization of the vascularization of various organoid models, such as those of the heart, kidneys, liver, and brain. As research progresses, these models are expected to be further refined for basic mechanistic studies and drug screening.[132]

In summary, as preclinical models, organoids hold potential for clinical application. They enhance the efficiency and precision of drug screening and reduce reliance on animal testing, thereby better simulating physiological responses within the human body. Since *ex vivo* PDOs retain their exact *in vivo* characteristics for only a limited time in culture, the challenge relies on making these culture systems more rapid and robust to achieve the clinical translatability of potential findings. This can be achieved by combining modern bioprinting, OoC, microfluidics, and gene-editing approaches.

GENE EDITING IN 3D ORGANOIDS

Generally, organoid models originate from induced PSCs (iPSCs) or ASCs. The development of disease-specific organoids due to gene mutations can take place at either the iPSC or the ASC level. Gene editing at the ASC level is particularly prevalent in tumor organoids. The transformation of wild-type (WT) organoids into tumor organoids via gene editing enables research into oncogene discovery, tumor genomic evolution, cancer stem cells, and oncogenic pathogens. Direct construction of organoids from cancer patient samples aids in studying the TME, drug screening, growth factors, and tumor heterogeneity.[6] This model addresses the heterogeneity in cancer causes and treatment responses seen in cancer patients.

Expanding beyond traditional organoids that have been extensively studied, such as those for the intestines, stomach, liver, and kidneys.[133–135] Hendriks and colleagues derived human brain organoids directly from human fetal brain tissue. They introduced TP53 mutations into a minority of the cells in these fetal brain organoids using CRISPR-Cas9 technology. Within 3 months, cells harboring TP53 gene defects had completely replaced the healthy cells in the organoid. To further investigate the link between brain tumors and gene mutations, the team utilized CRISPR-Cas9 to knock out three genes commonly mutated or inactivated in glioblastoma—TP53, PTEN, and NF1—observing the response of the model to existing tumor drugs.[136]

Currently, the majority of ASCs that are differentiated *in vitro* into organoids are epithelial cells with stem-like properties. Normal epithelial cells can be subjected to CRISPR gene editing to introduce oncogenic mutations or knock out key tumor suppressors. This process enables the *in vitro* simulation of cancer development, detailed carcinogenic mechanisms, and tumor genomic evolution. Comprehensive reviews have effectively summarized pre-2020 gene editing in organoids,[6,14] such as the impact of mutations in APC, KRAS, SMAD4, TP53, BRAF, and others on tumor development in organoid models. Notably, as human neurons are challenging to regenerate and obtain, it is possible to introduce oncogenic mutations into iPSCs or human embryonic stem cells (hESCs) to differentiate them into neoplastic brain organoids.[137–139]

Accordingly, PDOs offer a platform for cancer gene discovery and drug screening through gene-editing technology.[140] Drost et al. demonstrated that a deficiency in MLH1 causes CRC in organoids derived from human intestinal stem cells. This work was the first to use gene-editing technology on organoids for the screening of cancer genes.[141] A novel tumor-suppressor gene, BAP1, was discovered in CRISPR-Cas9-engineered liver organoids.[142] By enhancing the mutagenic capacity of CRISPR-Cas9 across the whole genome, screenings were performed on intestinal organoids comparing healthy and $APC^{-/-}$ organoids, identifying associations between Wnt and TGFβ signaling pathways.[143] Hirt et al. established PDAC organoids, reporting that missense mutations in ARID1A increase PDAC sensitivity to dasatinib and VE-821, among 1,172 drugs.[70] CRISPR-mediated introduction of multiple gene combinations has also helped

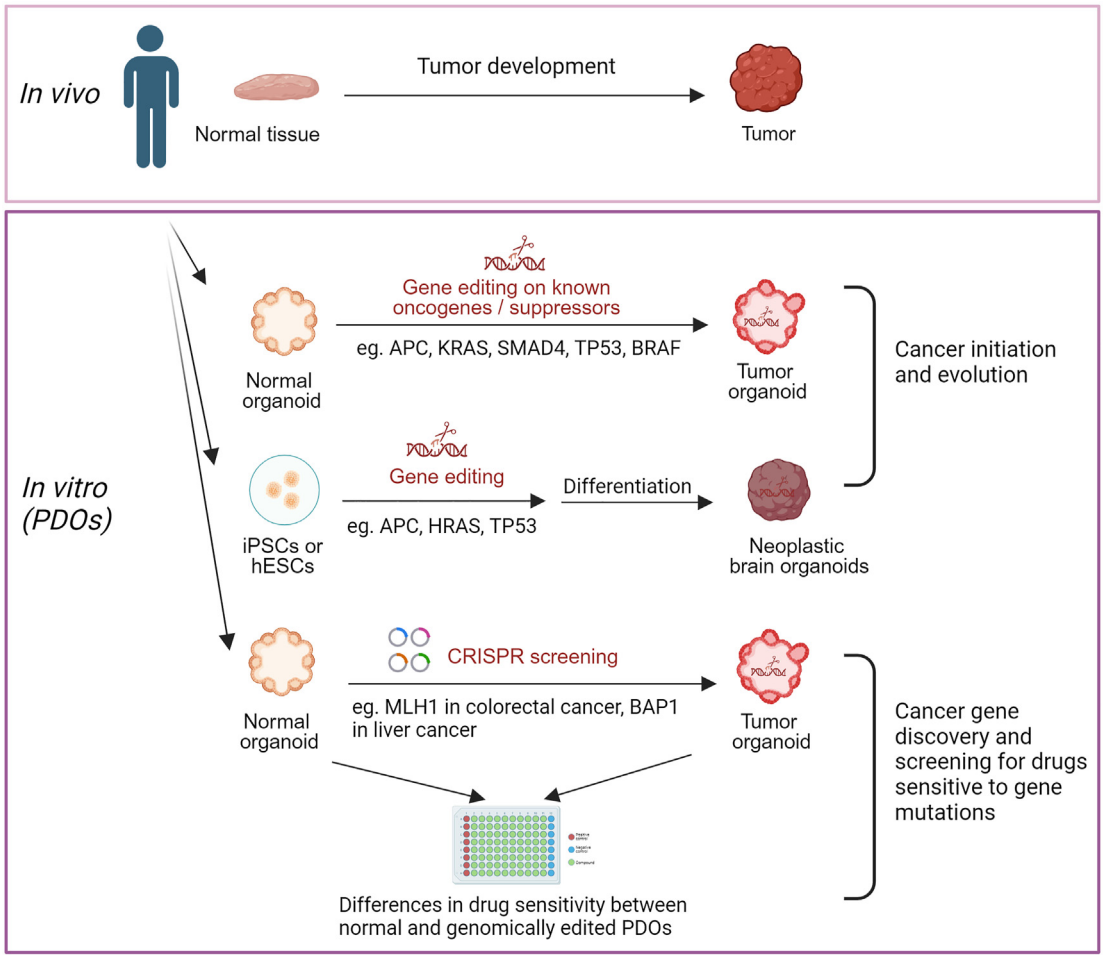

Figure 3. Gene editing in engineering PDOs for modeling tumor development and discovering cancer genes

Normal epithelial cells or organoids are amenable to CRISPR gene editing for the introduction of oncogenic mutations or the knockout of key tumor suppressors, such as APC, KRAS, SMAD4, TP53, and BRAF. Oncogenic mutations can be introduced into iPSCs or hESCs, differentiating them into neoplastic brain organoids. These methodologies enable the *in vitro* simulation of cancer development and the genomic evolution of tumors. CRISPR screening enables PDOs to provide a platform for the discovery of cancer genes and the screening of drugs through gene-editing technology. Created with BioRender.com.

address the plasticity of CRC stem cells after organoid transplantation in mice. By inserting inducible Cre into the LGR5 locus and introducing a multicolored Cre reporter gene, lineage tracing experiments on LGR5^{+} tumor cells have been performed.[144,145] Evidently, these preliminary studies demonstrate the impact of CRISPR screening in organoid research, suggesting that its application in organoid research will evolve rapidly. PDOs also provide platforms for developing the next generation of gene-editing therapies. For instance, using intestinal organoids from patients with DGAT1 deficiency, prime editing has shown greater precision than Cas9-mediated homology-directed repair. Prime editing, a versatile and precise gene-editing strategy, has been validated in intestinal organoids[146] (Figure 3).

MOLECULAR AND BIOCHEMICAL PROFILING

Molecular and biochemical profiling using PDOs has emerged as a pivotal tool to understand the complexities of tumor biology. Organoids successfully replicate the genetic and pathological phenotypes of tumors, offering significant insights into tumor behavior and treatment response.[147]

A critical advantage of utilizing PDOs for profiling is their ability to overcome the challenges often encountered when directly analyzing tumor tissues that are isolated from biopsies or surgical resections. Such challenges include the detection of DNA, RNA, proteins, or metabolic features. Typically, tumor cells may represent only a minor fraction of some patient-derived samples, leading to a potential contamination of the readout by signals from non-tumor cells. For instance, the presence of specific gene mutations in tumor cells can be obscured by overwhelming signals from non-tumor cells. Additionally, the quality of tumor samples can be compromised due to factors like necrotic areas common in many tumors or the adverse effects of prior treatments such as radiation or chemotherapy, which can significantly impact the accuracy of profiling. PDOs offer a solution to these issues by enriching tumor cells in

patient-derived samples, particularly viable ones, during the *in vitro* culture process. This enrichment results in a more pronounced presence of tumor-specific DNA mutations, RNA expressions, and tumor-associated protein expressions, thereby enhancing the reliability and feasibility of biochemical profiling. For example, measuring the metabolic status of PDOs becomes more viable due to this enrichment.

Notably, sequencing data from PDOs have shown superiority over standard formalin-fixed paraffin-embedded tissue pathology data. For instance, a recent study has demonstrated that next-generation sequencing (NGS) of PDAC PDOs can detect patient-specific KRAS or TP53 mutations, which were previously undetected in clinical NGS of primary tumors.[148] This highlights the enhanced sensitivity and specificity of PDO-based profiling. Further research involving a neuroendocrine neoplasm organoid library comprising 25 organoid lines was conducted, where genotypic analysis, including whole-genome sequencing (WGS), methylome, RNA-seq, assay for transposase accessible chromatin (ATAC)-seq, and phenotypic analysis, were performed. Compared with normal tissue organoids, PDOs showed alterations in TP53 and RB1 and upregulation of transcription factors such as ASCL1, NKX2–5, NEUROD1, POU2F3, thus effectively correlating genetic alterations with biological phenotypes. In addition, the study's findings on growth factor dependency provided valuable insights. Organoids with APC or CTNNB1 mutations demonstrated growth independence from Wnt and R-spondin, suggesting the potential ineffectiveness of WNT pathway inhibition in treating neuroendocrine neoplasms. Similarly, organoids with mutations in KRAS, BRAF, and NF1, but not in the epidermal growth factor receptor (EGFR) pathway, showed EGF-independent growth, potentially explaining the limited efficacy of EGFR-targeted therapies in neuroendocrine neoplasm treatment.[149]

Complementary to genomic studies, proteomic analyses have also contributed significantly to our understanding of tumor biology. A combined approach of transcriptomic and proteomic analyses can further unravel the mechanisms and biological pathways responsible for variations in protein expression.[150] Previous studies in murine organoids have analyzed the proteomic profile of PDAC and breast cancer, establishing correlations with metabolic pathways.[151,152] Recent proteomic analysis comparing human rectal cancer PDOs with normal organoids revealed changes in protein levels, with approximately 1% (78 types) of proteins showing an increase and about 4% (227 types) of proteins showing a decrease in PDOs.[34]

These studies collectively underscore the immense potential of PDOs as tools for molecular and biochemical profiling, providing a more accurate and comprehensive understanding of tumor biology and paving the way for more effective therapeutic strategies.

DRUG SCREENING

In contrast to typical 2D culture, the presence of a 3D environment in organoid cultures exposes cells to supplementary mechanical stimuli, such as stretching pressures and ECM stiffness changes.[153,154] By mimicking the TME, researchers are able to evaluate not only the viability of tumors but also their migratory and invasive capacities after being exposed to different compounds. This allows for a more exact portrayal of the conditions that occur *in vivo.*[155] Therefore, organoids and other 3D-based cell assays provide a more precise representation of biological references in drug discovery and screening.[155,156]

The presence of diverse characteristics and variations in cancer, both within individual patients and between different types of cancer, is commonly seen as a significant obstacle in the development of targeted treatments that are beneficial for each patient.[157] This heterogeneity also accounts for the wide range of responses to treatment observed among patients, including both primary and acquired resistance. Hence, the effective progress of personalized cancer treatments will rely on our capacity to methodically characterize and simulate disease heterogeneity.[157] Unlike conventional 2D monolayers or simple 3D spheres, organoids consist of multiple cell types and more accurately replicate the microarchitecture and mechanical properties of the real organ.[156,158,159] PDOs preserve key characteristics of the original tumor, including histology, biomarker protein expression, and genomic attributes such as copy-number variations and mutational landscapes.[36] Previous studies on PDOs derived from various organs demonstrated a noteworthy degree of resemblance between the PDOs and the original tumors in terms of their physical characteristics and patterns of genetic expression.[15,151,160–164] The results gained from comparing the outcomes of *ex vivo* experiments employing organoids with the responses of clinical trials indicate that PDOs have the ability to accurately replicate the responses of patients. Hence, the utilization of PDOs in personalized medicine endeavors shows potential for their actual implementation.[165,166]

The potential of PDOs in personalized medicine is increasingly recognized, offering a promising avenue for their practical application in this field. These organoids are not only instrumental in clinical applications but they also play a crucial role in understanding drug-genotype correlations[37,167–170] (Figure 4). A recent study demonstrated that PDOs efficiently evaluate patient-specific drug responses as *ex vivo* models in advanced breast cancer, with patients receiving PDO-sensitive treatments experiencing favorable clinical responses.[20] Similarly, the study conducted by Chen and colleagues, where a breast cancer whole-tumor cell culture (WTC) *ex vivo* model was established to perform drug profiling of a broad range of breast cancer therapies. They also performed a validation study where they mimicked the treatment regimens of 15 different breast cancer patients to their derived WTC model and found that their *ex vivo* model predicted clinical responses to therapy.[171]

Mo et al. discovered that the *in vitro* efficacy of PDOs treated with FOLFOX (folinic acid, fluorouracil, and oxaliplatin) or FOLFIRI (folinic acid, fluorouracil and irinotecan) correlates with progression-free survival of patients with colorectal liver metastasis (CRLM), highlighting the potential of PDOs in predicting the chemotherapy response of CRLM patients in clinical settings.[13] Hu et al. highlight the use of PDOs as a model to study human papillomavirus-related pre-cancerous cervical lesions and cervical cancer. The application of these PDOs could aid patients with drug resistance in finding more effective chemotherapy treatments, thereby enhancing their therapeutic

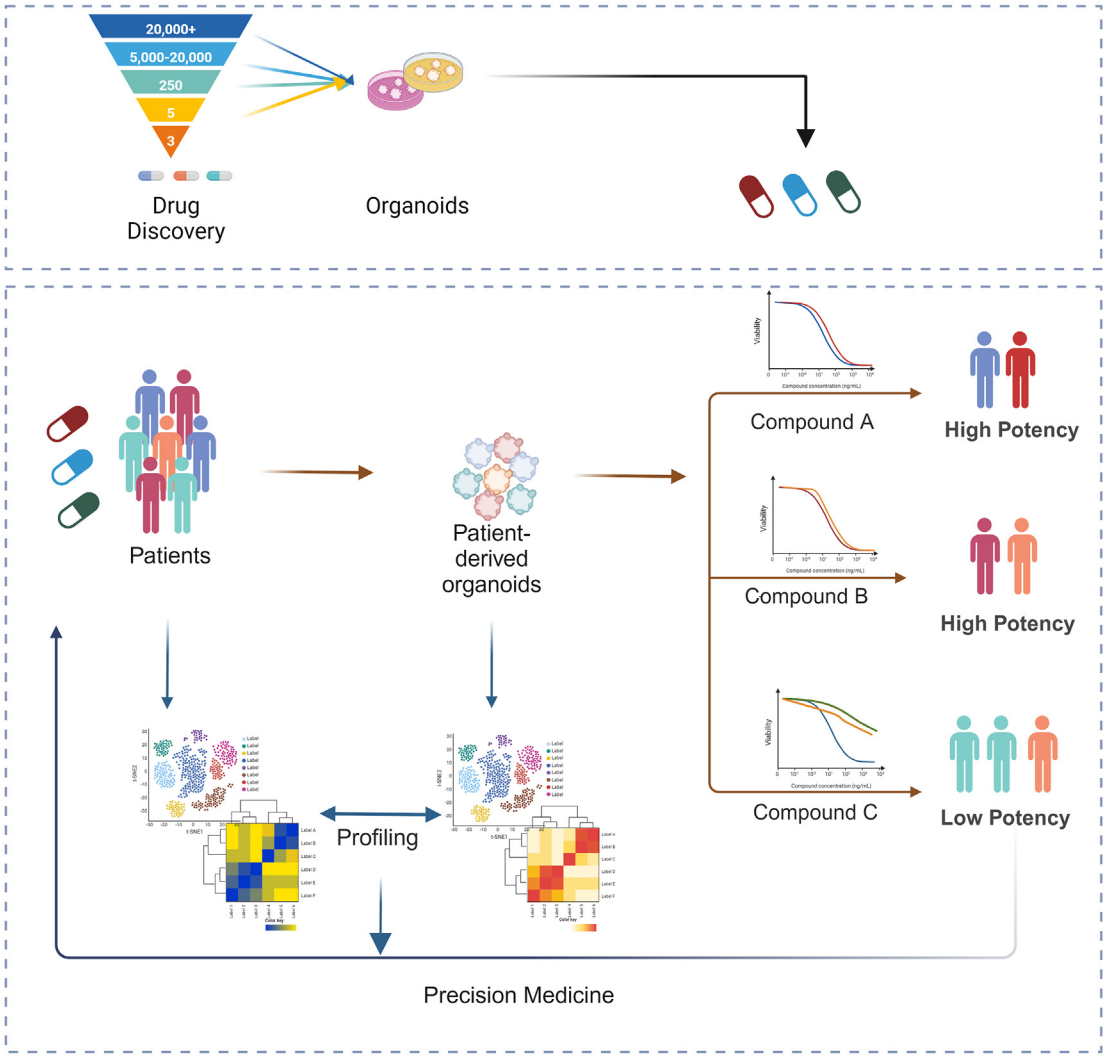

Figure 4. Applications of organoids and PDOs for drug discovery and precision medicine

Top: organoid models are utilized across various stages of the drug discovery process. Bottom: the focus shifts to PDOs, which are instrumental in developing tailored therapeutic strategies for individual patients. Through comprehensive -omics profiling and comparisons between patient samples and organoids, these approaches can facilitate the development of more accurate and personalized treatments. Created with BioRender.com.

outcomes.[172] Wang et al. used PDOs as an *in vitro* preclinical model for neoantigen prediction, and through multi-omics analysis, confirmed that PDOs preserve the neoantigen landscape of the original hepatobiliary tumors. By adding candidate peptides with human leukocyte antigen (HLA)-I-matched PBMCs, they generated reactive T cells, illustrating that PDOs are effective for the screening and quick validation of neoantigen peptides in upcoming precision immunotherapy.[46]

Ooft et al. found that the response of organoids derived from biopsies of metastatic CRC patients to the irinotecan-based therapies could be used to predict the responses of these patients treated with either irinotecan monotherapy or 5-fluorouracil (5-FU)-irinotecan combination therapy.[167] Millen et al. have established a biobank comprising patient-derived head and neck cancer organoids. These PDOs not only precisely mirror the treatment responses to adjuvant radiotherapy of the patients from whom they were derived but they can also be utilized for predicting new biomarkers in drug screening.[37]

The integration of CRISPR-Cas9 technology with organoid models has significantly advanced research in oncogenic transformation and tumorigenesis, shedding light on the mechanisms of drug responses and the influence of genetic alterations. Another notable development in this area is the establishment of "living" biobanks comprising PDO cultures.[173] These biobanks are pivotal in conducting extensive drug testing and identifying drug sensitivity profiles for distinct patient groups.[173–175] However, many challenges remain for the application of PDOs in drug screening due to limitations in these models and their culture conditions.[176] The current experimental protocols for PDOs remain relatively complex and challenging to establish routinely in standard biology laboratories. These challenges include ensuring representativeness and reproducibility, given the

significant variations among samples, and culture conditions. In addition, economic factors and sustainability are paramount considerations, as the costs for establishing and profiling PDOs are higher than those for conventional cell cultures. These aforementioned issues are particularly problematic for high-throughput drug screening, where the need for cost-effectiveness and scalability is crucial. Further adaptation and optimization of PDO protocols on a high-throughput scale are necessary to address these issues and enhance the reliability and efficiency of PDO-based drug screening.

Organoid models are also increasingly being recognized as valuable tools in later stages of both target-based drug discovery (TDD) and phenotype drug discovery (PDD).[156,177] However, these models frequently create obstacles when compared to simpler 2D cultures, such as a decreased throughput, increased difficulties in maintaining consistency among experiments, and prolonged time requirements. These challenges add complexity and increase costs in high-throughput screening applications.[153,156,158,159] Additionally, image-based screening methods face difficulties in capturing data from 3D cell-based assays due to the varying structures and sizes of spheres or organoids, which can hinder precise and comprehensive data collection. The utilization of organoids is more often applied on PDD, while their applicability in the initial stages of TDD is less common. However, recent studies provided a new possibility of using organoids at other stages of drug development.[178] Kourula et al. developed a bidirectional monolayer assay to enable human intestine-derived organoids to allow studies on drug disposition, metabolism, and intestinal toxicity.[80]

Although PDOs maintain a significantly higher degree of heterogeneity in comparison to other models, PDOs, which are formed from a sole region of a tumor, were also found to be inadequate in terms of their ability to accurately represent the results observed in a clinical setting in actual patients.[179] Therefore, collection of tissues from multiple regions (e.g., adjacent noncancerous tissue and tumor tissue) is required to generate a profile of the subpopulation that is more precise in terms of its heterogeneities.[161,162] The detailed genetic and epigenetic profile of patients and matched organoids will provide additional information and characteristics to evaluate treatment responses.

To date, the predominant methods for assessing the sensitivity of organoids to compounds have been cell viability assays, which rely on ATP measurements.[39,180] However, this approach has limitations, as it provides readouts from the global organoid population, failing to capture the responses of individual cell subgroups or provide temporal resolution of drug effects. To address these challenges, new image-based high-throughput screening methods and platforms have been developed. These techniques enable the evaluation of phenotypic changes in organoids and the real-time efficacy of compounds in organoid cultures.[181] Furthermore, the advancement of spatial transcriptomics is emerging as a promising tool, offering novel insights and enhanced capabilities in target deconvolution and the evaluation of drug actions.[168,182,183] This shift toward imaging-based methodologies and the integration of spatial transcriptomics and other omics approaches represents a significant advancement in the field of organoid-based drug screening.[182] For example, Legnini et al. developed a method combining optogenetics and gene perturbation technologies to control gene expression in organoids with precise spatial and temporal patterns. They successfully demonstrated this approach by locally activating Sonic Hedgehog (SHH) signaling in human neurodevelopment organoid models, revealing new insights into the role of SHH in neurodevelopment and highlighting the potential of this method for detailed studies in tissue patterning and cell fate determination.[182]

PDO-DERIVED BIOMARKERS

In the context of cancer therapy, a significant limitation is the lack of effective predictive biomarkers, highlighting the need to discover reliable biomarkers to predict response to therapy and potential side effects.[184,185] This advancement is critical for realizing the goals of personalized medicine and enhancing clinical outcomes.[186,187] Traditional biomarkers, sourced from patients, encompass a range of physiological indicators, including blood pressure, and can also be derived from biological samples like blood or tumor tissues.[188] These biomarkers often involve assessing the expression levels of specific factors in the blood or identifying gene mutations within tumor cells. The emphasis on personalized cancer treatment increasingly relies on genomic biomarkers.[189–191] In this regard, organoids, which can maintain the genomic profile of their parent tissue, have shown promising results.[27,37,76,192,193]

Genetic analysis of 1,977 cancer-related genes has revealed a strong correlation (0.89) between the DNA copy-number profiles of PDOs and their corresponding primary tumors.[27] Remarkably, PDOs have demonstrated the ability to stably maintain somatic mutations and transcriptomes even after extended culture periods of at least 6 months.[76] This stability renders them particularly useful in drug screening, linking genetic mutations with drug responses. For example, a study showed that a combination treatment of afatinib and simertinib effectively targeted organoids expressing WT KRAS within 72 h, while organoids expressing mutant KRAS exhibited a limited response in terms of cell-cycle arrest and cell death. Similarly, CRC PDOs, when implanted in immunodeficient mice, mirrored the drug responses observed *in vitro*, suggesting the potential of KRAS as a biomarker, albeit limited by the small sample size of the study.[194] In a separate larger study, gastric cancer PDOs with ARID1A mutations demonstrated increased sensitivity to the ATR inhibitor VE-822, a drug used in clinical trials.[76]

PDOs enable *in vitro* personalized treatment tests that are synchronized with the patient's condition. The research conducted by Wensink and colleagues, encompassing 17 studies across various cancer types such as colon, gastric, pancreatic, esophageal, melanoma, breast, ovarian, mesothelioma, glioblastoma, and head and neck cancers highlights the potential of PDOs in individualized tumor response tests.[17,19,35,38,48,63,74,195–205] This body of work underscores the considerable promise of PDOs as valuable tools to identify predictive biomarkers in cancer treatment. A standardized approach to analyzing these biomarkers focuses on three key aspects: analytical validity (accuracy, repeatability, and robustness of the test), clinical validity (correlation of the results with clinical outcomes), and clinical utility (the extent to which the use of predictive biomarkers improves treatment outcomes for patients in a cost-effective

manner).[195,206] In these studies, PDOs mirroring the cancer types of the patients underwent identical treatments *in vitro*, including both singular and combination drug trials, as well as radiotherapy or mixed treatment approaches. Five of the studies showed a significant correlation between PDO results from rectal, colorectal, gastric, and ovarian cancers and clinical outcomes. A trend toward correlation was observed in 12 studies involving various cancers like gastric, pancreatic, esophageal, melanoma, breast, ovarian, mesothelioma, glioblastoma, and head and neck cancers. Notably, two studies indicated that CRC PDOs treated with nivolumab plus ipilimumab or FOLFOX had no correlation with clinical outcomes.

A recent study confirmed that the response of head and neck squamous cell carcinoma (HNSCC) PDOs to radiotherapy correlates with the patients' responses. PDOs sensitive to radiotherapy indicated longer recurrence-free survival periods for the corresponding patients. The study also explored other biomarkers such as TP53 mutations, PIK3CA mutations, and CDKN2A loss. The TP53 mutation status in HNSCC PDOs was related to *in vitro* sensitivity to Nutlin-3a.[37] They also investigated PIK3CA in clinical trials, previously identified as a predictive biomarker for response to alpelisib in preclinical studies.[207,208] HNSCC PDOs with PIK3CA mutations did not show significantly higher sensitivity to alpelisib compared to PIK3CA WT PDOs. Introducing PIK3CA mutations (E545K) into normal HNSCC organoids via CRISPR resulted in increased sensitivity to alpelisib in these E545K mutant organoids compared to WT, but the difference was not significant.[37] CDKN2A is often co-deleted with methylthioadenosine phosphorylase, which has been proposed as a predictive biomarker for response to PRMT5 inhibitors.[209–211] Accordingly, HNSCC PDOs with CDKN2A loss exhibited increased sensitivity to PRMT5 inhibitors.[37] Another recent study utilized PDAC PDOs to predict the response of PDAC patients to FFX (fluorouracil, leucovorin, and irinotecan) treatment, which includes 5-FU, irinotecan, and oxaliplatin. The sensitivity of the PDOs to the three components of FFX correlated with a decrease in the tumor marker carbohydrate antigen 19-9 in the patients'' serum and a reduction in tumor volume.[71] Exome sequencing of microfluidic organoids by Choi et al. showed that some organoids have BRAF mutations, which upregulate the RAS-MEK-ERK pathway and promote cancer progression. Organoids with BRAF mutations demonstrated increased sensitivity to targeted therapies using RAF and MEK inhibitors compared to those with the WT BRAF, indicating that BRAF mutations could be used as biomarkers in patients receiving RAF and MEK inhibitors.[81] Through single-cell RNA-seq of hepatobiliary tumor organoids and testing 11 different tyrosine kinase inhibitors (TKIs), Zhao et al. discovered that the $CD44^{+}$ cancer stem cell population may be associated with PDO resistance to TKIs.[47]

Overall, biomarkers from PDOs have a potential role in predicting response to therapy, assessing safety at the *in vitro* level, fostering the development of personalized medicine, and advancing drug development.

PDO BIOBANKS

In recent years, the role of biobanks in cancer research has gained significant prominence, particularly with the advent of living biobanks and PDOs. These innovative biobanks provide researchers with more physiologically relevant cancer models, effectively bridging the gap between basic research and translational medicine.[14] In general, tumor biobanks mainly contain normal and tumor tissues and sometimes matched blood samples. However, such biobanks suffer from the limitation of being non-renewable and one-time use.[11] However, technological advancements have led to the development of organoid models based on 3D *in vitro* cell culture systems. Currently, several companies and institutes worldwide are collecting patient tissues to establish organoid biobanks,[212] such as the nonprofit technology group Hub (https://huborganoids.nl/)), utilized for research in regenerative medicine, disease pathogenesis, and drug screening (Figure 5).

In regenerative medicine, the mass culture of 3D organoids assists in addressing the donor source issue for organ transplantation. This advancement offers promising prospects for organ transplantation, particularly for patients requiring organ replacement following surgical removal due to conditions like cancer.[213] Somatic cells obtained from patients can be reprogrammed into iPSCs, which are then capable of being cultured *in vitro* to develop into tissue-specific organoids. Presently, organoids of various human tissues can be *in vitro* cultured from PSCs or adult human tissues. Successfully cultured organoids include mini-intestines,[79,214] mini-stomachs,[215,216] mini-brains,[217] mini-pancreases,[64] mini-prostates,[72] mini-lungs,[218] mini-kidneys,[219–222] and mini-livers.[223–225] Compared with traditional 2D single-layer cell cultures, 3D-cultured mini-organs exhibit a complex array of local cell types and intercellular network connections, closely mirroring the structure and function of actual organs. For example, *in vitro* cultured mini-livers demonstrate glycogen storage and low-density lipoprotein uptake capabilities[223]; mini-kidneys feature collecting duct structures and nephron configurations, suggesting potential in hemodialysis.[220] Additionally, mini-stomach tissues highly express the mucin marker MUC5AC and the epithelial tissue marker E-cadherin, while mini-intestinal tissues express the intestine-specific marker CDX2.[79,215] When human mini-stomach tissues were transplanted under the greater omentum of immunodeficient mice (NSG mice), these tissues continuously grew and developed into mature gastric epithelial tissues capable of secreting various hormones.[215] In 2021, the first successful transplantation of organoids onto human organs was reported.[223] This study involved transplanting bile duct organoids cultured *in vitro* into human livers under *ex vivo* conditions, thereby achieving the repair and regeneration of damaged bile ducts. This research initially cultured gallbladder-derived bile duct epithelial cells into organoids *in vitro* and transplanted them into a mouse model of liver bile duct disease, demonstrating the repair of damaged intrahepatic bile ducts. Researchers employed a normothermic perfusion system on human donor livers discarded for transplantation due to bile duct damage. This maintained their physiological functions for extended periods *ex vivo*. After transplanting gallbladder organoids into the intrahepatic bile duct, the grafts were retained within the bile duct chambers, forming connections with the recipient and achieving bile duct regeneration and improved bile properties. This finding confirms that organoids cultured in the laboratory

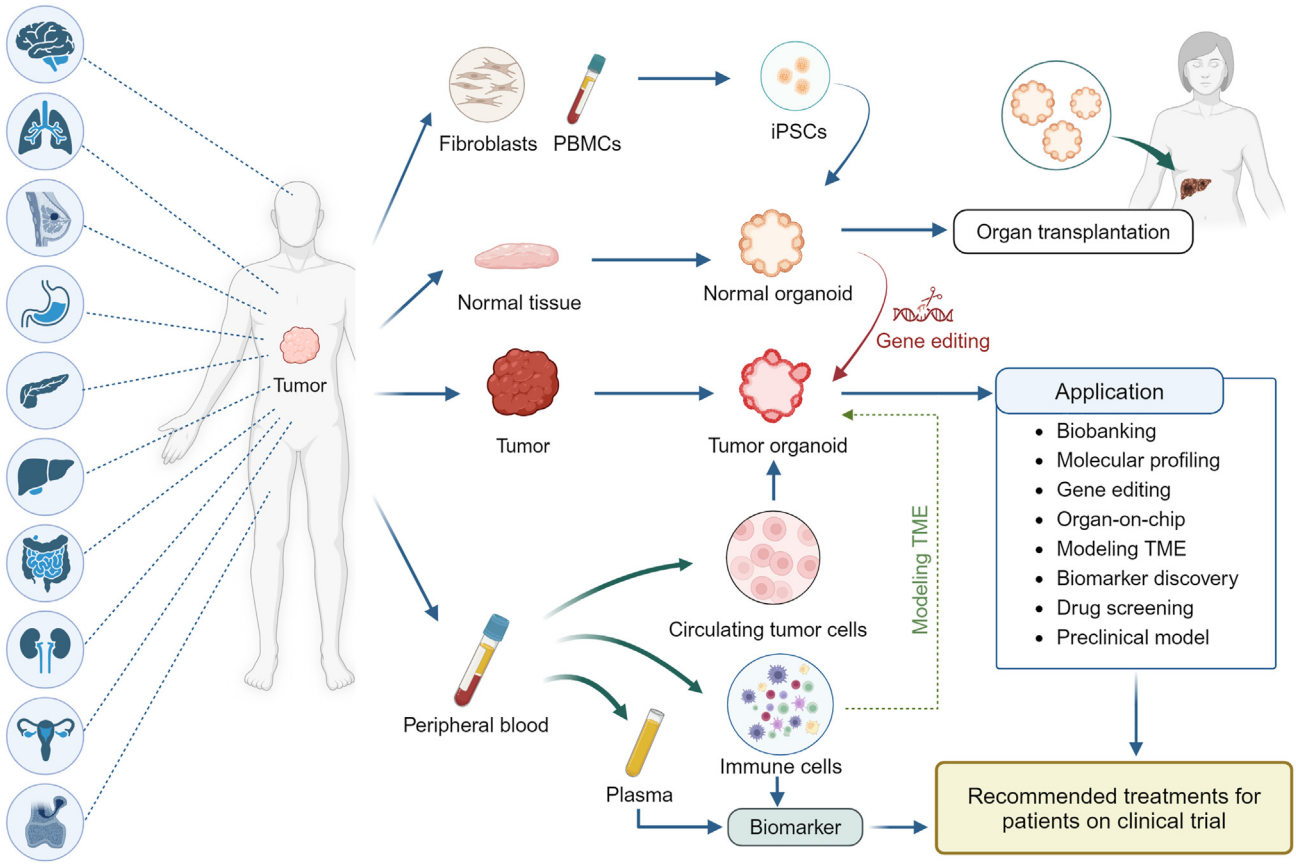

Figure 5. Establishment and application of PDO biobanks

The currently established biobanks of PDOs, sourced from various cancer types. These biobanks collect tumor tissue, paired normal tissue, and blood, with some also having patient-derived iPSCs that are reprogrammed from fibroblasts or PBMCs. Normal organoids derived from iPSCs or normal tissue offer potential in organ transplantation and can be transformed into tumor organoids through gene editing. Tumor organoids, derived either directly from tumor tissue or circulating tumor cells, offer numerous applications and assist in formulating treatment recommendations. Additionally, peripheral blood serves to isolate immune cells for culture with tumor organoids, aiding in modeling the TME. Plasma-derived biomarkers also play a crucial role in translational cancer research. Created with BioRender.com.

can be transplanted onto human organs and function effectively, laying the groundwork for the clinical application of organoid transplantation. However, the *ex vivo* cultivation of mini-organs is still in the exploratory stage and faces numerous challenges. For instance, certain organoids still lack key cell types of the respective organs, some cells are in early developmental stages and not yet mature, and there is an absence of necessary supporting structures like blood vessels and neural tissue required for organ transplantation. These issues present challenges to the application of organoid transplantation technology in therapeutic treatments.

PDOs can be established from various tumor cell sources, including surgical specimens and fine-needle biopsies, and have shown potential in reflecting the characteristics of primary tumors, particularly in terms of DNA sequences and methylation patterns.[4,14,226] The generation of PDO biobanks has been documented in a range of tumors, including those originating from the brain,[17,18] head and neck,[37,38,227] nasopharyngeal,[54] breast,[19] lung,[48–50] biliary tract,[228] liver,[44] kidneys,[41,42] esophagus,[82] stomach,[76,77] colon,[26–29,79,82] rectum,[74,79] pancreas,[63–66] prostate,[72,73] bladder,[16,229] ovaries,[56,57] neuroendocrine tumors,[55] upper tract urothelial carcinoma,[78] and CRC liver metastasis.[13] This demonstrates significant progress in the field of tumor biology and provides a valuable resource for research and personalized medicine. The recovery rates, survival efficiency, and growth after revival of these organoids *in vitro* have been reviewed previously.[230] Therefore, PDO biobanks significantly expand the types of patient samples that can be propagated and studied in the laboratory. These PDO biobanks are capable of reproducing the phenotype and genetic characteristics of target organs, offering new platforms for studying cancer development and progression, drug screening, and preclinical models, which were discussed in the previous sections.

In summary, organoid biobanks show immense potential not only in regenerative medicine but also in disease modeling research, such as cancer research. Future studies need to address the challenges and further explore the possible clinical applications of organoids, thereby advancing the field and leading to innovative therapeutic approaches.[231]

CONCLUSIONS

Cancer, as a leading global cause of mortality, is profoundly interesting within the scientific community, leading to an increased focus in cancer research, namely in the development of new cancer treatments.[232] Nevertheless, the molecular mechanisms of tumor development remain incompletely elucidated, and the intricacies of the *in vivo* microenvironment pose significant challenges in unraveling the essence of cancer and identifying effective therapeutic approaches.[233,234]

Recent studies have shown that PDOs have the potential to be used as tools for biomarker discovery, preclinical research, and drug development. Recent developments in organoid research have led to an improvement in the rates of establishing successful PDO cultures, which, coupled with advances in bioprinting techniques that lead to reduced handling times, make PDOs an attractive high-throughput and physiologically relevant tool to be used in a clinical setting. In the clinic, PDOs show the potential to be used as a proxy for cancer patient drug profiling and prediction of response to therapy *ex vivo*. By layering drug profiling data with different multi-omics analysis, PDOs can be useful tools to help make informed decisions on individualized cancer treatment regimens to tailor precision medicine.

The future trajectory will likely involve establishing PDOs efficiently and swiftly, ensuring a high success rate (currently ranging from 31% to 90%), and minimizing costs. These steps are pivotal in utilizing PDOs to optimally select patients for the most effective standard care treatment plans, marking significant strides toward personalized oncological care.[195]

Despite the existing challenges, the trend toward using organoids, which offer more physiologically relevant models, is anticipated to persist.[70,235–237] The choice of model in research will depend on the specific research question, available resources, and the desired level of physiological relevance. Advancements in new 3D bioprinting and culturing materials, gene-editing techniques, OoC technologies, iPSCs, and new computational and single-cell sequencing tools provide innovative strategies to overcome the obstacles associated with the application of organoids in drug screening and discovery.[238–240] Developments in PDO research take us a step toward fast and reliable precision medicine applications in cancer treatment.

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AUTHOR CONTRIBUTIONS

L.T. conceived the idea for the article. L.T. wrote the first version of the manuscript, with constructive input from W.C., B.Z., L.S., Q.Z., and P.Z., under supervision from A.L. Y.Y., L.T., and W.C. prepared the display items. P.F., B.X., Q.Z., Z.L., B.S.-L., and A.L. provided proofreading and input on later versions of the manuscript. All authors approved the final version of the article.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Rossi, G., Manfrin, A., and Lutolf, M.P. (2018). Progress and potential in organoid research. Nat. Rev. Genet. *19*, 671–687.
2. Lancaster, M.A., and Knoblich, J.A. (2014). Organogenesis in a dish: modeling development and disease using organoid technologies. Science *345*, 1247125.
3. Porter, R.J., Murray, G.I., and McLean, M.H. (2020). Current concepts in tumour-derived organoids. Br. J. Cancer *123*, 1209–1218.
4. Kretzschmar, K. (2021). Cancer research using organoid technology. J. Mol. Med. *99*, 501–515. https://doi.org/10.1007/s00109-020-01990-z.
5. Lancaster, M.A., and Huch, M. (2019). Disease modelling in human organoids. Dis. Model. Mech. *12*, dmm039347.
6. Lo, Y.H., Karlsson, K., and Kuo, C.J. (2020). Applications of Organoids for Cancer Biology and Precision Medicine. Nat. Cancer *1*, 761–773. https://doi.org/10.1038/s43018-020-0102-y.
7. Yuki, K., Cheng, N., Nakano, M., and Kuo, C.J. (2020). Organoid models of tumor immunology. Trends Immunol. *41*, 652–664.
8. Luckett, K.A., and Ganesh, K. (2023). Engineering the Immune Microenvironment into Organoid Models. Annu. Rev. Cancer Biol. *7*, 171–187. https://doi.org/10.1146/annurev-cancerbio-061421-040659.
9. Clevers, H., and Tuveson, D.A. (2019). Organoid Models for Cancer Research. Annu. Rev. Cancer Biol. *3*, 223–234. https://doi.org/10.1146/annurev-cancerbio-030518-055702.
10. Han, J.J. (2023). FDA Modernization Act 2.0 allows for alternatives to animal testing. Artif. Organs *47*, 449–450. https://doi.org/10.1111/aor.14503.
11. Li, H., Liu, H., and Chen, K. (2022). Living biobank-based cancer organoids: Prospects and challenges in cancer research. Cancer Biol. Med. *19*, 965–982.
12. Kim, S.C., Park, J.W., Seo, H.Y., Kim, M., Park, J.H., Kim, G.H., Lee, J.O., Shin, Y.K., Bae, J.M., Koo, B.K., et al. (2022). Multifocal Organoid Capturing of Colon Cancer Reveals Pervasive Intratumoral Heterogenous Drug Responses. Adv. Sci. *9*, e2103360. https://doi.org/10.1002/advs.202103360.
13. Mo, S., Tang, P., Luo, W., Zhang, L., Li, Y., Hu, X., Ma, X., Chen, Y., Bao, Y., He, X., et al. (2022). Patient-Derived Organoids from Colorectal Cancer with Paired Liver Metastasis Reveal Tumor Heterogeneity and Predict Response to Chemotherapy. Adv. Sci. *9*, e2204097. https://doi.org/10.1002/advs.202204097.
14. Tuveson, D., and Clevers, H. (2019). Cancer modeling meets human organoid technology. Science *364*, 952–955. https://doi.org/10.1126/science.aaw6985.
15. Lee, S.H., Hu, W., Matulay, J.T., Silva, M.V., Owczarek, T.B., Kim, K., Chua, C.W., Barlow, L.J., Kandoth, C., Williams, A.B., et al. (2018). Tumor Evolution and Drug Response in Patient-Derived Organoid Models of Bladder Cancer. Cell *173*, 515–528.e17. https://doi.org/10.1016/j.cell.2018.03.017.
16. Mullenders, J., de Jongh, E., Brousali, A., Roosen, M., Blom, J.P.A., Begthel, H., Korving, J., Jonges, T., Kranenburg, O., Meijer, R., and Clevers, H.C. (2019). Mouse and human urothelial cancer organoids: A tool for bladder cancer research. Proc. Natl. Acad. Sci. USA *116*, 4567–4574.
17. Jacob, F., Salinas, R.D., Zhang, D.Y., Nguyen, P.T.T., Schnoll, J.G., Wong, S.Z.H., Thokala, R., Sheikh, S., Saxena, D., Prokop, S., et al. (2020). A patient-derived glioblastoma organoid model and biobank recapitulates inter-and intra-tumoral heterogeneity. Cell *180*, 188–204.e22.
18. Hubert, C.G., Rivera, M., Spangler, L.C., Wu, Q., Mack, S.C., Prager, B.C., Couce, M., McLendon, R.E., Sloan, A.E., and Rich, J.N. (2016). A three-dimensional organoid culture system derived from human glioblastomas recapitulates the hypoxic gradients and cancer stem cell heterogeneity of tumors found in vivo. Cancer Res. *76*, 2465–2477.

19. Sachs, N., de Ligt, J., Kopper, O., Gogola, E., Bounova, G., Weeber, F., Balgobind, A.V., Wind, K., Gracanin, A., Begthel, H., et al. (2018). A living biobank of breast cancer organoids captures disease heterogeneity. Cell *172*, 373–386.e10.

20. Chen, P., Zhang, X., Ding, R., Yang, L., Lyu, X., Zeng, J., Lei, J.H., Wang, L., Bi, J., Shao, N., et al. (2021). Patient-Derived Organoids Can Guide Personalized-Therapies for Patients with Advanced Breast Cancer. Adv. Sci. *8*, e2101176. https://doi.org/10.1002/advs.202101176.

21. Chan, I.S., and Ewald, A.J. (2022). Organoid Co-culture Methods to Capture Cancer Cell-Natural Killer Cell Interactions. Methods Mol. Biol. *2463*, 235–250. https://doi.org/10.1007/978-1-0716-2160-8_17.

22. Dijkstra, K.K., Cattaneo, C.M., Weeber, F., Chalabi, M., van de Haar, J., Fanchi, L.F., Slagter, M., van der Velden, D.L., Kaing, S., Kelderman, S., et al. (2018). Generation of Tumor-Reactive T Cells by Co-culture of Peripheral Blood Lymphocytes and Tumor Organoids. Cell *174*, 1586–1598.e12. https://doi.org/10.1016/j.cell.2018.07.009.

23. Subtil, B., Iyer, K.K., Poel, D., Bakkerus, L., Gorris, M.A.J., Escalona, J.C., van den Dries, K., Cambi, A., Verheul, H.M.W., de Vries, I.J.M., and Tauriello, D.V.F. (2023). Dendritic cell phenotype and function in a 3D co-culture model of patient-derived metastatic colorectal cancer organoids. Front. Immunol. *14*, 1105244. https://doi.org/10.3389/fimmu.2023.1105244.

24. Kong, J.C.H., Guerra, G.R., Millen, R.M., Roth, S., Xu, H., Neeson, P.J., Darcy, P.K., Kershaw, M.H., Sampurno, S., Malaterre, J., et al. (2018). Tumor-Infiltrating Lymphocyte Function Predicts Response to Neoadjuvant Chemoradiotherapy in Locally Advanced Rectal Cancer. JCO Precis. Oncol. *2*, 1–15. https://doi.org/10.1200/po.18.00075.

25. Harter, M.F., Recaldin, T., Gerard, R., Avignon, B., Bollen, Y., Esposito, C., Guja-Jarosz, K., Kromer, K., Filip, A., Aubert, J., et al. (2024). Analysis of off-tumour toxicities of T-cell-engaging bispecific antibodies via donor-matched intestinal organoids and tumouroids. Nat. Biomed. Eng. *8*, 345–360. https://doi.org/10.1038/s41551-023-01156-5.

26. Fujii, M., Shimokawa, M., Date, S., Takano, A., Matano, M., Nanki, K., Ohta, Y., Toshimitsu, K., Nakazato, Y., Kawasaki, K., et al. (2016). A colorectal tumor organoid library demonstrates progressive loss of niche factor requirements during tumorigenesis. Cell Stem Cell *18*, 827–838.

27. Weeber, F., van de Wetering, M., Hoogstraat, M., Dijkstra, K.K., Krijgsman, O., Kuilman, T., Gadellaa-van Hooijdonk, C.G.M., van der Velden, D.L., Peeper, D.S., Cuppen, E.P.J.G., et al. (2015). Preserved genetic diversity in organoids cultured from biopsies of human colorectal cancer metastases. Proc. Natl. Acad. Sci. USA *112*, 13308–13311. https://doi.org/10.1073/pnas.1516689112.

28. Farin, H.F., Mosa, M.H., Ndreshkjana, B., Grebbin, B.M., Ritter, B., Menche, C., Kennel, K.B., Ziegler, P.K., Szabó, L., Bollrath, J., et al. (2023). Colorectal cancer organoid–stroma biobank allows subtype-specific assessment of individualized therapy responses. Cancer Discov. *13*, 2192–2211.

29. Luo, Z., Wang, B., Luo, F., Guo, Y., Jiang, N., Wei, J., Wang, X., Tseng, Y., Chen, J., Zhao, B., and Liu, J. (2023). Establishment of a large-scale patient-derived high-risk colorectal adenoma organoid biobank for high-throughput and high-content drug screening. BMC Med. *21*, 336.

30. Atanasova, V.S., de Jesus Cardona, C., Hejret, V., Tiefenbacher, A., Mair, T., Tran, L., Pfneissl, J., Draganić, K., Binder, C., Kabiljo, J., et al. (2023). Mimicking Tumor Cell Heterogeneity of Colorectal Cancer in a Patient-derived Organoid-Fibroblast Model. Cell. Mol. Gastroenterol. Hepatol. *15*, 1391–1419. https://doi.org/10.1016/j.jcmgh.2023.02.014.

31. Naruse, M., Ochiai, M., Sekine, S., Taniguchi, H., Yoshida, T., Ichikawa, H., Sakamoto, H., Kubo, T., Matsumoto, K., Ochiai, A., and Imai, T. (2021). Re-expression of REG family and DUOXs genes in CRC organoids by co-culturing with CAFs. Sci. Rep. *11*, 2077. https://doi.org/10.1038/s41598-021-81475-2.

32. Pinho, D., Santos, D., Vila, A., and Carvalho, S. (2021). Establishment of colorectal cancer organoids in microfluidic-based system. Micromachines *12*, 497.

33. Chen, J., Sun, H.-W., Yang, Y.-Y., Chen, H.-T., Yu, X.-J., Wu, W.-C., Xu, Y.-T., Jin, L.-L., Wu, X.-J., Xu, J., and Zheng, L. (2021). Reprogramming immunosuppressive myeloid cells by activated T cells promotes the response to anti-PD-1 therapy in colorectal cancer. Signal Transduct. Target. Ther. *6*, 4. https://doi.org/10.1038/s41392-020-00377-3.

34. Cristobal, A., van den Toorn, H.W.P., van de Wetering, M., Clevers, H., Heck, A.J.R., and Mohammed, S. (2017). Personalized Proteome Profiles of Healthy and Tumor Human Colon Organoids Reveal Both Individual Diversity and Basic Features of Colorectal Cancer. Cell Rep. *18*, 263–274. https://doi.org/10.1016/j.celrep.2016.12.016.

35. Ooft, S.N., Weeber, F., Dijkstra, K.K., McLean, C.M., Kaing, S., van Werkhoven, E., Schipper, L., Hoes, L., Vis, D.J., van de Haar, J., et al. (2019). Patient-derived organoids can predict response to chemotherapy in metastatic colorectal cancer patients. Sci. Transl. Med. *11*, eaay2574.

36. Verissimo, C.S., Overmeer, R.M., Ponsioen, B., Drost, J., Mertens, S., Verlaan-Klink, I., Gerwen, B.V., van der Ven, M., Wetering, M.v.d., Egan, D.A., et al. (2016). Targeting mutant RAS in patient-derived colorectal cancer organoids by combinatorial drug screening. Elife *5*, e18489. https://doi.org/10.7554/eLife.18489.

37. Millen, R., De Kort, W.W.B., Koomen, M., van Son, G.J.F., Gobits, R., Penning de Vries, B., Begthel, H., Zandvliet, M., Doornaert, P., Raaijmakers, C.P.J., et al. (2023). Patient-derived head and neck cancer organoids allow treatment stratification and serve as a tool for biomarker validation and identification. Med *4*, 290–310.e12. https://doi.org/10.1016/j.medj.2023.04.003.

38. Driehuis, E., Kolders, S., Spelier, S., Lõhmussaar, K., Willems, S.M., Devriese, L.A., de Bree, R., de Ruiter, E.J., Korving, J., Begthel, H., et al. (2019). Oral mucosal organoids as a potential platform for personalized cancer therapy. Cancer Discov. *9*, 852–871.

39. Driehuis, E., Kretzschmar, K., and Clevers, H. (2020). Establishment of patient-derived cancer organoids for drug-screening applications. Nat. Protoc. *15*, 3380–3409. https://doi.org/10.1038/s41596-020-0379-4.

40. Zhao, H., Jiang, E., and Shang, Z. (2021). 3D Co-culture of Cancer-Associated Fibroblast with Oral Cancer Organoids. J. Dent. Res. *100*, 201–208. https://doi.org/10.1177/0022034520956614.

41. Calandrini, C., Schutgens, F., Oka, R., Margaritis, T., Candelli, T., Mathijsen, L., Ammerlaan, C., van Ineveld, R.L., Derakhshan, S., de Haan, S., et al. (2020). An organoid biobank for childhood kidney cancers that captures disease and tissue heterogeneity. Nat. Commun. *11*, 1310.

42. Grassi, L., Alfonsi, R., Francescangeli, F., Signore, M., De Angelis, M.L., Addario, A., Costantini, M., Flex, E., Ciolfi, A., Pizzi, S., et al. (2019). Organoids as a new model for improving regenerative medicine and cancer personalized therapy in renal diseases. Cell Death Dis. *10*, 201.

43. Esser, L.K., Branchi, V., Leonardelli, S., Pelusi, N., Simon, A.G., Klümper, N., Ellinger, J., Hauser, S., Gonzalez-Carmona, M.A., Ritter, M., et al. (2020). Cultivation of clear cell renal cell carcinoma patient-derived organoids in an air-liquid interface system as a tool for studying individualized therapy. Front. Oncol. *10*, 1775.

44. Broutier, L., Mastrogiovanni, G., Verstegen, M.M., Francies, H.E., Gavarró, L.M., Bradshaw, C.R., Allen, G.E., Arnes-Benito, R., Sidorova, O., Gaspersz, M.P., et al. (2017). Human primary liver cancer–derived organoid cultures for disease modeling and drug screening. Nat. Med. *23*, 1424–1435.

45. Lim, J.T.C., Kwang, L.G., Ho, N.C.W., Toh, C.C.M., Too, N.S.H., Hooi, L., Benoukraf, T., Chow, P.K.H., Dan, Y.Y., Chow, E.K.H., et al. (2022). Hepatocellular carcinoma organoid co-cultures mimic angiocrine crosstalk to generate inflammatory tumor microenvironment. Biomaterials *284*, 121527. https://doi.org/10.1016/j.biomaterials.2022.121527.

46. Wang, W., Yuan, T., Ma, L., Zhu, Y., Bao, J., Zhao, X., Zhao, Y., Zong, Y., Zhang, Y., Yang, S., et al. (2022). Hepatobiliary Tumor Organoids Reveal HLA Class I Neoantigen Landscape and Antitumoral Activity of Neoantigen Peptide Enhanced with Immune Checkpoint Inhibitors. Adv. Sci. *9*, e2105810. https://doi.org/10.1002/advs.202105810.

47. Zhao, Y., Li, Z.X., Zhu, Y.J., Fu, J., Zhao, X.F., Zhang, Y.N., Wang, S., Wu, J.M., Wang, K.T., Wu, R., et al. (2021). Single-Cell Transcriptome Analysis Uncovers Intratumoral Heterogeneity and Underlying Mechanisms for Drug Resistance in Hepatobiliary Tumor Organoids. Adv. Sci. *8*, e2003897. https://doi.org/10.1002/advs.202003897.

48. Chalabi, M., Fanchi, L.F., Dijkstra, K.K., Van den Berg, J.G., Aalbers, A.G., Sikorska, K., Lopez-Yurda, M., Grootscholten, C., Beets, G.L., Snaebjornsson, P., et al. (2020). Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. Nat. Med. *26*, 566–576.

49. Sachs, N., Papaspyropoulos, A., Zomer-van Ommen, D.D., Heo, I., Böttinger, L., Klay, D., Weeber, F., Huelsz-Prince, G., Iakobachvili, N., Amatngalim, G.D., et al. (2019). Long-term expanding human airway organoids for disease modeling. The EMBO journal *38*, e100300.

50. Ebisudani, T., Hamamoto, J., Togasaki, K., Mitsuishi, A., Sugihara, K., Shinozaki, T., Fukushima, T., Kawasaki, K., Seino, T., Oda, M., et al. (2023). Genotype-phenotype mapping of a patient-derived lung cancer organoid biobank identifies NKX2-1-defined Wnt dependency in lung adenocarcinoma. Cell Rep. *42*, 112212.

51. Shin, T.H., Kim, M., Sung, C.O., Jang, S.J., and Jeong, G.S. (2019). A one-stop microfluidic-based lung cancer organoid culture platform for testing drug sensitivity. Lab Chip *19*, 2854–2865.

52. Jenkins, R.W., Aref, A.R., Lizotte, P.H., Ivanova, E., Stinson, S., Zhou, C.W., Bowden, M., Deng, J., Liu, H., Miao, D., et al. (2018). Ex vivo profiling of PD-1 blockade using organotypic tumor spheroids. Cancer Discov. *8*, 196–215.

53. Votanopoulos, K.I., Forsythe, S., Sivakumar, H., Mazzocchi, A., Aleman, J., Miller, L., Levine, E., Triozzi, P., and Skardal, A. (2020). Model of Patient-Specific Immune-Enhanced Organoids for Immunotherapy Screening: Feasibility Study. Ann. Surg Oncol. *27*, 1956–1967. https://doi.org/10.1245/s10434-019-08143-8.

54. Wang, X.-W., Xia, T.-L., Tang, H.-C., Liu, X., Han, R., Zou, X., Zhao, Y.-T., Chen, M.-Y., and Li, G. (2022). Establishment of a patient-derived organoid model and living biobank for nasopharyngeal carcinoma. Ann. Transl. Med. *10*, 526.

55. Kawasaki, K., Toshimitsu, K., Matano, M., Fujita, M., Fujii, M., Togasaki, K., Ebisudani, T., Shimokawa, M., Takano, A., Takahashi, S., et al. (2020). An organoid biobank of neuroendocrine neoplasms enables genotype-phenotype mapping. Cell *183*, 1420–1435.e21.

56. Kopper, O., De Witte, C.J., Lõhmussaar, K., Valle-Inclan, J.E., Hami, N., Kester, L., Balgobind, A.V., Korving, J., Proost, N., Begthel, H., et al. (2019). An organoid platform for ovarian cancer captures intra-and interpatient heterogeneity. Nat. Med. *25*, 838–849.

57. Hill, S.J., Decker, B., Roberts, E.A., Horowitz, N.S., Muto, M.G., Worley, M.J., Jr., Feltmate, C.M., Nucci, M.R., Swisher, E.M., Nguyen, H., et al. (2018). Prediction of DNA repair inhibitor response in short-term patient-derived ovarian cancer organoids. Cancer Discov. *8*, 1404–1421.

58. Knoblauch, M., Ma, T., Beirith, I., Koch, D., Hofmann, F., Heinrich, K., Aghamaliev, U., Sirtl, S., Westphalen, C.B., Nieß, H., et al. (2023). In-vitro model to mimic T cell subset change in human PDAC organoid co-culture. J. Cancer Res. Clin. Oncol. *149*, 13051–13064. https://doi.org/10.1007/s00432-023-05100-7.

59. Holokai, L., Chakrabarti, J., Lundy, J., Croagh, D., Adhikary, P., Richards, S.S., Woodson, C., Steele, N., Kuester, R., Scott, A., et al. (2020). Murine- and Human-Derived Autologous Organoid/Immune Cell Co-Cultures as Pre-Clinical Models of Pancreatic Ductal Adenocarcinoma. Cancers *12*, 3816. https://doi.org/10.3390/cancers12123816.

60. Chakrabarti, J., Koh, V., So, J.B.Y., Yong, W.P., and Zavros, Y. (2021). A Preclinical Human-Derived Autologous Gastric Cancer Organoid/Immune Cell Co-Culture Model to Predict the Efficacy of Targeted Therapies. J. Vis. Exp. *173*. https://doi.org/10.3791/61443.

61. Jiang, S., Deng, T., Cheng, H., Liu, W., Shi, D., Yuan, J., He, Z., Wang, W., Chen, B., Ma, L., et al. (2023). Macrophage-organoid co-culture model for identifying treatment strategies against macrophage-related gemcitabine resistance. J. Exp. Clin. Cancer Res. *42*, 199. https://doi.org/10.1186/s13046-023-02756-4.

62. Zhou, Z., Van der Jeught, K., Li, Y., Sharma, S., Yu, T., Moulana, I., Liu, S., Wan, J., Territo, P.R., Opyrchal, M., et al. (2023). A T Cell-Engaging Tumor Organoid Platform for Pancreatic Cancer Immunotherapy. Adv. Sci. *10*, e2300548. https://doi.org/10.1002/advs.202300548.

63. Tiriac, H., Belleau, P., Engle, D.D., Plenker, D., Deschênes, A., Somerville, T.D.D., Froeling, F.E.M., Burkhart, R.A., Denroche, R.E., Jang, G.-H., et al. (2018). Organoid profiling identifies common responders to chemotherapy in pancreatic cancer. Cancer Discov. *8*, 1112–1129.

64. Boj, S.F., Hwang, C.-I., Baker, L.A., Chio, I.I.C., Engle, D.D., Corbo, V., Jager, M., Ponz-Sarvise, M., Tiriac, H., Spector, M.S., et al. (2015). Organoid models of human and mouse ductal pancreatic cancer. Cell *160*, 324–338.

65. Seino, T., Kawasaki, S., Shimokawa, M., Tamagawa, H., Toshimitsu, K., Fujii, M., Ohta, Y., Matano, M., Nanki, K., Kawasaki, K., et al. (2018). Human pancreatic tumor organoids reveal loss of stem cell niche factor dependence during disease progression. Cell Stem Cell *22*, 454–467.e6.

66. Beato, F., Reverón, D., Dezsi, K.B., Ortiz, A., Johnson, J.O., Chen, D.-T., Ali, K., Yoder, S.J., Jeong, D., and Malafa, M. (2021). Establishing a Living Biobank of Patient-Derived Organoids of Intraductal Papillary Mucinous Neoplasms of the Pancreas. Lab. Invest. *101*, 204–217.

67. Kinny-Köster, B., Guinn, S., Tandurella, J.A., Mitchell, J.T., Sidiropoulos, D.N., Loth, M., Lyman, M.R., Pucsek, A.B., Seppälä, T.T., and Cherry, C. (2022). Inflammatory Signaling in Pancreatic Cancer Transfers Between a Single-cell RNA Sequencing Atlas and Co-Culture. Preprint at bioRxiv. https://doi.org/10.1101/2022.07.14.500096.

68. Schuth, S., Le Blanc, S., Krieger, T.G., Jabs, J., Schenk, M., Giese, N.A., Büchler, M.W., Eils, R., Conrad, C., and Strobel, O. (2022). Patient-specific modeling of stroma-mediated chemoresistance of pancreatic cancer using a three-dimensional organoid-fibroblast co-culture system. J. Exp. Clin. Cancer Res. *41*, 312. https://doi.org/10.1186/s13046-022-02519-7.

69. Tsai, S., McOlash, L., Palen, K., Johnson, B., Duris, C., Yang, Q., Dwinell, M.B., Hunt, B., Evans, D.B., Gershan, J., and James, M.A. (2018). Development of primary human pancreatic cancer organoids, matched stromal and immune cells and 3D tumor microenvironment models. BMC Cancer *18*, 335. https://doi.org/10.1186/s12885-018-4238-4.

70. Hirt, C.K., Booij, T.H., Grob, L., Simmler, P., Toussaint, N.C., Keller, D., Taube, D., Ludwig, V., Goryachkin, A., Pauli, C., et al. (2022). Drug screening and genome editing in human pancreatic cancer organoids identifies drug-gene interactions and candidates for off-label treatment. Cell Genom. *2*, 100095. https://doi.org/10.1016/j.xgen.2022.100095.

71. Seppälä, T.T., Zimmerman, J.W., Suri, R., Zlomke, H., Ivey, G.D., Szabolcs, A., Shubert, C.R., Cameron, J.L., Burns, W.R., Lafaro, K.J., et al. (2022). Precision medicine in pancreatic cancer: patient-derived organoid pharmacotyping is a predictive biomarker of clinical treatment response. Clin. Cancer Res. *28*, 3296–3307.

72. Gao, D., Vela, I., Sboner, A., Iaquinta, P.J., Karthaus, W.R., Gopalan, A., Dowling, C., Wanjala, J.N., Undvall, E.A., Arora, V.K., et al. (2014). Organoid cultures derived from patients with advanced prostate cancer. Cell *159*, 176–187.

73. Beshiri, M.L., Tice, C.M., Tran, C., Nguyen, H.M., Sowalsky, A.G., Agarwal, S., Jansson, K.H., Yang, Q., McGowen, K.M., Yin, J., et al. (2018). A PDX/organoid biobank of advanced prostate cancers captures genomic and phenotypic heterogeneity for disease modeling and therapeutic screening. Clin. Cancer Res. *24*, 4332–4345.

74. Yao, Y., Xu, X., Yang, L., Zhu, J., Wan, J., Shen, L., Xia, F., Fu, G., Deng, Y., Pan, M., et al. (2020). Patient-derived organoids predict chemoradiation responses of locally advanced rectal cancer. Cell Stem Cell *26*, 17–26.e6.

75. Chakrabarti, J., Holokai, L., Syu, L., Steele, N., Chang, J., Dlugosz, A., and Zavros, Y. (2018). Mouse-Derived Gastric Organoid and Immune Cell Co-culture for the Study of the Tumor Microenvironment.

Methods Mol. Biol. *1817*, 157–168. https://doi.org/10.1007/978-1-4939-8600-2_16.

76. Yan, H.H.N., Siu, H.C., Law, S., Ho, S.L., Yue, S.S.K., Tsui, W.Y., Chan, D., Chan, A.S., Ma, S., Lam, K.O., et al. (2018). A comprehensive human gastric cancer organoid biobank captures tumor subtype heterogeneity and enables therapeutic screening. Cell Stem Cell *23*, 882–897.e11.
77. Nanki, K., Toshimitsu, K., Takano, A., Fujii, M., Shimokawa, M., Ohta, Y., Matano, M., Seino, T., Nishikori, S., Ishikawa, K., et al. (2018). Divergent routes toward Wnt and R-spondin niche independency during human gastric carcinogenesis. Cell *174*, 856–869.e17.
78. Li, Z., Xu, H., Gong, Y., Chen, W., Zhan, Y., Yu, L., Sun, Y., Li, A., He, S., Guan, B., et al. (2022). Patient-Derived Upper Tract Urothelial Carcinoma Organoids as a Platform for Drug Screening. Adv. Sci. *9*, e2103999. https://doi.org/10.1002/advs.202103999.
79. Van de Wetering, M., Francies, H.E., Francis, J.M., Bounova, G., Iorio, F., Pronk, A., van Houdt, W., van Gorp, J., Taylor-Weiner, A., Kester, L., et al. (2015). Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell *161*, 933–945.
80. Kourula, S., Derksen, M., Jardi, F., Jonkers, S., van Heerden, M., Verboven, P., Theuns, V., Van Asten, S., Huybrechts, T., Kunze, A., et al. (2023). Intestinal organoids as an in vitro platform to characterize disposition, metabolism, and safety profile of small molecules. Eur. J. Pharm. Sci. *188*, 106481. https://doi.org/10.1016/j.ejps.2023.106481.
81. Choi, D., Gonzalez-Suarez, A.M., Dumbrava, M.G., Medlyn, M., de Hoyos-Vega, J.M., Cichocki, F., Miller, J.S., Ding, L., Zhu, M., Stybayeva, G., et al. (2024). Microfluidic Organoid Cultures Derived from Pancreatic Cancer Biopsies for Personalized Testing of Chemotherapy and Immunotherapy. Adv. Sci. *11*, e2303088. https://doi.org/10.1002/advs.202303088.
82. Sato, T., Stange, D.E., Ferrante, M., Vries, R.G.J., Van Es, J.H., Van Den Brink, S., Van Houdt, W.J., Pronk, A., Van Gorp, J., Siersema, P.D., and Clevers, H. (2011). Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology *141*, 1762–1772.
83. Neal, J.T., Li, X., Zhu, J., Giangarra, V., Grzeskowiak, C.L., Ju, J., Liu, I.H., Chiou, S.H., Salahudeen, A.A., Smith, A.R., et al. (2018). Organoid Modeling of the Tumor Immune Microenvironment. Cell *175*, 1972–1988.e16. https://doi.org/10.1016/j.cell.2018.11.021.
84. Whiteside, T.L. (2008). The tumor microenvironment and its role in promoting tumor growth. Oncogene *27*, 5904–5912.
85. Anderson, N.M., and Simon, M.C. (2020). The tumor microenvironment. Curr. Biol. *30*, R921–R925.
86. Joyce, J.A., and Fearon, D.T. (2015). T cell exclusion, immune privilege, and the tumor microenvironment. Science *348*, 74–80.
87. Wang, M., Zhao, J., Zhang, L., Wei, F., Lian, Y., Wu, Y., Gong, Z., Zhang, S., Zhou, J., Cao, K., et al. (2017). Role of tumor microenvironment in tumorigenesis. J. Cancer *8*, 761–773.
88. Quail, D.F., and Joyce, J.A. (2013). Microenvironmental regulation of tumor progression and metastasis. Nat. Med. *19*, 1423–1437.
89. Spano, D., and Zollo, M. (2012). Tumor microenvironment: a main actor in the metastasis process. Clin. Exp. Metastasis *29*, 381–395.
90. Wu, T., and Dai, Y. (2017). Tumor microenvironment and therapeutic response. Cancer letters *387*, 61–68.
91. Bejarano, L., Jordão, M.J.C., and Joyce, J.A. (2021). Therapeutic targeting of the tumor microenvironment. Cancer Discov. *11*, 933–959.
92. Flores-Torres, S., Dimitriou, N.M., Pardo, L.A., Kort-Mascort, J., Pal, S., Peza-Chavez, O., Kuasne, H., Berube, J., Bertos, N., Park, M., et al. (2023). Bioprinted Multicomponent Hydrogel Co-culture Tumor-Immune Model for Assessing and Simulating Tumor-Infiltrated Lymphocyte Migration and Functional Activation. ACS Appl. Mater. Interfaces *15*, 33250–33262. https://doi.org/10.1021/acsami.3c02995.
93. Neo, S.Y., Oliveira, M.M.S., Tong, L., Chen, Y., Chen, Z., Cismas, S., Burduli, N., Malmerfelt, A., Teo, J.K.H., Lam, K.-P., et al. (2024). Natural killer cells drive 4-1BBL positive uveal melanoma towards EMT and metastatic disease. J. Exp. Clin. Cancer Res. *43*, 13. https://doi.org/10.1186/s13046-023-02917-5.
94. Buonfiglioli, A., and Hambardzumyan, D. (2021). Macrophages and microglia: the cerberus of glioblastoma. Acta Neuropathol. Commun. *9*, 54. https://doi.org/10.1186/s40478-021-01156-z.
95. Tong, L., Jiménez-Cortegana, C., Tay, A.H.M., Wickström, S., Galluzzi, L., and Lundqvist, A. (2022). NK cells and solid tumors: therapeutic potential and persisting obstacles. Mol. Cancer *21*, 206. https://doi.org/10.1186/s12943-022-01672-z.
96. Xiao, W., Pahlavanneshan, M., Eun, C.Y., Zhang, X., DeKalb, C., Mahgoub, B., Knaneh-Monem, H., Shah, S., Sohrabi, A., Seidlits, S.K., and Hill, R. (2022). Matrix stiffness mediates pancreatic cancer chemoresistance through induction of exosome hypersecretion in a cancer associated fibroblasts-tumor organoid biomimetic model. Matrix Biol. *14*, 100111. https://doi.org/10.1016/j.mbplus.2022.100111.
97. Potente, M., and Carmeliet, P. (2017). The Link Between Angiogenesis and Endothelial Metabolism. Annu. Rev. Physiol. *79*, 43–66. https://doi.org/10.1146/annurev-physiol-021115-105134.
98. Harney, A.S., Arwert, E.N., Entenberg, D., Wang, Y., Guo, P., Qian, B.-Z., Oktay, M.H., Pollard, J.W., Jones, J.G., and Condeelis, J.S. (2015). Real-time imaging reveals local, transient vascular permeability, and tumor cell intravasation stimulated by TIE2hi macrophage–derived VEGFA. Cancer Discov. *5*, 932–943.
99. Kerbel, R.S. (2008). Tumor angiogenesis. N. Engl. J. Med. *358*, 2039–2049.
100. Huinen, Z.R., Huijbers, E.J.M., van Beijnum, J.R., Nowak-Sliwinska, P., and Griffioen, A.W. (2021). Anti-angiogenic agents — overcoming tumour endothelial cell anergy and improving immunotherapy outcomes. Nat. Rev. Clin. Oncol. *18*, 527–540. https://doi.org/10.1038/s41571-021-00496-y.
101. Hong, H.K., Yun, N.H., Jeong, Y.L., Park, J., Doh, J., Lee, W.Y., and Cho, Y.B. (2021). Establishment of patient-derived organotypic tumor spheroid models for tumor microenvironment modeling. Cancer Med. *10*, 5589–5598. https://doi.org/10.1002/cam4.4114.
102. Lai, B.F.L., Lu, R.X.Z., Davenport Huyer, L., Kakinoki, S., Yazbeck, J., Wang, E.Y., Wu, Q., Zhang, B., and Radisic, M. (2021). A well plate-based multiplexed platform for incorporation of organoids into an organ-on-a-chip system with a perfusable vasculature. Nat. Protoc. *16*, 2158–2189. https://doi.org/10.1038/s41596-020-00490-1.
103. Low, L.A., Mummery, C., Berridge, B.R., Austin, C.P., and Tagle, D.A. (2021). Organs-on-chips: into the next decade. Nat. Rev. Drug Discov. *20*, 345–361. https://doi.org/10.1038/s41573-020-0079-3.
104. Novak, R., Ingram, M., Marquez, S., Das, D., Delahanty, A., Herland, A., Maoz, B.M., Jeanty, S.S.F., Somayaji, M.R., Burt, M., et al. (2020). Robotic fluidic coupling and interrogation of multiple vascularized organ chips. Nat. Biomed. Eng. *4*, 407–420. https://doi.org/10.1038/s41551-019-0497-x.
105. Monteduro, A.G., Rizzato, S., Caragnano, G., Trapani, A., Giannelli, G., and Maruccio, G. (2023). Organs-on-chips technologies - A guide from disease models to opportunities for drug development. Biosens. Bioelectron. *231*, 115271. https://doi.org/10.1016/j.bios.2023.115271.
106. Kalot, R., Mhanna, R., and Talhouk, R. (2022). Organ-on-a-chip platforms as novel advancements for studying heterogeneity, metastasis, and drug efficacy in breast cancer. Pharmacol. Ther. *237*, 108156. https://doi.org/10.1016/j.pharmthera.2022.108156.
107. Geyer, M., Gaul, L.M., D Agosto, S.L., Corbo, V., and Queiroz, K. (2023). The tumor stroma influences immune cell distribution and recruitment in a PDAC-on-a-chip model. Front. Immunol. *14*, 1155085. https://doi.org/10.3389/fimmu.2023.1155085.
108. Hassell, B.A., Goyal, G., Lee, E., Sontheimer-Phelps, A., Levy, O., Chen, C.S., and Ingber, D.E. (2017). Human Organ Chip Models Recapitulate Orthotopic Lung Cancer Growth, Therapeutic Responses, and Tumor

Dormancy In Vitro. Cell Rep. *21*, 508–516. https://doi.org/10.1016/j.celrep.2017.09.043.

109. Carvalho, M.R., Yan, L.P., Li, B., Zhang, C.H., He, Y.L., Reis, R.L., and Oliveira, J.M. (2023). Gastrointestinal organs and organoids-on-a-chip: advances and translation into the clinics. Biofabrication *15*. https://doi.org/10.1088/1758-5090/acf8fb.

110. Yi, H.G., Jeong, Y.H., Kim, Y., Choi, Y.J., Moon, H.E., Park, S.H., Kang, K.S., Bae, M., Jang, J., Youn, H., et al. (2019). A bioprinted human-glioblastoma-on-a-chip for the identification of patient-specific responses to chemoradiotherapy. Nat. Biomed. Eng. *3*, 509–519. https://doi.org/10.1038/s41551-019-0363-x.

111. Yu, Y., Zhou, T., and Cao, L. (2023). Use and application of organ-on-a-chip platforms in cancer research. J. Cell Commun. Signal. *17*, 1163–1179. https://doi.org/10.1007/s12079-023-00790-7.

112. Delon, L.C., Guo, Z., Oszmiana, A., Chien, C.C., Gibson, R., Prestidge, C., and Thierry, B. (2019). A systematic investigation of the effect of the fluid shear stress on Caco-2 cells towards the optimization of epithelial organ-on-chip models. Biomaterials *225*, 119521. https://doi.org/10.1016/j.biomaterials.2019.119521.

113. Ning, L., Shim, J., Tomov, M.L., Liu, R., Mehta, R., Mingee, A., Hwang, B., Jin, L., Mantalaris, A., Xu, C., et al. (2022). A 3D Bioprinted in vitro Model of Neuroblastoma Recapitulates Dynamic Tumor-Endothelial Cell Interactions Contributing to Solid Tumor Aggressive Behavior. Adv. Sci. *9*, e2200244. https://doi.org/10.1002/advs.202200244.

114. Li, W., Mao, S., Khan, M., Zhang, Q., Huang, Q., Feng, S., and Lin, J.M. (2019). Responses of Cellular Adhesion Strength and Stiffness to Fluid Shear Stress during Tumor Cell Rolling Motion. ACS Sens. *4*, 1710–1715. https://doi.org/10.1021/acssensors.9b00678.

115. Park, T.E., Mustafaoglu, N., Herland, A., Hasselkus, R., Mannix, R., Fitz-Gerald, E.A., Prantil-Baun, R., Watters, A., Henry, O., Benz, M., et al. (2019). Hypoxia-enhanced Blood-Brain Barrier Chip recapitulates human barrier function and shuttling of drugs and antibodies. Nat. Commun. *10*, 2621. https://doi.org/10.1038/s41467-019-10588-0.

116. Bai, H., and Ingber, D.E. (2022). What Can an Organ-on-a-Chip Teach Us About Human Lung Pathophysiology? Physiology *37*, 0. https://doi.org/10.1152/physiol.00012.2022.

117. Wise, H.C., and Solit, D.B. (2019). Precision Oncology: Three Small Steps Forward. Cancer Cell *35*, 825–826. https://doi.org/10.1016/j.ccell.2019.05.009.

118. Ingber, D.E. (2022). Human organs-on-chips for disease modelling, drug development and personalized medicine. Nat. Rev. Genet. *23*, 467–491. https://doi.org/10.1038/s41576-022-00466-9.

119. Miller, C.P., Tsuchida, C., Zheng, Y., Himmelfarb, J., and Akilesh, S. (2018). A 3D Human Renal Cell Carcinoma-on-a-Chip for the Study of Tumor Angiogenesis. Neoplasia *20*, 610–620. https://doi.org/10.1016/j.neo.2018.02.011.

120. Hu, Y., Sui, X., Song, F., Li, Y., Li, K., Chen, Z., Yang, F., Chen, X., Zhang, Y., Wang, X., et al. (2021). Lung cancer organoids analyzed on microwell arrays predict drug responses of patients within a week. Nat. Commun. *12*, 2581. https://doi.org/10.1038/s41467-021-22676-1.

121. Wang, Y., Gao, Y., Pan, Y., Zhou, D., Liu, Y., Yin, Y., Yang, J., Wang, Y., and Song, Y. (2023). Emerging trends in organ-on-a-chip systems for drug screening. Acta Pharm. Sin. B *13*, 2483–2509. https://doi.org/10.1016/j.apsb.2023.02.006.

122. Ma, C., Peng, Y., Li, H., and Chen, W. (2021). Organ-on-a-Chip: A New Paradigm for Drug Development. Trends Pharmacol. Sci. *42*, 119–133. https://doi.org/10.1016/j.tips.2020.11.009.

123. Deng, S., Li, C., Cao, J., Cui, Z., Du, J., Fu, Z., Yang, H., and Chen, P. (2023). Organ-on-a-chip meets artificial intelligence in drug evaluation. Theranostics *13*, 4526–4558. https://doi.org/10.7150/thno.87266.

124. Porter, R.J., Murray, G.I., and McLean, M.H. (2020). Current concepts in tumour-derived organoids. Br. J. Cancer *123*, 1209–1218. https://doi.org/10.1038/s41416-020-0993-5.

125. Mak, I.W., Evaniew, N., and Ghert, M. (2014). Lost in translation: animal models and clinical trials in cancer treatment. Am. J. Transl. Res. *6*, 114–118.

126. Ramli, M.N.B., Lim, Y.S., Koe, C.T., Demircioglu, D., Tng, W., Gonzales, K.A.U., Tan, C.P., Szczerbinska, I., Liang, H., Soe, E.L., et al. (2020). Human Pluripotent Stem Cell-Derived Organoids as Models of Liver Disease. Gastroenterology *159*, 1471–1486.e12. https://doi.org/10.1053/j.gastro.2020.06.010.

127. Hendriks, D., Brouwers, J.F., Hamer, K., Geurts, M.H., Luciana, L., Massalini, S., López-Iglesias, C., Peters, P.J., Rodríguez-Colman, M.J., Chuva de Sousa Lopes, S., et al. (2023). Engineered human hepatocyte organoids enable CRISPR-based target discovery and drug screening for steatosis. Nat. Biotechnol. *41*, 1567–1581. https://doi.org/10.1038/s41587-023-01680-4.

128. Rallabandi, H.R., Yang, H., Oh, K.B., Lee, H.C., Byun, S.J., and Lee, B.R. (2020). Evaluation of Intestinal Epithelial Barrier Function in Inflammatory Bowel Diseases Using Murine Intestinal Organoids. Tissue Eng. Regen. Med. *17*, 641–650. https://doi.org/10.1007/s13770-020-00278-0.

129. Zhou, J., Li, C., Sachs, N., Chiu, M.C., Wong, B.H.Y., Chu, H., Poon, V.K.M., Wang, D., Zhao, X., Wen, L., et al. (2018). Differentiated human airway organoids to assess infectivity of emerging influenza virus. Proc. Natl. Acad. Sci. USA *115*, 6822–6827. https://doi.org/10.1073/pnas.1806308115.

130. Lamers, M.M., Beumer, J., van der Vaart, J., Knoops, K., Puschhof, J., Breugem, T.I., Ravelli, R.B.G., Paul van Schayck, J., Mykytyn, A.Z., Duimel, H.Q., et al. (2020). SARS-CoV-2 productively infects human gut enterocytes. Science *369*, 50–54. https://doi.org/10.1126/science.abc1669.

131. Monteil, V., Kwon, H., Prado, P., Hagelkrüys, A., Wimmer, R.A., Stahl, M., Leopoldi, A., Garreta, E., Hurtado Del Pozo, C., Prosper, F., et al. (2020). Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2. Cell *181*, 905–913.e7. https://doi.org/10.1016/j.cell.2020.04.004.

132. Tang, X.Y., Wu, S., Wang, D., Chu, C., Hong, Y., Tao, M., Hu, H., Xu, M., Guo, X., and Liu, Y. (2022). Human organoids in basic research and clinical applications. Signal Transduct. Target. Ther. *7*, 168. https://doi.org/10.1038/s41392-022-01024-9.

133. Dao, V., Yuki, K., Lo, Y.H., Nakano, M., and Kuo, C.J. (2022). Immune organoids: from tumor modeling to precision oncology. Trends Cancer *8*, 870–880. https://doi.org/10.1016/j.trecan.2022.06.001.

134. Xu, H., Jiao, D., Liu, A., and Wu, K. (2022). Tumor organoids: applications in cancer modeling and potentials in precision medicine. J. Hematol. Oncol. *15*, 58. https://doi.org/10.1186/s13045-022-01278-4.

135. Yuki, K., Cheng, N., Nakano, M., and Kuo, C.J. (2020). Organoid Models of Tumor Immunology. Trends Immunol. *41*, 652–664. https://doi.org/10.1016/j.it.2020.06.010.

136. Hendriks, D., Pagliaro, A., Andreatta, F., Ma, Z., van Giessen, J., Massalini, S., López-Iglesias, C., van Son, G.J.F., DeMartino, J., Damen, J.M.A., et al. (2024). Human fetal brain self-organizes into long-term expanding organoids. Cell *187*, 712–732.e38. https://doi.org/10.1016/j.cell.2023.12.012.

137. Bian, S., Repic, M., Guo, Z., Kavirayani, A., Burkard, T., Bagley, J.A., Krauditsch, C., and Knoblich, J.A. (2018). Genetically engineered cerebral organoids model brain tumor formation. Nat. Methods *15*, 631–639. https://doi.org/10.1038/s41592-018-0070-7.

138. Sturm, D., Orr, B.A., Toprak, U.H., Hovestadt, V., Jones, D.T.W., Capper, D., Sill, M., Buchhalter, I., Northcott, P.A., Leis, I., et al. (2016). New Brain Tumor Entities Emerge from Molecular Classification of CNS-PNETs. Cell *164*, 1060–1072. https://doi.org/10.1016/j.cell.2016.01.015.

139. Ogawa, J., Pao, G.M., Shokhirev, M.N., and Verma, I.M. (2018). Glioblastoma model using human cerebral organoids. Cell Rep. *23*, 1220–1229.

140. Hendriks, D., Clevers, H., and Artegiani, B. (2020). CRISPR-Cas Tools and Their Application in Genetic Engineering of Human Stem Cells and

Organoids. Cell Stem Cell *27*, 705–731. https://doi.org/10.1016/j.stem.2020.10.014.

141. Drost, J., van Boxtel, R., Blokzijl, F., Mizutani, T., Sasaki, N., Sasselli, V., de Ligt, J., Behjati, S., Grolleman, J.E., van Wezel, T., et al. (2017). Use of CRISPR-modified human stem cell organoids to study the origin of mutational signatures in cancer. Science *358*, 234–238. https://doi.org/10.1126/science.aao3130.

142. Artegiani, B., van Voorthuijsen, L., Lindeboom, R.G.H., Seinstra, D., Heo, I., Tapia, P., López-Iglesias, C., Postrach, D., Dayton, T., Oka, R., et al. (2019). Probing the Tumor Suppressor Function of BAP1 in CRISPR-Engineered Human Liver Organoids. Cell Stem Cell *24*, 927–943.e6. https://doi.org/10.1016/j.stem.2019.04.017.

143. Michels, B.E., Mosa, M.H., Streibl, B.I., Zhan, T., Menche, C., Abou-El-Ardat, K., Darvishi, T., Członka, E., Wagner, S., Winter, J., et al. (2020). Pooled In Vitro and In Vivo CRISPR-Cas9 Screening Identifies Tumor Suppressors in Human Colon Organoids. Cell Stem Cell *26*, 782–792.e7. https://doi.org/10.1016/j.stem.2020.04.003.

144. Cortina, C., Turon, G., Stork, D., Hernando-Momblona, X., Sevillano, M., Aguilera, M., Tosi, S., Merlos-Suárez, A., Stephan-Otto Attolini, C., Sancho, E., and Batlle, E. (2017). A genome editing approach to study cancer stem cells in human tumors. EMBO Mol. Med. *9*, 869–879. https://doi.org/10.15252/emmm.201707550.

145. Shimokawa, M., Ohta, Y., Nishikori, S., Matano, M., Takano, A., Fujii, M., Date, S., Sugimoto, S., Kanai, T., and Sato, T. (2017). Visualization and targeting of LGR5(+) human colon cancer stem cells. Nature *545*, 187–192. https://doi.org/10.1038/nature22081.

146. Schene, I.F., Joore, I.P., Oka, R., Mokry, M., van Vugt, A.H.M., van Boxtel, R., van der Doef, H.P.J., van der Laan, L.J.W., Verstegen, M.M.A., van Hasselt, P.M., et al. (2020). Prime editing for functional repair in patient-derived disease models. Nat. Commun. *11*, 5352. https://doi.org/10.1038/s41467-020-19136-7.

147. Bock, C., Boutros, M., Camp, J.G., Clarke, L., Clevers, H., Knoblich, J.A., Liberali, P., Regev, A., Rios, A.C., Stegle, O., et al. (2021). The organoid cell atlas. Nat. Biotechnol. *39*, 13–17.

148. Seppälä, T.T., Zimmerman, J.W., Suri, R., Zlomke, H., Ivey, G.D., Szabolcs, A., Shubert, C.R., Cameron, J.L., Burns, W.R., Lafaro, K.J., et al. (2022). Precision Medicine in Pancreatic Cancer: Patient-Derived Organoid Pharmacotyping Is a Predictive Biomarker of Clinical Treatment Response. Clin. Cancer Res. *28*, 3296–3307. https://doi.org/10.1158/1078-0432.Ccr-21-4165.

149. Kawasaki, K., Toshimitsu, K., Matano, M., Fujita, M., Fujii, M., Togasaki, K., Ebisudani, T., Shimokawa, M., Takano, A., Takahashi, S., et al. (2020). An Organoid Biobank of Neuroendocrine Neoplasms Enables Genotype-Phenotype Mapping. Cell *183*, 1420–1435.e21. https://doi.org/10.1016/j.cell.2020.10.023.

150. Gonneaud, A., Asselin, C., Boudreau, F., and Boisvert, F.M. (2017). Phenotypic Analysis of Organoids by Proteomics. Proteomics *17*. https://doi.org/10.1002/pmic.201700023.

151. Boj, S.F., Hwang, C.I., Baker, L.A., Chio, I.I.C., Engle, D.D., Corbo, V., Jager, M., Ponz-Sarvise, M., Tiriac, H., Spector, M.S., et al. (2015). Organoid models of human and mouse ductal pancreatic cancer. Cell *160*, 324–338. https://doi.org/10.1016/j.cell.2014.12.021.

152. Williams, K.E., Lemieux, G.A., Hassis, M.E., Olshen, A.B., Fisher, S.J., and Werb, Z. (2016). Quantitative proteomic analyses of mammary organoids reveals distinct signatures after exposure to environmental chemicals. Proc. Natl. Acad. Sci. USA *113*, E1343–E1351. https://doi.org/10.1073/pnas.1600645113.

153. Wang, Y., and Jeon, H. (2022). 3D cell cultures toward quantitative high-throughput drug screening. Trends Pharmacol. Sci. *43*, 569–581. https://doi.org/10.1016/j.tips.2022.03.014.

154. Wang, H., Brown, P.C., Chow, E.C.Y., Ewart, L., Ferguson, S.S., Fitzpatrick, S., Freedman, B.S., Guo, G.L., Hedrich, W., Heyward, S., et al. (2021). 3D cell culture models: Drug pharmacokinetics, safety assessment, and regulatory consideration. Clin. Transl. Sci. *14*, 1659–1680. https://doi.org/10.1111/cts.13066.

155. Booij, T.H., Price, L.S., and Danen, E.H.J. (2019). 3D Cell-Based Assays for Drug Screens: Challenges in Imaging, Image Analysis, and High-Content Analysis. SLAS Discov. *24*, 615–627. https://doi.org/10.1177/2472555219830087.

156. Francies, H.E., Barthorpe, A., McLaren-Douglas, A., Barendt, W.J., and Garnett, M.J. (2019). Drug Sensitivity Assays of Human Cancer Organoid Cultures. Methods Mol. Biol. *1576*, 339–351. https://doi.org/10.1007/7651_2016_10.

157. Weeber, F., Ooft, S.N., Dijkstra, K.K., and Voest, E.E. (2017). Tumor Organoids as a Pre-clinical Cancer Model for Drug Discovery. Cell Chem. Biol. *24*, 1092–1100. https://doi.org/10.1016/j.chembiol.2017.06.012.

158. Vlachogiannis, G., Hedayat, S., Vatsiou, A., Jamin, Y., Fernández-Mateos, J., Khan, K., Lampis, A., Eason, K., Huntingford, I., Burke, R., et al. (2018). Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. Science *359*, 920–926. https://doi.org/10.1126/science.aao2774.

159. de Witte, C.J., Espejo Valle-Inclan, J., Hami, N., Lõhmussaar, K., Kopper, O., Vreuls, C.P.H., Jonges, G.N., van Diest, P., Nguyen, L., Clevers, H., et al. (2020). Patient-Derived Ovarian Cancer Organoids Mimic Clinical Response and Exhibit Heterogeneous Inter- and Intrapatient Drug Responses. Cell Rep. *31*, 107762. https://doi.org/10.1016/j.celrep.2020.107762.

160. Bertotti, A., Migliardi, G., Galimi, F., Sassi, F., Torti, D., Isella, C., Corà, D., Di Nicolantonio, F., Buscarino, M., Petti, C., et al. (2011). A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov. *1*, 508–523. https://doi.org/10.1158/2159-8290.CD-11-0109.

161. Nanki, Y., Chiyoda, T., Hirasawa, A., Ookubo, A., Itoh, M., Ueno, M., Akahane, T., Kameyama, K., Yamagami, W., Kataoka, F., and Aoki, D. (2020). Patient-derived ovarian cancer organoids capture the genomic profiles of primary tumours applicable for drug sensitivity and resistance testing. Sci. Rep. *10*, 12581. https://doi.org/10.1038/s41598-020-69488-9.

162. Li, X., Nadauld, L., Ootani, A., Corney, D.C., Pai, R.K., Gevaert, O., Cantrell, M.A., Rack, P.G., Neal, J.T., Chan, C.W.M., et al. (2014). Oncogenic transformation of diverse gastrointestinal tissues in primary organoid culture. Nat. Med. *20*, 769–777. https://doi.org/10.1038/nm.3585.

163. Dye, B.R., Hill, D.R., Ferguson, M.A.H., Tsai, Y.H., Nagy, M.S., Dyal, R., Wells, J.M., Mayhew, C.N., Nattiv, R., Klein, O.D., et al. (2015). In vitro generation of human pluripotent stem cell derived lung organoids. Elife *4*, e05098. https://doi.org/10.7554/eLife.05098.

164. Hattori, N. (2014). Cerebral organoids model human brain development and microcephaly. Mov. Disord. *29*, 185. https://doi.org/10.1002/mds.25740.

165. Love, J.R., and Karthaus, W.R. (2024). Next-Generation Modeling of Cancer Using Organoids. Cold Spring Harb. Perspect. Med. *14*, a041380. https://doi.org/10.1101/cshperspect.a041380.

166. LeSavage, B.L., Suhar, R.A., Broguiere, N., Lutolf, M.P., and Heilshorn, S.C. (2022). Next-generation cancer organoids. Nat. Mater. *21*, 143–159. https://doi.org/10.1038/s41563-021-01057-5.

167. Ooft, S.N., Weeber, F., Dijkstra, K.K., McLean, C.M., Kaing, S., van Werkhoven, E., Schipper, L., Hoes, L., Vis, D.J., van de Haar, J., et al. (2019). Patient-derived organoids can predict response to chemotherapy in metastatic colorectal cancer patients. Sci. Transl. Med. *11*, eaay2574. https://doi.org/10.1126/scitranslmed.aay2574.

168. Betge, J., Rindtorff, N., Sauer, J., Rauscher, B., Dingert, C., Gaitantzi, H., Herweck, F., Srour-Mhanna, K., Miersch, T., Valentini, E., et al. (2022). The drug-induced phenotypic landscape of colorectal cancer organoids. Nat. Commun. *13*, 3135. https://doi.org/10.1038/s41467-022-30722-9.

169. Done, A.J., and Birkeland, A.C. (2023). Organoids as a tool in drug discovery and patient-specific therapy for head and neck cancer. Cell Rep. Med. *4*, 101087. https://doi.org/10.1016/j.xcrm.2023.101087.

170. Grossman, J.E., Muthuswamy, L., Huang, L., Akshinthala, D., Perea, S., Gonzalez, R.S., Tsai, L.L., Cohen, J., Bockorny, B., Bullock, A.J., et al. (2022). Organoid Sensitivity Correlates with Therapeutic Response in Patients with Pancreatic Cancer. Clin. Cancer Res. *28*, 708–718. https://doi.org/10.1158/1078-0432.CCR-20-4116.

171. Chen, X., Sifakis, E.G., Robertson, S., Neo, S.Y., Jun, S.-H., Tong, L., Hui Min, A.T., Lövrot, J., Hellgren, R., Margolin, S., et al. (2023). Breast cancer patient-derived whole-tumor cell culture model for efficient drug profiling and treatment response prediction. Proc. Natl. Acad. Sci. USA *120*, e2209856120. https://doi.org/10.1073/pnas.2209856120.

172. Hu, B., Wang, R., Wu, D., Long, R., Fan, J., Hu, Z., Hu, X., Ma, D., Li, F., Sun, C., and Liao, S. (2024). A Promising New Model: Establishment of Patient-Derived Organoid Models Covering HPV-Related Cervical Pre-Cancerous Lesions and Their Cancers. Adv. Sci. *11*, e2302340. https://doi.org/10.1002/advs.202302340.

173. Yan, H.H.N., Siu, H.C., Law, S., Ho, S.L., Yue, S.S.K., Tsui, W.Y., Chan, D., Chan, A.S., Ma, S., Lam, K.O., et al. (2018). A Comprehensive Human Gastric Cancer Organoid Biobank Captures Tumor Subtype Heterogeneity and Enables Therapeutic Screening. Cell Stem Cell *23*, 882–897.e11. https://doi.org/10.1016/j.stem.2018.09.016.

174. Botti, G., Di Bonito, M., and Cantile, M. (2021). Organoid biobanks as a new tool for pre-clinical validation of candidate drug efficacy and safety. Int. J. Physiol. Pathophysiol. Pharmacol. *13*, 17–21.

175. Zhou, Z., Cong, L., and Cong, X. (2021). Patient-Derived Organoids in Precision Medicine: Drug Screening, Organoid-on-a-Chip and Living Organoid Biobank. Front. Oncol. *11*, 762184. https://doi.org/10.3389/fonc.2021.762184.

176. Tebon, P.J., Wang, B., Markowitz, A.L., Davarifar, A., Tsai, B.L., Krawczuk, P., Gonzalez, A.E., Sartini, S., Murray, G.F., Nguyen, H.T.L., et al. (2023). Drug screening at single-organoid resolution via bioprinting and interferometry. Nat. Commun. *14*, 3168. https://doi.org/10.1038/s41467-023-38832-8.

177. Vincent, F., Nueda, A., Lee, J., Schenone, M., Prunotto, M., and Mercola, M. (2022). Phenotypic drug discovery: recent successes, lessons learned and new directions. Nat. Rev. Drug Discov. *21*, 899–914. https://doi.org/10.1038/s41573-022-00472-w.

178. Matsui, T., and Shinozawa, T. (2021). Human Organoids for Predictive Toxicology Research and Drug Development. Front. Genet. *12*, 767621. https://doi.org/10.3389/fgene.2021.767621.

179. Roerink, S.F., Sasaki, N., Lee-Six, H., Young, M.D., Alexandrov, L.B., Behjati, S., Mitchell, T.J., Grossmann, S., Lightfoot, H., Egan, D.A., et al. (2018). Intra-tumour diversification in colorectal cancer at the single-cell level. Nature *556*, 457–462. https://doi.org/10.1038/s41586-018-0024-3.

180. Phan, N., Hong, J.J., Tofig, B., Mapua, M., Elashoff, D., Moatamed, N.A., Huang, J., Memarzadeh, S., Damoiseaux, R., and Soragni, A. (2019). A simple high-throughput approach identifies actionable drug sensitivities in patient-derived tumor organoids. Commun. Biol. *2*, 78. https://doi.org/10.1038/s42003-019-0305-x.

181. Schuster, B., Junkin, M., Kashaf, S.S., Romero-Calvo, I., Kirby, K., Matthews, J., Weber, C.R., Rzhetsky, A., White, K.P., and Tay, S. (2020). Automated microfluidic platform for dynamic and combinatorial drug screening of tumor organoids. Nat. Commun. *11*, 5271. https://doi.org/10.1038/s41467-020-19058-4.

182. Legnini, I., Emmenegger, L., Zappulo, A., Rybak-Wolf, A., Wurmus, R., Martinez, A.O., Jara, C.C., Boltengagen, A., Hessler, T., Mastrobuoni, G., et al. (2023). Spatiotemporal, optogenetic control of gene expression in organoids. Nat. Methods *20*, 1544–1552. https://doi.org/10.1038/s41592-023-01986-w.

183. Li, C., Fleck, J.S., Martins-Costa, C., Burkard, T.R., Themann, J., Stuempflen, M., Peer, A.M., Vertesy, Á., Littleboy, J.B., Esk, C., et al. (2023). Single-cell brain organoid screening identifies developmental defects in autism. Nature *621*, 373–380. https://doi.org/10.1038/s41586-023-06473-y.

184. Sawyers, C.L. (2008). The cancer biomarker problem. Nature *452*, 548–552.

185. Kern, S.E. (2012). Why your new cancer biomarker may never work: recurrent patterns and remarkable diversity in biomarker failures. Cancer Res. *72*, 6097–6101.

186. La Thangue, N.B., and Kerr, D.J. (2011). Predictive biomarkers: a paradigm shift towards personalized cancer medicine. Nat. Rev. Clin. Oncol. *8*, 587–596.

187. Kamel, H.F.M., and Al-Amodi, H.S.A.B. (2017). Exploitation of gene expression and cancer biomarkers in paving the path to era of personalized medicine. Dev. Reprod. Biol. *15*, 220–235.

188. Califf, R.M. (2018). Biomarker definitions and their applications. Exp. Biol. Med. *243*, 213–221.

189. Letai, A. (2017). Functional precision cancer medicine—moving beyond pure genomics. Nat. Med. *23*, 1028–1035.

190. Majewski, I.J., and Bernards, R. (2011). Taming the dragon: genomic biomarkers to individualize the treatment of cancer. Nat. Med. *17*, 304–312.

191. Simon, R. (2011). Genomic biomarkers in predictive medicine. An interim analysis. EMBO Mol. Med. *3*, 429–435.

192. Nanki, Y., Chiyoda, T., Hirasawa, A., Ookubo, A., Itoh, M., Ueno, M., Akahane, T., Kameyama, K., Yamagami, W., Kataoka, F., and Aoki, D. (2020). Patient-derived ovarian cancer organoids capture the genomic profiles of primary tumours applicable for drug sensitivity and resistance testing. Sci. Rep. *10*, 12581.

193. Usman, O.H., Zhang, L., Xie, G., Kocher, H.M., Hwang, C.-i., Wang, Y.J., Mallory, X., and Irianto, J. (2022). Genomic heterogeneity in pancreatic cancer organoids and its stability with culture. NPJ Genom. Med. *7*, 71.

194. Verissimo, C.S., Overmeer, R.M., Ponsioen, B., Drost, J., Mertens, S., Verlaan-Klink, I., Gerwen, B.v., van der Ven, M., Wetering, M.v.d., Egan, D.A., et al. (2016). Targeting mutant RAS in patient-derived colorectal cancer organoids by combinatorial drug screening. Elife *5*, e18489.

195. Wensink, G.E., Elias, S.G., Mullenders, J., Koopman, M., Boj, S.F., Kranenburg, O.W., and Roodhart, J.M.L. (2021). Patient-derived organoids as a predictive biomarker for treatment response in cancer patients. npj Precis. Oncol. *5*, 30. https://doi.org/10.1038/s41698-021-00168-1.

196. Ganesh, K., Wu, C., O'Rourke, K.P., Szeglin, B.C., Zheng, Y., Sauvé, C.-E.G., Adileh, M., Wasserman, I., Marco, M.R., Kim, A.S., et al. (2019). A rectal cancer organoid platform to study individual responses to chemoradiation. Nat. Med. *25*, 1607–1614.

197. Narasimhan, V., Wright, J.A., Churchill, M., Wang, T., Rosati, R., Lannagan, T.R.M., Vrbanac, L., Richardson, A.B., Kobayashi, H., Price, T., et al. (2020). Medium-throughput drug screening of patient-derived organoids from colorectal peritoneal metastases to direct personalized therapy. Clin. Cancer Res. *26*, 3662–3670.

198. Vlachogiannis, G., Hedayat, S., Vatsiou, A., Jamin, Y., Fernández-Mateos, J., Khan, K., Lampis, A., Eason, K., Huntingford, I., Burke, R., et al. (2018). Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. Science *359*, 920–926.

199. Steele, N.G., Chakrabarti, J., Wang, J., Biesiada, J., Holokai, L., Chang, J., Nowacki, L.M., Hawkins, J., Mahe, M., Sundaram, N., et al. (2019). An organoid-based preclinical model of human gastric cancer. Cell. Mol. Gastroenterol. Hepatol. *7*, 161–184.

200. Sharick, J.T., Walsh, C.M., Sprackling, C.M., Pasch, C.A., Pham, D.L., Esbona, K., Choudhary, A., Garcia-Valera, R., Burkard, M.E., McGregor, S.M., et al. (2020). Metabolic heterogeneity in patient tumor-derived organoids by primary site and drug treatment. Front. Oncol. *10*.

201. Li, X., Francies, H.E., Secrier, M., Perner, J., Miremadi, A., Galeano-Dalmau, N., Barendt, W.J., Letchford, L., Leyden, G.M., Goffin, E.K., et al. (2018). Organoid cultures recapitulate esophageal adenocarcinoma

heterogeneity providing a model for clonality studies and precision therapeutics. Nat. Commun. *9*, 2983.

202. Votanopoulos, K.I., Forsythe, S., Sivakumar, H., Mazzocchi, A., Aleman, J., Miller, L., Levine, E., Triozzi, P., and Skardal, A. (2020). Model of patient-specific immune-enhanced organoids for immunotherapy screening: feasibility study. Ann. Surg Oncol. *27*, 1956–1967.
203. Phan, N., Hong, J.J., Tofig, B., Mapua, M., Elashoff, D., Moatamed, N.A., Huang, J., Memarzadeh, S., Damoiseaux, R., and Soragni, A. (2019). A simple high-throughput approach identifies actionable drug sensitivities in patient-derived tumor organoids. Commun. Biol. *2*, 78.
204. de Witte, C.J., Valle-Inclan, J.E., Hami, N., Lõhmussaar, K., Kopper, O., Vreuls, C.P.H., Jonges, G.N., van Diest, P., Nguyen, L., and Clevers, H. (2020). Patient-derived ovarian cancer organoids mimic clinical response and exhibit heterogeneous inter-and intrapatient drug responses. Cell Rep. *31*, 107762.
205. Mazzocchi, A.R., Rajan, S.A.P., Votanopoulos, K.I., Hall, A.R., and Skardal, A. (2018). In vitro patient-derived 3D mesothelioma tumor organoids facilitate patient-centric therapeutic screening. Sci. Rep. *8*, 2886.
206. Simon, R. (2010). Clinical trial designs for evaluating the medical utility of prognostic and predictive biomarkers in oncology. Per. Med. *7*, 33–47.
207. Juric, D., Rodon, J., Tabernero, J., Janku, F., Burris, H.A., Schellens, J.H.M., Middleton, M.R., Berlin, J., Schuler, M., Gil-Martin, M., et al. (2018). Phosphatidylinositol 3-kinase α–selective inhibition with alpelisib (BYL719) in PIK3CA-altered solid tumors: results from the first-in-human study. J. Clin. Oncol. *36*, 1291–1299.
208. Fritsch, C., Huang, A., Chatenay-Rivauday, C., Schnell, C., Reddy, A., Liu, M., Kauffmann, A., Guthy, D., Erdmann, D., De Pover, A., et al. (2014). Characterization of the novel and specific PI3Kα inhibitor NVP-BYL719 and development of the patient stratification strategy for clinical trials. Mol. Cancer Ther. *13*, 1117–1129.
209. Marjon, K., Cameron, M.J., Quang, P., Clasquin, M.F., Mandley, E., Kunii, K., McVay, M., Choe, S., Kernytsky, A., Gross, S., et al. (2016). MTAP deletions in cancer create vulnerability to targeting of the MAT2A/PRMT5/RIOK1 axis. Cell Rep. *15*, 574–587.
210. Kryukov, G.V., Wilson, F.H., Ruth, J.R., Paulk, J., Tsherniak, A., Marlow, S.E., Vazquez, F., Weir, B.A., Fitzgerald, M.E., Tanaka, M., et al. (2016). MTAP deletion confers enhanced dependency on the PRMT5 arginine methyltransferase in cancer cells. Science *351*, 1214–1218.
211. Mavrakis, K.J., McDonald, E.R., 3rd, Schlabach, M.R., Billy, E., Hoffman, G.R., deWeck, A., Ruddy, D.A., Venkatesan, K., Yu, J., McAllister, G., et al. (2016). Disordered methionine metabolism in MTAP/CDKN2A-deleted cancers leads to dependence on PRMT5. Science *351*, 1208–1213. https://doi.org/10.1126/science.aad5944.
212. Li, H., Liu, H., and Chen, K. (2022). Living biobank-based cancer organoids: prospects and challenges in cancer research. Cancer Biol. Med. *19*, 965–982. https://doi.org/10.20892/j.issn.2095-3941.2021.0621.
213. Lechler, R.I., Sykes, M., Thomson, A.W., and Turka, L.A. (2005). Organ transplantation—how much of the promise has been realized? Nat. Med. *11*, 605–613. https://doi.org/10.1038/nm1251.
214. Yoshida, S., Miwa, H., Kawachi, T., Kume, S., and Takahashi, K. (2020). Generation of intestinal organoids derived from human pluripotent stem cells for drug testing. Sci. Rep. *10*, 5989.
215. McCracken, K.W., Catá, E.M., Crawford, C.M., Sinagoga, K.L., Schumacher, M., Rockich, B.E., Tsai, Y.-H., Mayhew, C.N., Spence, J.R., Zavros, Y., and Wells, J.M. (2014). Modelling human development and disease in pluripotent stem-cell-derived gastric organoids. Nature *516*, 400–404.
216. Barker, N., Huch, M., Kujala, P., van de Wetering, M., Snippert, H.J., van Es, J.H., Sato, T., Stange, D.E., Begthel, H., van den Born, M., et al. (2010). Lgr5+ ve stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. Cell Stem Cell *6*, 25–36.
217. Lancaster, M.A., and Knoblich, J.A. (2014). Generation of cerebral organoids from human pluripotent stem cells. Nat. Protoc. *9*, 2329–2340.
218. Louie, S.M., Moye, A.L., Wong, I.G., Lu, E., Shehaj, A., Garcia-de-Alba, C., Ararat, E., Raby, B.A., Lu, B., Paschini, M., et al. (2022). Progenitor potential of lung epithelial organoid cells in a transplantation model. Cell Rep. *39*, 110662.
219. Schutgens, F., Rookmaaker, M.B., Margaritis, T., Rios, A., Ammerlaan, C., Jansen, J., Gijzen, L., Vormann, M., Vonk, A., Viveen, M., et al. (2019). Tubuloids derived from human adult kidney and urine for personalized disease modeling. Nat. Biotechnol. *37*, 303–313.
220. Uchimura, K., Wu, H., Yoshimura, Y., and Humphreys, B.D. (2020). Human pluripotent stem cell-derived kidney organoids with improved collecting duct maturation and injury modeling. Cell Rep. *33*, 108514.
221. Takasato, M., Er, P.X., Chiu, H.S., and Little, M.H. (2016). Generation of kidney organoids from human pluripotent stem cells. Nat. Protoc. *11*, 1681–1692.
222. Morizane, R., and Bonventre, J.V. (2017). Generation of nephron progenitor cells and kidney organoids from human pluripotent stem cells. Nat. Protoc. *12*, 195–207.
223. Sampaziotis, F., Muraro, D., Tysoe, O.C., Sawiak, S., Beach, T.E., Godfrey, E.M., Upponi, S.S., Brevini, T., Wesley, B.T., Garcia-Bernardo, J., et al. (2021). Cholangiocyte organoids can repair bile ducts after transplantation in the human liver. Science *371*, 839–846.
224. Hu, H., Gehart, H., Artegiani, B., LÖpez-Iglesias, C., Dekkers, F., Basak, O., van Es, J., Chuva de Sousa Lopes, S.M., Begthel, H., Korving, J., et al. (2018). Long-term expansion of functional mouse and human hepatocytes as 3D organoids. Cell *175*, 1591–1606.e19.
225. Huch, M., Gehart, H., Van Boxtel, R., Hamer, K., Blokzijl, F., Verstegen, M.M.A., Ellis, E., Van Wenum, M., Fuchs, S.A., de Ligt, J., et al. (2015). Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell *160*, 299–312.
226. Joshi, R., Castro De Moura, M., Piñeyro, D., Alvarez-Errico, D., Arribas, C., and Esteller, M. (2020). The DNA methylation landscape of human cancer organoids available at the American type culture collection. Epigenetics *15*, 1167–1177.
227. Driehuis, E., Kretzschmar, K., and Clevers, H. (2020). Establishment of patient-derived cancer organoids for drug-screening applications. Nat. Protoc. *15*, 3380–3409.
228. Nasar, N., McIntyre, S.M.H., Kalvin, H.L., Gonen, M., Lecomte, N., Karnoub, E., Hong, J., Soares, K., Balachandran, V.P., and Drebin, J.A. (2024). Patient-derived Biliary Tract Cancer Organoid Biobank: Is Personalized Medicine Utilizing Organoids Feasible for Patients with Cholangiocarcinoma? (American Society of Clinical Oncology).
229. Lee, S.H., Hu, W., Matulay, J.T., Silva, M.V., Owczarek, T.B., Kim, K., Chua, C.W., Barlow, L.J., Kandoth, C., Williams, A.B., et al. (2018). Tumor evolution and drug response in patient-derived organoid models of bladder cancer. Cell *173*, 515–528.e17.
230. Xie, X., Li, X., and Song, W. (2023). Tumor organoid biobank-new platform for medical research. Sci. Rep. *13*, 1819. https://doi.org/10.1038/s41598-023-29065-2.
231. Schneemann, S.A., Boers, S.N., van Delden, J.J.M., Nieuwenhuis, E.E.S., Fuchs, S.A., and Bredenoord, A.L. (2020). Ethical challenges for pediatric liver organoid transplantation. Sci. Transl. Med. *12*, eaau8471.
232. Siegel, R.L., Miller, K.D., Fuchs, H.E., and Jemal, A. (2022). Cancer statistics, 2022. CA. Cancer J. Clin. *72*, 7–33. https://doi.org/10.3322/caac.21708.
233. Mullard, A. (2020). Addressing cancer's grand challenges. Nat. Rev. Drug Discov. *19*, 825–826. https://doi.org/10.1038/d41573-020-00202-0.
234. de Visser, K.E., and Joyce, J.A. (2023). The evolving tumor microenvironment: From cancer initiation to metastatic outgrowth. Cancer Cell *41*, 374–403. https://doi.org/10.1016/j.ccell.2023.02.016.
235. Yang, S., Hu, H., Kung, H., Zou, R., Dai, Y., Hu, Y., Wang, T., Lv, T., Yu, J., and Li, F. (2023). Organoids: The current status and biomedical applications. MedComm (2020) *4*, e274. https://doi.org/10.1002/mco2.274.

236. Feng, W., Schriever, H., Jiang, S., Bais, A., Wu, H., Kostka, D., and Li, G. (2022). Computational profiling of hiPSC-derived heart organoids reveals chamber defects associated with NKX2-5 deficiency. Commun. Biol. *5*, 399. https://doi.org/10.1038/s42003-022-03346-4.

237. Yao, Y., Xu, X., Yang, L., Zhu, J., Wan, J., Shen, L., Xia, F., Fu, G., Deng, Y., Pan, M., et al. (2020). Patient-Derived Organoids Predict Chemoradiation Responses of Locally Advanced Rectal Cancer. Cell Stem Cell *26*, 17–26.e6. https://doi.org/10.1016/j.stem.2019.10.010.

238. Vandana, J.J., Manrique, C., Lacko, L.A., and Chen, S. (2023). Human pluripotent-stem-cell-derived organoids for drug discovery and evaluation. Cell Stem Cell *30*, 571–591. https://doi.org/10.1016/j.stem.2023.04.011.

239. Xue, Y., Seiler, M.J., Tang, W.C., Wang, J.Y., Delgado, J., McLelland, B.T., Nistor, G., Keirstead, H.S., and Browne, A.W. (2021). Retinal organoids on-a-chip: a micro-millifluidic bioreactor for long-term organoid maintenance. Lab Chip *21*, 3361–3377. https://doi.org/10.1039/d1lc00011j.

240. Yi, S.A., Zhang, Y., Rathnam, C., Pongkulapa, T., and Lee, K.B. (2021). Bioengineering Approaches for the Advanced Organoid Research. Adv. Mater. *33*, e2007949. https://doi.org/10.1002/adma.202007949.