Cancer Treatment Reviews 143 (2026) 103072

Contents lists available at ScienceDirect

Cancer Treatment Reviews

journal homepage: www.elsevier.com/locate/ctrv

Ex vivo drug screening on patient-derived tumor material to advance functional precision in oncology: an overview on current approaches and unresolved challenges

Zoltán Spiró [a,b,*], Amin El-Heliebi [c], Maximilian J. Mair [a], Thomas R. Pieber [b], Barbara Prietl [b], Sabine Spiegl-Kreinecker [d,e], Stefanie Stanzer [b], Adelheid Wöhrer [f], Matthias Preusser [a], Anna Sophie Berghoff [a]

[a] *Division of Oncology, Department of Medicine I, Medical University of Vienna, Vienna, Austria*
[b] *Precision Medicine Technologies, CBmed GmbH, Graz, Austria*
[c] *Gottfried Schatz Research Center, Division of Cell Biology, Histology and Embryology, Medical University of Graz, Graz, Austria*
[d] *Department of Neurosurgery, Johannes Kepler University, Linz, Kepler University Hospital Linz, Linz, Austria*
[e] *Clinical Research Institute for Neurosciences, Johannes Kepler University and Kepler University Hospital Linz, Linz, Austria*
[f] *Department of Pathology, Neuropathology and Molecular Pathology, Medical University of Innsbruck, Innsbruck, Austria*

ARTICLE INFO

Keywords:
Functional precision oncology
Patient-derived cells
Patient-derived organoids
Drug screening

ABSTRACT

The advent of mutation-informed targeted therapies has transformed medical oncology, delivering durable responses in several cancer types. However, this success has not been universal. Tumors such as glioblastoma have largely remained unresponsive to genomics-guided personalized treatments, and in many cancers, the correlation between genetic alterations and therapeutic response remains inconsistent.

To address these limitations, functional precision oncology – based on *ex vivo* drug screening using patient-derived tumor cells – has emerged as a compelling complementary approach. By directly testing drug responses in tumor cells, this strategy seeks to bypass the shortcomings of purely genomic prediction models, particularly in malignancies that have proven refractory to current targeted approaches.

This review outlines the state-of-the-art methodologies for patient-derived cell-based drug screening, examining their application across various tumor types and highlighting the current challenges and opportunities in implementing functional precision medicine in clinical oncology.

Introduction

Over the past two decades, oncology has undergone a profound transformation, largely driven by the rise of targeted therapies and immune-modulating agents. By tailoring treatments to the molecular profile of each patient's tumor, outcomes have significantly improved in several cancer types, including breast cancer, non-small cell lung cancer (NSCLC), melanoma, colorectal cancer, and various hematologic malignancies [1–5]. These advances, enabled by next-generation sequencing (NGS) and the identification of actionable genetic alterations, have reshaped therapeutic strategies across oncology.

However, the benefits of this genomic revolution have not been evenly distributed. Some solid tumors, such as pancreatic ductal adenocarcinoma, ovarian cancer, and glioblastoma, a highly aggressive primary brain cancer, have seen little progress despite extensive biological profiling [6–14]. In such cases, the predictive power of genomics alone has proven insufficient to guide effective therapy.

To address these limitations, there is growing interest in functional approaches that assess drug efficacy directly on cells originating from the patient. Functional precision oncology (FPO) bridges this gap by using *ex vivo* drug testing on patient-derived cells (PDCs) to identify individualized therapeutic vulnerabilities in real time. This review explores the current landscape of patient-derived cell models for functional drug screening, highlighting available technologies, clinical applications, and the potential impact on treatment decision-making.

* Corresponding author at: Division of Oncology, Department of Medicine I, Medical University of Vienna, Vienna, Austria.
E-mail address: zoltan.spiro@meduniwien.ac.at (Z. Spiró).

https://doi.org/10.1016/j.ctrv.2025.103072
Received 17 November 2025; Received in revised form 17 December 2025; Accepted 18 December 2025
Available online 22 December 2025

Patient-derived models and methodologies

Forty-eight recent studies, summarized in Table 1, present a compelling case for the use of *ex vivo* patient-derived tumor models to guide therapy. At the heart of FPO is the idea that tumor cells isolated from patient material can be used to identify effective treatments – bridging the gap between genomic predictions and the individual drug response of the patient. Fig. 1 displays an overview of the various methods being discussed, while the different aspects of these models are summarized in Table 2.

Patient-Derived 2D cancer cell models for personalized treatment

Recent studies have highlighted the potential of patient-derived two-dimensional (2D) cell cultures as predictive models for personalized cancer therapy [15–22]. While 2D cultures are valuable for initial drug screening and preserving patient-specific tumor traits, they come with significant limitations. These models lack the complex three-dimensional architecture, cell–cell interactions, and tumor microenvironment components (such as stromal and immune cells) found in actual tumors. As a result, they often fail to replicate key aspects of *in vivo* drug responses, including resistance mechanisms that arise from spatial organization and cellular heterogeneity. Additionally, prolonged culture can lead to genetic drift and loss of cellular heterogeneity, potentially reducing the predictive power over time. For these reasons, 2D cultures are increasingly used in combination with more advanced models, such as organoids or patient-derived xenografts, to create more robust preclinical pipelines [23].

Collectively, these studies underscore that while patient-derived 2D cultures provide a fast, cost-effective, and scalable platform for personalized oncology, their limitations must be acknowledged and addressed through integrative modeling approaches.

Spheroids and tumor cell clusters: simplicity and speed

Tumor spheroids and patient-derived multicellular clusters present a fast and accessible alternative to matrix-embedded organoids, offering a streamlined approach for modeling cancer biology and drug response. These models are typically generated under low-attachment conditions without extracellular matrix scaffolds, enabling rapid 3D cell aggregation; an advantage for integrating FPO into time-sensitive clinical settings such as glioblastoma, breast cancer, colorectal cancer, gastric cancer and various pediatric cancers [24–31].

Across multiple studies, a consistent theme emerges: spheroid cultures balance operational simplicity with the capacity to preserve key tumor properties. Methodological advances including serum-free culture conditions and automation have enhanced reproducibility and throughput while retaining stem-like features critical for modeling tumor behavior. Moreover, several investigations highlight how integrating omics technologies and spatial transcriptomics into spheroid systems can address intratumoral heterogeneity and clonal evolution under treatment stress, partially compensating for the lack of architectural complexity typical of these models [25,27,30].

Recent innovations have further improved translational potential. High-throughput screening platforms based on spheroids now incorporate automated liquid handling and real-time imaging, allowing dynamic, multiparametric assessment of drug efficacy rather than reliance on endpoint viability alone. These technical refinements expand the scope of pharmacologic profiling and support identification of patient-specific vulnerabilities, including actionable targets like JAK3, HER2, and FGFR3 in metastatic brain tumors [26,27].

Several authors emphasize the need for careful benchmarking of spheroid models. While their speed is invaluable for clinical decision-making, especially in metastatic or rapidly progressing cancers, fidelity to original tumor histopathology and genomics must be continuously evaluated to ensure translational relevance [29]. Ongoing clinical efforts, such as the randomized, prospective phase 2 ATTRACT (Advanced brain Tumor TheRApy Clinical Trial) study, evaluating glioblastoma spheroids for screening blood–brain barrier-penetrating agents, reflect the growing confidence in these models as pragmatic tools for individualized therapy selection – even as they remain structurally simpler than full organoids [24].

Nevertheless, spheroids present significant limitations. As also discussed in previous reviews [32,33], these models lack stromal and immune cells, do not form organized tissue structures and often show variability in morphology and size, leading to challenges in assay reproducibility and data interpretation. Drug diffusion into larger spheroids may be uneven, which can skew sensitivity profiles and misrepresent therapeutic efficacy. Moreover, the reliance on bulk metabolic viability assays (such as those based on ATP levels) further obscures cell type–specific responses and can mask the presence of resistant subclones.

Still, for rapid turnaround and high-throughput screening spheroids remain a highly useful and increasingly refined option. When paired with appropriate methodological controls and complementary profiling tools (eg. NGS), they can deliver clinically relevant insights within actionable timeframes.

Organoids: fidelity and scalability

Organoid models have emerged as a foundational tool in FPO due to their ability to faithfully replicate the molecular, histological, and genetic characteristics of primary tumors. Derived from patient tissue and cultured in three-dimensional extracellular matrix environments, organoids consistently demonstrate high genomic fidelity, preserving key mutations and maintaining subtype identity, tumor heterogeneity, and microenvironmental features. These qualities enable them to serve as reliable surrogates for patient tumors in drug response testing and therapeutic development in various tumor entities [23,25,34–46].

Multiple studies across various cancer types, including gastric, glioblastoma, colorectal, lung, ovarian, and biliary tract cancers, consistently show that organoids retain hallmark oncogenic mutations (e.g., *TP53*, *EGFR*, *PIK3CA*), epigenetic signatures (e.g., *MGMT* promoter methylation), and structural features of their parental tumor. Transcriptomic and genomic analyses have confirmed that these models closely mirror the primary tumors not only at the genetic level but also in preserving histological and spatial organization, even after long-term culture or cryopreservation [34,36–39,41].

A significant strength of organoid systems is their relatively high success rate of establishment across tumor types. Our pooled analysis across gastric and colorectal cancer as well as glioblastoma suggests a consistent 60–90 % success benchmark, depending on tumor entity, sample quality and culture conditions. However, this performance is not uniform across all cancers. In particular, organoid generation remains challenging in pediatric tumors, where reported establishment rates are substantially lower and the above success benchmarks do not apply. These limitations likely reflect differences in cell of origin and developmental context, as organoid systems rely on stem or progenitor cell architectures that are often absent or unstable in childhood malignancies. In addition, the prolonged culture times required for organoid expansion constrain their clinical utility in rapidly progressive diseases, especially in the relapsed pediatric setting, where patients may clinically deteriorate before functional results become available (reviewed in [47]).

Several platforms have expanded upon the traditional organoid format to enhance scalability and applicability. For example, microorganospheres and integrated dual-platform systems combining 2D and 3D models have enabled high-throughput or rapid drug screening, preserving functional heterogeneity and stromal components essential for modeling treatment response [23,40,48]. More specifically, Lenin et al. [23] applied a pipeline, where patient-derived glioma stem–like cells were allocated to parallel 2D and 3D workflows. High-throughput

Table 1
Characteristics of included studies reporting FPO drug testing in patient-derived models. A structured search of the PubMed database was performed to identify relevant studies published up to 31 August 2025. The studies were included if they reported patient-sample–based functional drug testing with sufficient methodological information to extract at least tumor type, model system, drug panel composition, screening mode, and readout. The table summarizes, for each study, the tumor entity, number of patient samples screened, patient-derived model type, drug panel characteristics, screening strategy, primary response endpoint, and study context. To facilitate comparison across disease contexts, studies are grouped by tumor type.

Tumor Type	First Author	Year	Reference	# of patient samples used for drug screen	patient-derived model type	Drug Panel: Chemo, Targeted or both (#)	Drug Screening Mode (monotherapy or combination)	Viability Readout Type	Study Type
Blood	Schmid	2024	63	24	2D culture	Targeted (6)	Monotherapy	imaging: pharmacoscopy	Prospective Pilot Study (n = 17 treatments)
	Heinemann	2022	60	66	2D culture	Targeted (136)	Monotherapy	imaging: pharmacoscopy	Retrospective
	Kazianka	2025	61	14	2D culture	Both (112)	Monotherapy	imaging: pharmacoscopy	Phase 2 Three-Arm (n = 7 treatments)
	Kornauth	2022	57	143	2D culture	Targeted (139)	Monotherapy	imaging: pharmacoscopy	Phase 2 Single Arm (n = 56)
	Malani	2022	18	186	2D culture	Both (515)	Monotherapy	metabolic	Phase 2 Single Arm (n = 37)
	Snijder	2017	58	68	2D culture	both (139)	Combination	imaging: pharmacoscopy	Retrospective + Phase 2 Single Arm (n = 17)
	Spinner	2020	20	54	2D culture	both (74)	Both	metabolic	Feasibility Pilot Study + Prospective Observational
Colorectal	Ding	2022	40	8	organospheres	Both (119)	Monotherapy	metabolic	Prospective Observational
	He	2023	41	42	organoids	Chemo (3)	Combination	metabolic	Prospective Observational
	Mertens	2023	42	36	organoids	Both (414)	Monotherapy	imaging: circularity and size	Retrospective
	Narasimhan	2020	43	19	organoids	Both (49)	Monotherapy	metabolic	Prospective Pilot Study (n = 2 treatments)
	Ooft	2021	44	25	organoids	targeted (8)	Monotherapy	growth rate inhibition	Phase 2 Single Arm (n = 6)
	Tan	2023	46	104	organoids	Both (9)	Combination	imaging: size	Feasibility Pilot Study + Prospective Observational
Gastric	Zhao	2024	38	41	organoids	Chemo (6 drugs)	Monotherapy	metabolic	Prospective Observational
Gastric, Colorectal, Breast	Yin	2020	28	28	spheroids	Both (22)	Monotherapy	metabolic	Prospective Pilot Study (n = 1 treatment)
Pancreatic, Colorectal	Almstedt	2022	52	11	PDX − zebrafish	targeted (1)	Monotherapy	imaging: tumor size	Preclinical
Biliary tract	Ren	2023	36	61	organoids	Chemo (7)	Monotherapy	metabolic	Prospective Observational
Glioblastoma	Berghoff	2025	24	120	spheroids	Both (28)	Monotherapy	metabolic	Phase 2 Two-Arm (n = 120 − in progress)
	Chadwick	2020	25	15	spheroids and organoids	Both (3)	Monotherapy	metabolic	Retrospective
	Charbonneau	2023	53	14	PDX − chicken embryo CAM	chemo (2)	Monotherapy	imaging: tumor size	Preclinical
	Gagg	2024	26	18	spheroids	Both (35)	Monotherapy	imaging: immunostaining	Feasibility Pilot Study
	Jacob	2020	34	58	organoids	Both (4)	Monotherapy	imaging: size	Feasibility Pilot Study
	Lee	2024	62	27	2D + 3D cultures	Both (132)	Monotherapy	imaging: pharmacoscopy	Retrospective + Prospective Observational
Glioblastoma	Lenin	2021	23	2	2D culture and organoids	Targeted (65)	Monotherapy	metabolic	Preclinical
	Ntafoulis	2023	16	66	2D culture	Chemo (1)	Monotherapy	metabolic	Retrospective
	Ntafoulis	2024	65	Glioblastoma	3	2D culture	Chemo (107)	Monotherapy	metabolic
	Ranjan	2023	19	78	2D culture	Chemo (9)	Combination	metabolic	Phase 2 Two-Arm (n = 43)
	Rattliff	2022	45	4	organoids	Both (41)	Monotherapy	imaging: microtubule + nuclei	Pilot Study

(*continued on next page*)

Table 1 (*continued*)

Tumor Type	First Author	Year	Reference	# of patient samples used for drug screen	patient-derived model type	Drug Panel: Chemo, Targeted or both (#)	Drug Screening Mode (monotherapy or combination)	Viability Readout Type	Study Type
	Verduin	2023	37	10	organoids	both (2)	Monotherapy	metabolic	Prospective Observational
	Yi	2019	50	7	chip-based	Both (4)	Monotherapy	metabolic	Retrospective
Brain metastases	Jeising	2024	27	26	spheroids	Both (267)	Monotherapy	metabolic	Preclinical
	Zhu	2022	51	19	organotypic culture	Both (114)	Monotherapy	Image-based: cell proliferation	Feasibility Pilot Study
High grade gliomas	Posthoorn-Verheul	2025	22	16	2D culture	Both (21)	Monotherapy	metabolic	Pilot Study
IDH1-mutant glioma	Verheul	2021	17	12	2D culture	Both (107)	Monotherapy	metabolic	Retrospective
Lung	Kim	2021	35	5	organoids	Targeted (9)	Monotherapy	metabolic	Retrospective
	Kim	2024	15	139	2D culture	Chemo (64)	Monotherapy	metabolic	Prospective Pilot Study (n = 4 treatments)
Melanoma	Flørenes	2019	55	38	spheroids and PDX in mice	targeted (1)	Monotherapy	metabolic	Prospective Observational
Squamos Cell Carcinoma	Nykänen	2021	66	1	2D culture	both (193)	Monotherapy	imaging: immunostaining	Prospective Pilot Study (n = 1 treatments)
Ovarian	de Witte	2020	39	23	organoids	Both (15)	Combination	metabolic	Feasibility Pilot Study
Various (adult)	Lee	2018	29	462	spheroids	Targeted (60)	Monotherapy	metabolic	Retrospective
Various (adult)	Pauli	2017	48	4	organoids	Both (160)	Combination	metabolic	Retrospective
Various (adult)	Wegmann	2024	64	105	2D culture	Both (101)	Monotherapy	imaging: pharmacoscopy	Prospective Observational
Medulloblastoma	Zhou	2024	31	1	spheroids	Both (172)	Monotherapy	metabolic	Feasibility Pilot Study
pediatric AML	Haladik	2025	59	45	2D culture	both (115)	Both	imaging: pharmacoscopy	Retrospective
Various (Pediatric)	Gatzweiler	2022	54	3	spheroids and PDX in zebrafish	Both (76)	Monotherapy	metabolic (spheroids) and imaging: tumor volume (zebrafish)	Preclinical
Various (Pediatric)	Lau	2022	56	17	spheroids and PDX in mice	Both (111)	Both	metabolic (spheroids) and imaging: tumor size (mice)	Prospective Observational
Various (Pediatric)	Peterziel	2022	30	65	spheroids	Both (78)	Monotherapy	metabolic	Prospective Observational
Various (Pediatric)	Acanda de la Rocha	2024	21	21	2D culture	Both (125)	Monotherapy	metabolic	Prospective Pilot Study (n = 6 treatments)

drug sensitivity testing in 2D cultures enabled rapid prioritization of clinically relevant compounds based on dose–response viability profiles. In parallel, matched three-dimensional glioblastoma organoids were generated and exposed to temozolomide-based chemoradiotherapy, followed by a recovery phase to model therapy-resistant disease. Compounds identified as active in the 2D screen were subsequently tested in these treatment-conditioned organoids, allowing assessment of drug efficacy in a three-dimensional, resistance-enriched context that better reflects *in vivo* tumor biology. In a related study [48], patient tumor samples across various entities were evaluated using multiple complementary functional platforms in parallel, including 2D cell cultures, 3D organoid systems, and *in vivo* models, to assess drug sensitivity across different biological contexts. Therapeutic candidates showing concordant responses across 2D and 3D models were prioritized, with cross-model agreement used to increase confidence in clinical relevance and guide individualized treatment selection.

Organoid-based drug screening has not only validated known treatment responses but also uncovered new vulnerabilities and combinatorial strategies, such as the synergistic inhibition of MAPK and microtubule pathways in colorectal cancer [42]. These insights highlight the role of organoids in drug repurposing and optimization of combination therapies. Biobanking initiatives further support large-scale screening efforts while preserving subtype-specific behaviors and inter-patient variability, facilitating broader implementation in personalized oncology [41,43].

Perhaps most compelling is the growing body of evidence linking *in vitro* organoid drug response to real-world clinical outcomes. In colorectal cancer, organoid drug sensitivity has shown predictive accuracy rates of up to ~ 80 % for standard chemotherapies, including fluoropyrimidine, oxaliplatin, and irinotecan, and has helped identify patients with limited benefit from these regimens [40,43,44,46]. Similarly, glioblastoma and gastric cancer organoids have been used to predict patient response to platinum-based agents and targeted therapies, offering valuable insights even in recurrent or treatment-resistant settings [34,38,45]. Across studies, organoids have consistently distinguished responders from non-responders with high specificity in retrospective analyses; however, validation in a prospective randomized trial is still lacking.

Importantly, the versatility of organoid models has been demonstrated across multiple solid tumor types. Studies show their robustness in modeling therapy resistance, tumor evolution, and treatment efficacy in diverse indications, including difficult-to-model cancers like biliary tract tumors [36]. The collective evidence underscores the translational potential of organoids to bridge the gap between genomic profiling and

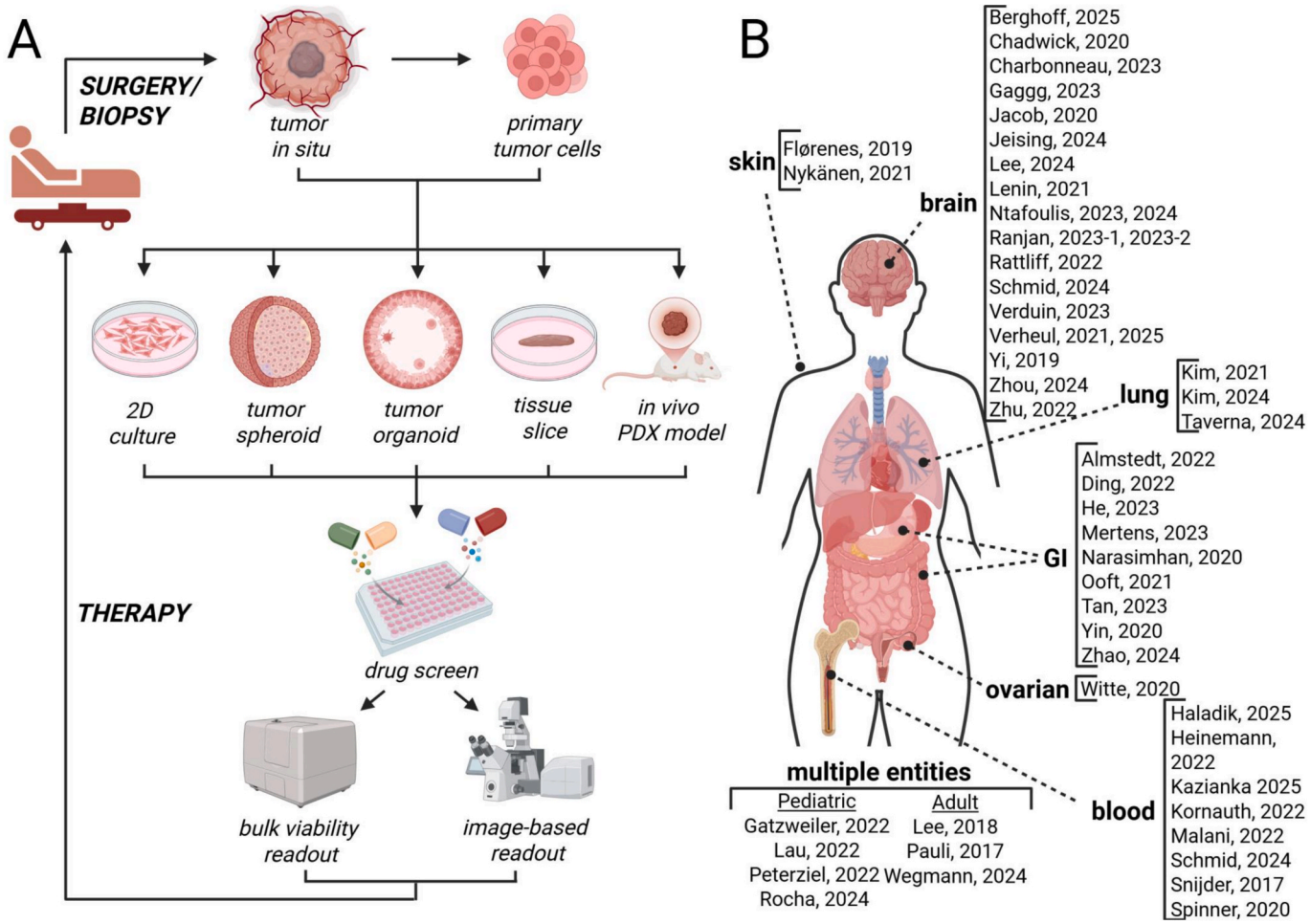

Fig. 1. (A) Schematic workflow of drug screening using patient-derived models. (B) Summary of cited research papers categorized by tumor indication. Figure 1 was created using BioRender.

functional therapeutic prediction, marking a significant advancement in personalized cancer care [36,39,48].

The timeline for establishing patient-derived organoids (PDOs) remains a significant barrier to their adoption as a basis for clinical decision-making in oncology, particularly in cancers with aggressive biological behavior, like high-grade gliomas or metastatic colorectal cancer. While drug sensitivity testing itself can be completed relatively quickly – often within 1–2 weeks once organoids are established – the upfront culture and expansion process is typically far slower. For instance, in glioblastoma models, organoids required up to two months and three passages before drug testing could be initiated [37], a timeframe incompatible with the clinical management of these tumors. Similarly, while some platforms like micro-organospheres enable drug response assessment within 14 days of biopsy [40], they are structurally and technically distinct from traditional PDO workflows. Rapid dual-platform pipelines that combine 2D and 3D models have shown promise for glioblastoma, delivering drug sensitivity results within 13–21 days of tissue acquisition [23,45]. For colorectal and gastric cancers, more mature organoid pipelines allow for complete drug screening within 4–7 weeks, timelines that – though slower – may still be compatible with certain clinical treatment windows [38,41,46].

Furthermore, standard organoid protocols typically lack immune and stromal components, which are critical for predicting responses to immunotherapy or microenvironment-modulated treatments. The reliance on commercially available extracellular matrix products also increases cost and batch variability, limiting adaptation to high-throughput screening platforms. Emerging protocols aim to incorporate immune and stromal co-cultures as well as synthetic extracellular matrices to improve microenvironmental fidelity and throughput [49].

In summary, while organoid models offer a high-fidelity and scalable platform for drug screening, considerations regarding culture success rates, time requirements, and the incorporation of microenvironmental factors are essential for their clinical integration. Ongoing innovations in organoid technology and analytics continue to address these barriers, expanding the reach and impact of FPO.

Advanced 3D models and in vivo systems

To better replicate the complexity of human tumors and their microenvironments, recent research has emphasized the development of advanced 3D models and *in vivo* systems that enable physiologically relevant assessment of drug responses. Across multiple studies, common themes include the integration of patient-derived material, biomimetic scaffolds, and real-time imaging to improve the fidelity of preclinical modeling.

A variety of 3D culture systems have been designed to mimic key tumor characteristics, such as hypoxia, vascularization, and invasive behavior. These include organotypic brain slice cultures, glioblastoma-on-a-chip technologies, and stem-like cell-derived tumor models, all of which have shown promise in capturing tumor heterogeneity and predicting treatment outcomes more reliably than traditional 2D systems. These models consistently support longitudinal tracking of tumor behavior and therapy response, offering a scalable approach for

Table 2
This table summarizes commonly used FPO model systems, highlighting qualitative differences in complexity, turnaround time, robustness, resemblance to the primary tumor, and clinical applicability. Model choice depends on tumor type and clinical context: 3D systems are predominantly used in solid and brain tumors to preserve tissue architecture, whereas 2D primary cell cultures are widely applied and clinically validated in hematologic malignancies due to rapid turnaround and high assay success rates. Immune components (e.g., autologous immune cells or cytokine supplementation) can be incorporated into several of the listed platforms but are not shown as a separate model class.

Model Type	Model Source	Advantages	Disadvantages	Turnaround Time	Robustness	Resemblance to Primary Tumor	Applicability in Clinical Decision Making
2D primary cell cultures (eg. hematologic malignancies)	Cell suspension	Rapid setup; high assay success rate; compatible with large drug libraries; clinically validated in leukemias	Limited architectural context; reduced modeling of niche interactions unless stromal co-culture is used	5–10 days	High; reproducible functional responses across samples	Moderate; captures functional drug sensitivity but limited structural fidelity	High; used in prospective leukemia trials and functional MTBs – demonstrated clinical benefit (eg. Kornauth and Ranjan et al)
Spheroids & tumor cell clusters (solid/brain tumors)	Cell suspension	Rapid setup; high throughput; automation-compatible; retains stem-like features; suitable for time-sensitive settings	Limited architectural complexity; may lack full histological fidelity; requires benchmarking	3–4 weeks	Moderate; improving with serum-free protocols and automation	Moderate; retains key tumor biology with reduced structure	Emerging; suitable for aggressive cancers with limited validation
Organoids (solid tumors)	Cell suspension	High fidelity to patient tumors; preserves histology and heterogeneity; predictive accuracy	Longer establishment time; scalability challenges; sample-quality dependent; fails to work in childhood cancers	8–10 weeks	High; reproducible across tumor types	High; preserves histology, genetics, and transcriptomic profiles	High; used in real-world precision oncology settings; Low in childhood cancers
Advanced 3D & *in vivo*-like systems	Tissue of origin (eg. organotypic slice cultures)	Captures microenvironmental complexity; models resistance mechanisms	Labor-intensive; low throughput; infrastructure intensive	Variable	High for specific biological questions	High; includes architecture and microenvironment	Moderate; primarily research-oriented

personalized drug testing, particularly in challenging contexts such as pediatric or low-mutation tumors [25,31,50,51].

Likewise, bioprinting and brain-mimetic matrices have been harnessed to produce platforms that recapitulate crucial pathological features of aggressive cancers like glioblastoma, enabling individualized testing of chemoradiotherapy and combination regimens. The convergence of spatial microenvironmental cues – such as oxygen gradients and extracellular matrix composition – within these systems has been shown to recreate resistance niches and predict patient-specific therapeutic responses, further bridging the gap between *in vitro* testing and clinical application [25,50].

In vivo models remain essential for assessing tumor dynamics in an organismal context. Scalable and cost-effective platforms such as the chicken embryo chorioallantoic membrane (CAM) model and zebrafish xenografts have gained traction due to their ability to support rapid engraftment of patient-derived cells, vascularization, and functional drug testing. These systems provide real-time insights into tumor proliferation and therapeutic efficacy, and they are sensitive enough to detect inter-patient variability in treatment response, positioning them as valuable components of preclinical precision oncology pipelines [52–56].

Together, these studies illustrate a shared emphasis on capturing clinically relevant tumor behavior and enhancing the predictive power of preclinical testing. By leveraging biomimetic engineering, patient-derived materials, and high-throughput *in vivo* platforms, these models contribute significantly to the refinement of individualized cancer treatment strategies [25,31,50–54].

Immune-Inclusive models and single-cell readouts

Recent advances in functional precision medicine have increasingly integrated immune-inclusive co-culture systems and single-cell imaging readouts, offering refined insights into tumor behavior and drug responsiveness. Several studies converge on the utility of pharmacoscopy, a high-content imaging method that enables *ex vivo* drug testing on short-term cultures consisting of tumor and other (eg. immune) cells [57–64]. By distinguishing malignant from non-malignant populations and quantifying cell death at the single-cell level, this approach provides a granular view of drug effects, including on immune modulation.

Across hematologic and solid malignancies, pharmacoscopy has been shown to support clinically actionable decisions. These studies illustrate how functional responses align with patient-specific phenotypes and immune profiles, identifying resistance mechanisms and guiding individualized treatments that correlate with improved outcomes, including prolonged event-free survival. The method's ability to stratify therapy based on tumor-intrinsic and microenvironmental features underscores its clinical relevance [57–59,62,63].

Further extending the reach of immune-inclusive functional profiling, recent work has demonstrated the feasibility of applying similar pipelines to malignant serous effusions. Here, high-throughput molecular and phenotypic assays have been combined to preserve transcriptomic fidelity and identify patient-specific vulnerabilities, even in fluid-derived samples. These studies highlight the value of integrating functional response with omics data to uncover mechanisms of resistance and potential new targets [64].

Despite its promise, the implementation of pharmacoscopy remains resource-intensive. Challenges include dependency on fresh, viable samples, complex imaging infrastructure, and computational pipelines, with typical turnaround times exceeding 10 days. Nevertheless, the collective evidence supports its adaptability across cancer types and sample sources, as well as its potential to bridge molecular features with phenotypic drug sensitivity in a clinically meaningful timeframe [57–59,61–64].

Tumor entities and clinical translation

The versatility of FPO is reflected in its application across a diverse array of tumor entities, each posing unique biological and clinical challenges. While glioblastoma remains the focal point of many proof-of-concept studies due to its resistance to genomics-driven therapy,

FPMO has also made significant inroads in colorectal, gastric, biliary, lung, and pediatric cancers. Fig. 2 shows the number of patients that were treated based on PDC-based drug screening across tumor entities.

Haematological malignancies: precision through Function

Recent advances in FPO have demonstrated substantial clinical benefit across hematologic malignancies, particularly for patients who have exhausted standard treatment options. Because the need to replicate the complex 3D architecture of the tumor microenvironment is less stringent than in solid tumors, hematology has provided a fertile ground where many innovative approaches and novel therapeutic modalities were pioneered before later being translated into solid tumors. Central to these approaches, *ex vivo* drug sensitivity testing, which enables direct assessment of how patient-derived tumor cells respond to therapeutic compounds. These platforms, ranging from high-throughput flow cytometry to image-based single-cell analysis, consistently delivered clinically actionable results within a short turnaround of 5 to 15 days, allowing timely integration into treatment planning [18,20,57,60,63].

Across multiple studies involving relapsed or refractory hematologic malignancies, functional profiling guided therapy in approximately 39–57 % of enrolled patients and led to improved outcomes in a majority of those treated. Around 54 % of patients who received matched therapy experienced a progression-free survival (PFS) benefit of at least 1.3-fold compared with their prior regimen, and 40 % of responders showed exceptional responses (PFS $>$ 3 $\times$ baseline) [57,58]. Importantly, the ongoing EXALT-2 trial is a randomized study benchmarking pharmacoscopy-based single-cell functional precision medicine and comprehensive genomic profiling (CGP) against physician's choice in relapsed/refractory hematologic malignancies [61]. Early feasibility data demonstrate that both approaches reliably generate actionable treatment options with scFPM offering particularly rapid turnaround, and the field is eagerly awaiting the final results to inform future clinical implementation.

Crucially, *ex vivo* assay results proved highly predictive of clinical response. For instance, one platform achieved a positive predictive value of 0.92 and an overall accuracy of 0.85 in predicting treatment responses in myeloid neoplasms [20]. Therapies selected using high-efficacy *ex vivo* scores were associated with significantly higher remission rates and longer survival than physician-selected alternatives [18,63].

Altogether, these findings underscore the value of integrating functional precision medicine into routine oncological care, particularly for patients facing limited options. By capturing tumor-specific drug vulnerabilities irrespective of genotype, these approaches offer a powerful, individualized path forward in managing refractory malignancies.

Glioblastoma: A model of clinical Urgency

Glioblastoma stands as one of the most aggressive and therapeutically resistant solid tumors. Its hallmark features – profound genomic heterogeneity, diffuse infiltration, and remarkable biological plasticity – undermine the efficacy of most single-target therapies. This clinical intractability has made glioblastoma a proving ground for FPO, which emphasizes phenotype-driven treatment selection based on live-cell drug responses.

To address the limitations of conventional molecular stratification, several platforms have emerged that model glioblastoma *ex vivo* while preserving key microenvironmental and architectural features. A notable example is the development of a four-dimensional (4D) bioprinted array system that enables the culture of patient-derived glioblastoma spheroids. By maintaining tissue architecture and enabling multiplexed readouts including histology, proliferation, and viability, this platform facilitates more nuanced assessments of drug response than bulk assays alone [25].

Expanding on tissue fidelity, the GliExP platform leveraged freshly resected glioblastoma tissue from 18 patients to conduct high-throughput drug screening across 35 compounds [26]. By incorporating multi-region sampling, GliExP preserved intratumoral heterogeneity and glioma stem cell (GSC) characteristics, while uniquely enabling functional comparisons between primary and recurrent tumor regions demonstrating an essential capability given the frequent clinical challenge of treating recurrent glioblastoma.

Large-scale efforts to correlate *ex vivo* drug sensitivity with patient outcomes are also underway. In a study of 66 patients with newly diagnosed glioblastoma, temozolomide sensitivity measured via a short-term 2D culture system correlated significantly with both progression-free and overall survival [16]. While retrospective, the scale and clinical outcome associations provided one of the strongest validations to date of the prognostic utility of functional assays in glioblastoma.

Crucially, evidence from randomized clinical trials has begun to

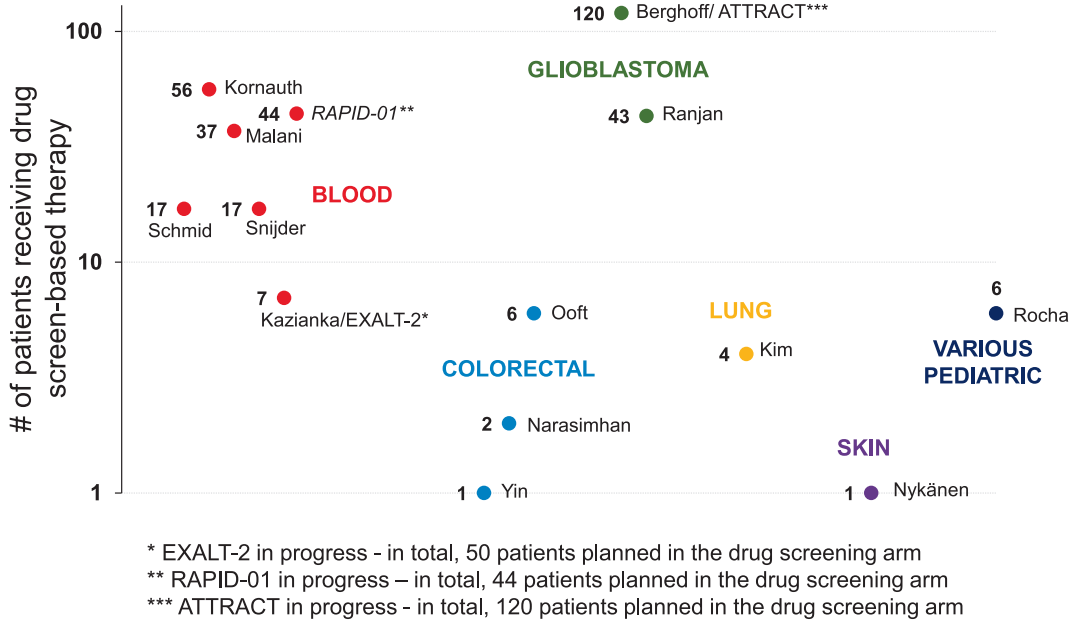

Fig. 2. Number of patients receiving drug-screening–based therapy recommendations in previous and current studies.

emerge. The ChemoID trial, a prospective randomized controlled study involving 78 patients with recurrent glioblastoma, demonstrated that patients receiving treatment guided by cancer stem cell sensitivity assays had significantly improved progression-free and overall survival compared to those treated at the physician's discretion [19]. According to the interim efficacy analysis, the median survival in the ChemoID assay-guided group was 12.5 months (n = 43, 95 % CI, 10.2–14.7), whereas it was 9 months (n = 35, 95 % CI, 4.2–13.8) in the physicians' choice group (p = 0.010). This marks one of the few trials in glioblastoma to translate *ex vivo* drug response into tangible clinical benefit.

FPO also opens doors to drug repurposing. A recent study screened CNS-penetrant compounds using patient-derived tumor spheroids and identified omacetaxine mepesuccinate, a protein synthesis inhibitor, as a potent anti-glioma agent [65]. Its efficacy was confirmed across three orthotopic glioblastoma patient-derived xenograft (PDX) models, with pharmacokinetic studies verifying CNS penetration. This approach not only personalizes therapy but accelerates the repositioning of approved drugs for high-need indications.

Innovative model systems further broaden the translational relevance of FPO. A microfluidic glioblastoma-on-a-chip platform reconstituted patient-specific tumor microenvironments by incorporating vascular endothelial cells and decellularized brain matrix, enabling real-time monitoring of therapy response under conditions that more closely mimic *in vivo* physiology [50]. Similarly, platforms combining 2D and 3D patient-derived *in vitro* models have been used to establish robust pipelines for preclinical assessment of drug efficacy, accounting for the structural and cellular complexity of glioblastoma [23].

Complementing these biological advances are computational frameworks that integrate single-cell phenotyping with pharmacologic profiling. In one of the largest studies to date, researchers screened over 2,500 drug responses in 27 patient-derived glioblastoma samples, identifying neuroactive compounds with selective efficacy against GSC-enriched populations [62]. This strategy stresses the potential of integrating systems biology with FPO to uncover novel vulnerabilities.

Adding to this landscape, Ratliff et al. [45] established a patient-derived glioblastoma organoid platform for functional drug profiling. This system preserved key features of individual patient tumors and demonstrated the feasibility of identifying effective treatments within a clinically relevant timeframe of approximately two weeks. By screening a panel of 41 FDA-approved drugs, the study identified potential treatment options for three out of four patients, highlighting the platform's potential to complement molecular profiling in personalized therapy selection.

The field is moving toward prospective validation at scale. The ATTRACT (Advanced brain Tumor TheRApy Clinical Trial) trial [24] represents the first randomized phase 2 study using targeted therapeutics to test functional drug sensitivity-guided therapy in glioblastoma at diagnosis. Enrolling 240 patients across multiple centers, the trial compares standard-of-care treatment to regimens selected following evaluation of drug response via an ATP-luminescence viability assay applied to PDCs. With overall survival as the primary endpoint and secondary measures including progression free survival and quality of life, ATTRACT may establish the clinical value of FPO in neuro-oncology.

Colorectal Cancer: Organoids meet the Clinic

Colorectal cancer (CRC) has emerged as a fertile ground for applying PDO models in functional precision medicine, largely due to the high culture success rates and standardized chemotherapy regimens available for this disease.

In efforts to correlate *ex vivo* drug response with clinical outcomes, Ding et al. [40] generated a living biobank of CRC organospheres and stratified them based on oxaliplatin sensitivity. Their results showed that organoid-derived oxaliplatin resistance was associated with poorer patient responses to oxaliplatin-based regimens, demonstrating the predictive promise of PDO platforms.

Further support comes from a cohort study involving metastatic CRC patients, where high concordance between organoid response profiles and actual clinical progression was found, particularly when sampling from liver and lung metastases [42]. The study developed a high-content imaging assay to accurately distinguish cytostatic from cytotoxic effects in colorectal cancer PDOs. Using a drug-repurposing screen of 414 compounds, the authors identified microtubule-targeting agents, particularly vinorelbine, as the most effective partners to combine with EGFR/MEK inhibition. The combination converted otherwise cytostatic responses into robust apoptosis across more than 20 PDO models, regardless of RAS/BRAF status or tumor stage, and showed strong tumor-suppressive activity in mouse xenografts. These findings provide a strong preclinical rationale for clinical testing of vinorelbine with MAPK pathway inhibitors in metastatic RAS-mutant colorectal cancer.

Demonstrating feasibility at a clinical timescale, a study streamlined the entire organoid-based screening process – from biopsy to drug response readout – within a 7-week period [46]. This protocol achieved 85 % accuracy in predicting therapeutic benefit across standard CRC regimens, underlining the practicality of using PDOs within real-world therapeutic windows.

The SENSOR trial offered a prospective evaluation of PDOs in guiding treatment decisions [44]. In this study, 31 organoids were successfully generated from 57 biopsies of 61 metastatic CRC patients. Of those, 25 were tested *in vitro*, with 19 showing drug sensitivity. Based on PDO results, six patients received targeted therapies (vistusertib or capivasertib), though none experienced clinical responses. These findings point to the operational feasibility of drug screening-guided treatment using PDOs but also underscore the current translational gap in predicting *in vivo* efficacy.

Expanding the evidence base, CRC PDOs could reliably identify individualized drug sensitivities in end-stage patients, supporting their use in late-line treatment personalization [41].

Skin cancer: Ex vivo sensitivity into clinical benefit

Two studies demonstrate the clinical utility of PDC models for drug screening in skin cancer, using short-term cultures from fresh tumor biopsies to assess personalized drug sensitivities [55,66]. A high-content, image-based single-cell drug profiling platform was applied to a patient with metastatic squamous cell carcinoma, identifying strong sensitivity to HER2-targeted therapies [66]. Clinically, the patient was first treated with trastuzumab emtansine (T-DM1), achieving a partial radiographic response with sustained tumor shrinkage for over 10 months. Upon disease progression, a new biopsy was screened and Afatinib plus lapatinib was selected based on updated drug profiling, which again led to measurable tumor control. This case exemplifies dynamic treatment adaptation through repeated PDC screening. In the other study, the authors developed a 3D spheroid-based *ex vivo* assay on 38 melanoma lymph node metastases and demonstrated a robust correlation between drug response and mutation status [55]. Among 21 $BRAF^{V600E}$-mutant tumors tested, 12 (57 %) responded to vemurafenib in the *ex vivo* assay, closely mirroring clinical response rates reported for BRAF inhibitors in patients. None of the BRAF-wildtype tumors showed sensitivity, while several NRAS-mutated tumors exhibited paradoxical increases in viability, suggesting enhanced signaling rather than inhibition. Additionally, when compared with corresponding PDXs, the *ex vivo* assay retained predictive accuracy. These findings show that functional testing using PDCs offers a rapid, cost-effective, and clinically actionable complement to genomics for guiding therapy decisions in melanoma, including initial treatment and management at recurrence.

Gastric, biliary, and lung cancers: expanding indications

The use of organoids for FPO continues to expand into less tractable solid tumors such as gastric, biliary, and lung cancers, each with distinct

challenges and opportunities for integration into clinical decision-making.

In gastric cancer, PDOs were successfully established from 57 of 73 tumor samples, achieving a 78 % success rate [38]. These organoids retained both histopathological features and genomic integrity of the original tumors. Chemosensitivity screening revealed varied responses across standard agents such as 5-FU and oxaliplatin. Among 12 patients treated with chemotherapy, 91.7 % (11 patients) exhibited clinical outcomes consistent with *ex vivo* PDO results. These drug response patterns were also validated in organoid-derived xenograft models, and gene expression analyses further identified biomarker panels predictive of drug sensitivity and resistance.

In biliary tract cancers (BTC), where standard therapies often lack efficacy and genomic guidance is limited, a living biobank of 61 PDOs was derived from intrahepatic, extrahepatic, and gallbladder cancers [36]. Drug testing across seven standard agents demonstrated wide interpatient variability in response. Clinical correlation in a prospective subgroup of 13 patients revealed that PDO-based predictions matched treatment responses in 12 cases (92.3 %). Importantly, the organoid data were validated in xenograft models, and transcriptomic analysis revealed proliferation and stemness signatures associated with successful culture and drug sensitivity, providing a rare clinically validated framework for BTC.

In lung cancer, two complementary studies underscore the emerging utility of both 3D PDO and 2D PDC systems in predicting targeted therapy responses. A large-scale organoid study involving 84 advanced lung adenocarcinoma cases demonstrated that PDOs preserved key somatic mutations and were able to replicate individual patient responses to tyrosine kinase inhibitors, including complex cases with atypical EGFR and BRAF co-mutations [35]. PDOs (n = 5) were also used to test investigational drugs against ERBB2 and RET alterations, supporting their translational value.

Separately, a PDC-based screening platform using 139 advanced NSCLC samples showed strong correlations between *in vitro* drug responses and clinical outcomes in patients receiving targeted therapies [15]. Notably, in EGFR- or ALK-positive NSCLC, patients whose PDCs were non-responsive *in vitro* had significantly shorter progression-free survival (3.4 vs. 11.8 months) and lower response rates to targeted therapies. The platform also demonstrated predictive utility: four patients with either wild-type EGFR or uncommon EGFR-mutant NSCLC received EGFR inhibitor treatment guided by favorable PDC responses, and two of them achieved remarkable clinical benefit.

Together, these studies illustrate the growing feasibility and clinical relevance of FPO approaches in diverse cancer types, offering faster turnaround, enhanced biological fidelity, and, increasingly, prospective validation in real-world treatment settings.

Pediatric oncology: toward real-world application

Pediatric cancers represent a high-need domain within precision oncology, where functional profiling approaches are beginning to complement traditional molecular diagnostics. Recent efforts illustrate growing feasibility and early clinical utility across a variety of pediatric malignancies.

The INFORM program stands as one of the largest international pediatric precision oncology initiatives, incorporating both molecular and functional diagnostics. In a two-year pilot study, short-term drug sensitivity profiling (DSP) was implemented in fresh tumor tissue cultures from 132 pediatric samples across seven countries [30]. Of these, 89 samples met viability thresholds, and 69 (78 %) passed quality control. Using a panel of 75–78 clinically relevant drugs, the study successfully identified actionable vulnerabilities – including in cases without high-evidence molecular targets. Integration into molecular tumor boards demonstrated that *ex vivo* functional data could be processed and returned within a median of three weeks, enabling real-time clinical decision-making.

In parallel to these efforts, Lau et al. [56] developed a high-throughput functional precision medicine platform tailored specifically for pediatric solid tumors. This platform utilized *ex vivo* drug screening of patient-derived tumor tissue fragments, achieving a high success rate in maintaining tumor architecture and cellular heterogeneity. The study demonstrated robust drug response predictions correlating with clinical outcomes, including in rare and refractory pediatric cancers. By enabling rapid functional profiling within clinically actionable timeframes, this platform represents a meaningful step forward in personalizing therapy selection for pediatric oncology patients.

In a separate prospective feasibility study, patient-derived drug sensitivity testing was combined with genomic profiling in 25 children with relapsed or refractory cancers [21]. Drug testing was completed in 21 patients, with treatment recommendations returned for 76 % of cases. Notably, 6 patients received drug screen-guided therapy, with 5 of them (83 %) showing improved PFS compared to their prior lines of treatment. The median turnaround for functional testing was 10 days, which is substantially faster than genomic profiling, making it a practical option for dynamic clinical scenarios where treatment reassessment is urgent.

Further advancing the methodology, a stem-like cell-derived 3D screening platform was developed specifically for sonic hedgehog (SHH)-subtype medulloblastoma [31]. This model preserved tumor subtype fidelity and stemness markers while enabling high-throughput drug screening. From a library of 172 compounds, the S6K1 inhibitor PF4708671 was identified as a selective vulnerability for SHH-driven medulloblastoma. The agent demonstrated efficacy *in vitro* and in orthotopic mouse models, with minimal effects on normal neural stem cells, supporting its translational relevance. Importantly, the model enabled differentiation between tumor-specific and off-target toxicities, a crucial feature in the pediatric context.

Very recently, pharmacoscopy-based drug sensitivity has been applied in combination with multi-omics molecular profiling on 45 pediatric AML samples on a retrospective manner [59]. Using a library of 115 drugs in monotherapy setting as well as in combination, the authors identified clinically relevant targeted treatment options such as venetoclax (BCL2 inhibitor), and FLT3 inhibitors. Importantly, pharmacoscopy not only validated known vulnerabilities (e.g., venetoclax sensitivity in certain AML subtypes) but also uncovered novel patient-specific sensitivities, demonstrating its potential to guide personalized therapeutic strategies in pediatric AML.

Together, these studies underscore the growing translational readiness of functional profiling in pediatric oncology. They highlight the logistical feasibility, clinical relevance, and subtype-specific refinement possible with organoid and stem-like cell platforms in this traditionally underserved population.

Discussion, challenges and future directions

Although FPO has advanced significantly, the clinical utility in routine care remains a matter of research. Overcoming interconnected challenges in standardization, clinical feasibility, biological fidelity, data interpretation, and clinical validation, all of which require aligned progress in research, infrastructure, and policy need to be addressed in future.

1. Technical Standardization and Assay Design

(i) Variability in Tissue Processing, Culture Conditions, and Assay Formats.

FPO is hindered by significant variability across institutions in tissue processing, culture conditions, drug panels, and assay formats. Even minor methodological differences, such as changes in media composition, matrix type, or cell line passage number, can substantially alter drug response outcomes. Variability in tissue quality, particularly in cases with limited or fragile samples like pediatric brain tumors, further

affects assay viability and fidelity. These challenges underscore the need for standardized protocols, shared criteria for assay success, internal controls, and consistent thresholds for classifying drug responses.

(ii) Drug Library Composition: Clinical Utility versus Discovery Depth.

Drug libraries used in FPO platforms vary widely in scope, composition, and intended application, with important implications for feasibility and translational relevance. Clinically oriented libraries typically consist of approved or late-stage investigational agents tested at clinically achievable concentrations. These panels are designed to maximize interpretability in tumor board settings and facilitate regulatory and ethical approval for treatment recommendations. For example, pediatric precision oncology programs incorporating ex vivo drug sensitivity testing have employed libraries comprising 75 clinically relevant compounds [30].

In contrast, discovery-oriented FPO efforts frequently employ larger libraries ranging from several hundred to several thousand compounds, enabling systematic identification of novel vulnerabilities, genotype–-phenotype associations, and drug combinations [18,57]. Large-scale *ex vivo* profiling initiatives in hematologic malignancies have used panels of over 100 targeted inhibitors across hundreds of patient samples, while in ovarian cancer for instance, high-throughput datasets include several thousand compounds [67]. Although these approaches are powerful for hypothesis generation and drug development, substantial down-selection is required before results can be translated into clinically actionable recommendations. Thus, library size and composition should be viewed as a strategic design choice rather than a technical limitation.

(iii) Endpoint Selection and Readout Strategies

The choice of endpoint measurement is a critical determinant of interpretability and predictive value in FPO assays. ATP-based viability assays are widely used because they are sensitive, scalable, and compatible with high-throughput screening. ATP content however reflects cellular metabolic activity rather than cell death *per se* and may therefore overestimate drug efficacy for agents that induce cytostasis or metabolic reprogramming without durable tumor control.

Apoptosis- or cytotoxicity-based readouts offer greater mechanistic specificity but are often more sensitive to timing, cell type, and assay conditions, complicating cross-platform standardization. Comparative studies in three-dimensional cell-line based tumor models have demonstrated that different endpoint modalities can yield discordant sensitivity profiles, underscoring the importance of aligning readouts with the intended clinical question [68,69].

Imaging-based endpoints, including pharmacoscopy, spheroid or organoid size, morphology, and invasion metrics, provide spatial and temporal resolution and can distinguish cytostatic from cytotoxic effects. However, these approaches require robust image acquisition, segmentation, and quality control pipelines and are less amenable to rapid clinical deployment.

Beyond the choice of assay, data interpretation strategies also differ. Transversal approaches identify outlier drug responses relative to a cohort distribution, which is valuable in heterogeneous datasets, whereas longitudinal or baseline-referenced analyses quantify treatment effects relative to untreated growth, conceptually analogous to RECIST assessments. Each strategy has inherent limitations, and combined reporting of cohort-normalized sensitivity scores and baseline-referenced effect sizes may offer the most clinically informative representation.

(2) Turnaround Time, Clinical Feasibility, and Molecular Tumor Boards

(i) Time Constraints and Workflow Optimization

To influence treatment in real-world settings, FPO assays must produce actionable results within clinically relevant timeframes. Conventional spheroid models require 3–4 weeks and organoid models 8–10 weeks, timelines that are impractical for aggressive cancers. Some platforms, however, have demonstrated the potential for faster drug screening, producing viable data in just 9 to 14 days. These faster approaches can support timely tumor board decisions but remain the exception rather than the norm. Technologies like microfluidics, miniaturized high-throughput assays, and modular automation, along with the use of context-specific drug panels, offer promising avenues to reduce assay complexity and speed up results without compromising interpretability [21,70].

(ii) Integration of FPO into molecular tumor board decision-making

Molecular Tumor Boards increasingly must arbitrate between genotype-derived actionability (NGS) and phenotype-derived vulnerability (FPO/*ex vivo* drug response). NGS is often prioritized because it links to approved targeted drugs and guideline tiers; however, actionable alterations do not always translate into response due to pathway redundancy, downstream re-wiring, tumor heterogeneity, and non-genetic resistance. Functional profiling can complement this by testing actual drug sensitivity in patient-derived material and can reveal vulnerabilities not obvious from genomics alone or deprioritize targets that appear actionable but are functionally ineffective in the tested context [18].

Combining transcriptomic data with functional screening has been postulated as particularly effective in identifying druggable escape routes and predicting combination efficacy. Embedding real-time data integration into clinical workflows, supported by machine learning models that synthesize diverse assay outputs, offers a pathway to more actionable and scalable decision-making. However, for these tools to gain regulatory acceptance, they must meet rigorous standards for transparency, cross-validation, and clinical interpretability [36,71,72].

(iii) Biological completeness and tumor microenvironment fidelity

Many FPO systems rely on tumor cells cultured in isolation, omitting stromal, vascular, and immune components that play a crucial role in modulating drug responses, particularly in immunotherapy contexts. More integrative approaches, such as 3D tumor slice cultures and co-culture systems, preserve native architecture and some elements of the tumor microenvironment, enhancing physiological relevance.

Lung cancer models incorporating immune profiling and co-culture techniques have also enabled limited ex vivo immunotherapy testing. However, maintaining functional immune populations and achieving scalable, reproducible platforms remain significant technical challenges. Tumor-on-chip systems and immune-enhanced organoids represent ongoing efforts to address these gaps, though throughput and consistency are still limiting factors [30,73].

3. Clinical Validation, Study Design, and Regulatory Pathways

(i) Current Evidence Base and Its Limitations

Despite promising feasibility studies, FPO still lacks clinical validation needed for widespread adoption. Most existing evidence comes from non-randomized studies reporting correlations between ex vivo drug sensitivity and patient outcomes across various tumor types. In glioblastoma and hematological malignancies, patients receiving drug screen–guided therapies have shown improved response rates and survival outcomes, suggesting real-world benefit [19,57].

However, because these studies are observational or retrospective, they do not allow causal inference regarding whether FPO-guided therapy improves patient outcomes. Observed associations between ex vivo sensitivity and clinical response must therefore be interpreted cautiously and as hypothesis-generating.

(ii) Pathways to demonstrating clinical utility

Clinical evaluation of FPO platforms typically progresses through staged evidence generation. Early-phase prospective observational studies are commonly used to assess assay success rates, turnaround time, and concordance between ex vivo drug sensitivity and clinical response under standard treatments. These designs are particularly valuable in rare or refractory cancers, where conventional trials may be difficult to conduct, but they do not establish clinical benefit because treatment decisions are not dictated by functional assay results.

To move beyond feasibility and correlation, interventional designs in which treatment selection is explicitly informed by FPO results are required. Because classical randomized strategy trials are resource-intensive and may face ethical, logistical, or accrual challenges in advanced disease settings, several FPO studies have adopted alternative approaches. These include enrichment designs or intra-patient comparisons, such as progression-free survival on FPO-guided therapy relative to prior therapy (eg. PFS2/PFS1 ratios) [57,63]. While pragmatic and attractive in heavily pretreated populations, these endpoints are sensitive to time-dependent biases, regression to the mean, and biological evolution across therapy lines, and they lack validation as surrogates for overall survival or quality-of-life benefit.

Adaptive and platform trials that allow dynamic modification of treatment assignments based on accumulating functional and clinical data offer a potential compromise between rigor and feasibility. However, these designs introduce additional statistical complexity and depend critically on predefined sensitivity thresholds, decision rules, and governance structures.

Ultimately, definitive demonstration of clinical utility requires prospective trials in which outcomes under FPO-guided therapy are compared against appropriate control strategies.

(iii) IVDR-Regulation and Compliance Considerations

The EU In Vitro Diagnostic Regulation (IVDR 2017/746) raises the bar for any diagnostic used to inform treatment decisions, which becomes especially relevant when FPO outputs are used to assign therapies in prospective interventional trials. Key IVDR expectations include clearly defined intended use, evidence for analytical and clinical performance, risk management, quality systems, and traceability and documentation across the testing workflow. These requirements are particularly challenging for FPO platforms because assays are complex, multi-parametric, sensitive to pre-analytical variables such as fresh tissue logistics, and often lack standardized reference materials or universally accepted clinical performance endpoints for functional response readouts.

A major practical distinction is the health institution ("in-house") exemption (Article 5(5)) versus commercial platforms. Academic or hospital-based FPO assays may be used under Article 5(5) only if the test is manufactured and used within the same health institution, justified by unmet patient needs not addressed by an equivalent CE-marked device, and supported by appropriate quality management and documentation obligations as clarified in MDCG guidance. This pathway may be compatible with single-center clinical use but becomes challenging for multicenter trials or cross-site service models. For further information, we refer readers to the original legislative text of Regulation (EU) 2017/746, available via EUR-Lex (https://eur-lex.europa.eu/legal-content/EL/TXT/?uri=CELEX:32017R0746).

Conclusion

FPO complements gene panel or whole genome sequencing by revealing actionable vulnerabilities even in tumors without targetable mutations. For it to become a standard part of oncology practice, solutions are needed to standardize workflows, shorten turnaround times, incorporate microenvironmental elements, and improve data synthesis. Crucially, randomized controlled trials are needed to confirm that drug screening-guided treatments improve survival, quality of life, or cost-effectiveness relative to genomic or standard-of-care approaches. Only with such evidence can FPO earn widespread clinical and regulatory acceptance as well as reimbursement by statutory health insurances.

Methods

The literature search was conducted using PubMed with the search terms "functional precision oncology", "drug screen", "patient-derived cells", "patient-derived organoids" and "patient-derived models" with a cut-off date of August 2025. The first version of the manuscript was carefully curated and checked by multiple co-authors. It subsequently underwent a language edit and minor adaptation assisted by ChatGPT. The authors take full responsibility for the content and interpretation.

Authors contributions

Z.S. and A.S.B conceptualized the review and wrote the main manuscript text; Z.S. prepared figures 1. and 2. and tables 1. and 2.; Z.S., A.E.H., M.J.M., T.R.P., B.P., S.S.K., S.S., A.W., M.P., A.S.B reviewed, edited and approved the manuscript.

Ethics declaration

Human Ethics and Consent to Participate declarations: not applicable.

Funding

Z.S. is funded by the Clinical Research Groups Program (LBG_KFG_22_07) of the Ludwig Boltzmann Gesellschaft with funds from the Austrian Federal Ministry of Education, Science and Research.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: ZS is a previous employee of Boehringer Ingelheim and Turbine AI and a current employee of CBmed GmbH. AEH is a previous employee of CBmed GmbH and has received honoraria for lectures from the for-profit company Illumina. MJM has received research support from Bristol-Myers Squibb and travel support from Pierre Fabre. TRP is the CSO of CBmed GmbH, received research support from AstraZeneca, Novo Nordisk Foundation and Sanofi, and has received honoraria for consulting and lectures from Novo Nordisk, Sanofi, Eli Lilly and Arecor . BP is a current employee of CBmed GmbH. SSK declares having no conflict of interest. SS is a current employee of CBmed GmbH. AW is a previous employee of CBmed GmbH . MP has received honoraria for lectures, consultation or advisory board participation from the following for-profit companies: Bayer, Bristol-Myers Squibb, Novartis, Gerson Lehrman Group (GLG), CMC Contrast, GlaxoSmithKline, Mundipharma, Roche, BMJ Journals, MedMedia, Astra Zeneca, AbbVie, Lilly, Medahead, Daiichi Sankyo, Sanofi, Merck Sharp & Dome, Tocagen, Adastra, Gan & Lee Pharmaceuticals, Janssen, Servier, Miltenyi, Boehringer-Ingelheim, Telix, Medscape.. ASB has research support from Daiichi Sankyo, Roche and honoraria for lectures, consultation or advisory board participation from Roche, Bristol-Myers Squibb, Merck, Daiichi Sankyo, AstraZeneca, CeCaVa, Seagen, Alexion and Servier as well as travel support from Roche, Amgen and AbbVie.

References

[1] Bardia A, et al. *Elacestrant in ER+, HER2- Metastatic Breast Cancer with ESR1-Mutated Tumors: Subgroup analyses from the phase III EMERALD Trial by prior Duration of Endocrine Therapy plus CDK4/6 Inhibitor and in Clinical Subgroups.* Clin Cancer Res 2024;30(19):4299–309.

[2] Bekaii-Saab TS, et al. *Impact of Anti-EGFR Therapies on HER2-positive Metastatic Colorectal Cancer: a Systematic Literature Review and Meta-Analysis of Clinical Outcomes*. Oncologist 2023;28(10):885–93.
[3] Flaherty KT, et al. *Improved survival with MEK inhibition in BRAF-mutated melanoma*. N Engl J Med 2012;367(2):107–14.
[4] Papaemmanuil E, et al. *Genomic Classification and Prognosis in Acute Myeloid Leukemia*. N Engl J Med 2016;374(23):2209–21.
[5] Huang Q, et al. *Advances in molecular pathology and therapy of non-small cell lung cancer*. Signal Transduct Target Ther 2025;10(1):186.
[6] Bernhard C, et al. *A coregulatory influence map of glioblastoma heterogeneity and plasticity*. npj Precis Oncol 2025;9(1):110.
[7] Nomura M, et al. *The multilayered transcriptional architecture of glioblastoma ecosystems*. Nat Genet 2025;57(5):1155–67.
[8] Yabo YA, Niclou SP, Golebiewska A. *Cancer cell heterogeneity and plasticity: a paradigm shift in glioblastoma*. Neuro Oncol 2022;24(5):669–82.
[9] Espona-Fiedler M, et al. *Overcoming therapy resistance in pancreatic cancer: New insights and future directions*. Biochem Pharmacol 2024;229:116492.
[10] Fang YT, et al. *Recent advances in targeted therapy for pancreatic adenocarcinoma*. World J Gastrointest Oncol 2023;15(4):571–95.
[11] Hosein AN, et al. *Translational advances in pancreatic ductal adenocarcinoma therapy*. Nat Cancer 2022;3(3):272–86.
[12] Katayama ES, et al. *A comprehensive analysis of clinical trials in pancreatic cancer: what is coming down the pike?* Oncotarget 2020;11(38):3489–501.
[13] Liberto JM, et al. Current and Emerging Methods for Ovarian Cancer Screening and Diagnostics: A Comprehensive Review. Cancers (Basel), 2022; 14(12).
[14] Wang G, et al. *Ovarian cancer targeted therapy: current landscape and future challenges*. Front Oncol 2025;15:1535235.
[15] Kim S, et al. *Drug Response of Patient-Derived Lung Cancer Cells Predicts Clinical Outcomes of Targeted Therapy*. Cancers (Basel) 2024;16(4).
[16] Ntafoulis I, et al. *Ex vivo drug sensitivity screening predicts response to temozolomide in glioblastoma patients and identifies candidate biomarkers*. Br J Cancer 2023;129(8):1327–38.
[17] Verheul C, et al. *Generation, characterization, and drug sensitivities of 12 patient-derived IDH1-mutant glioma cell cultures*. Neurooncol Adv 2021;3(1).
[18] Malani D, et al. *Implementing a Functional Precision Medicine Tumor Board for Acute Myeloid Leukemia*. Cancer Discov 2022;12(2):388–401.
[19] Ranjan T, et al. *Cancer stem cell assay-guided chemotherapy improves survival of patients with recurrent glioblastoma in a randomized trial*. Cell Rep Med 2023;4(5):101025.
[20] Spinner MA, et al. *Ex vivo drug screening defines novel drug sensitivity patterns for informing personalized therapy in myeloid neoplasms*. Blood Adv 2020;4(12):2768–78.
[21] Acanda De La Rocha AM, et al. *Feasibility of functional precision medicine for guiding treatment of relapsed or refractory pediatric cancers*. Nat Med 2024;30(4):990–1000.
[22] Posthoorn-Verheul C, et al. *Optimized culturing yields high success rates and preserves molecular heterogeneity, enabling personalized screening for high-grade gliomas*. npj Precis Oncol 2025;9(1):156.
[23] Lenin S, et al. *A Drug Screening Pipeline using 2D and 3D Patient-Derived In Vitro Models for Pre-Clinical Analysis of Therapy Response in Glioblastoma*. Int J Mol Sci 2021;22(9).
[24] Berghoff AS, et al. *Personalized targeted glioblastoma therapies by ex vivo drug screening: Study protocol of the Advanced brain Tumor TheRApy Clinical Trial (ATTRACT)*. Neurooncol Adv 2025;7(1).
[25] Chadwick M, et al. *Rapid processing and drug evaluation in glioblastoma patient-derived organoid models with 4D*. Bioprinted Arrays iScience 2020;23(8):101365.
[26] Gagg H, et al. *Ex-vivo drug screening of surgically resected glioma stem cells to replace murine avatars and provide personalise cancer therapy for glioblastoma patients*. F1000Res 2023;12:954.
[27] Jeising S, et al. *A clinically compatible in vitro drug-screening platform identifies therapeutic vulnerabilities in primary cultures of brain metastases*. J Neurooncol 2024;169(3):613–23.
[28] Yin S, et al. *Patient-derived tumor-like cell clusters for drug testing in cancer therapy*. Sci Transl Med 2020;12(549).
[29] Lee JK, et al. *Pharmacogenomic landscape of patient-derived tumor cells informs precision oncology therapy*. Nat Genet 2018;50(10):1399–411.
[30] Peterziel H, et al. *Drug sensitivity profiling of 3D tumor tissue cultures in the pediatric precision oncology program INFORM*. npj Precis Oncol 2022;6(1):94.
[31] Zhou L, et al. *High-throughput neural stem cell-based drug screening identifies S6K1 inhibition as a selective vulnerability in sonic hedgehog-medulloblastoma*. Neuro Oncol 2024;26(9):1685–99.
[32] Liu L, et al. *Patient-derived organoid (PDO) platforms to facilitate clinical decision making*. J Transl Med 2021;19(1):40.
[33] Wang J, et al. *Patient-Derived Tumor Organoids: New Progress and Opportunities to Facilitate Precision Cancer Immunotherapy*. Front Oncol 2022;12:872531.
[34] Jacob F, et al. *A Patient-Derived Glioblastoma Organoid Model and Biobank Recapitulates Inter- and Intra-tumoral Heterogeneity*. Cell 2020;180(1):188–204 e22.
[35] Kim SY, et al. *Modeling Clinical responses to Targeted Therapies by Patient-Derived Organoids of Advanced Lung Adenocarcinoma*. Clin Cancer Res 2021;27(15):4397–409.
[36] Ren X, et al. *Personalized drug screening in patient-derived organoids of biliary tract cancer and its clinical application*. Cell Rep Med 2023;4(11):101277.
[37] Verduin M, et al. *Patient-derived glioblastoma organoids reflect tumor heterogeneity and treatment sensitivity*. Neurooncol Adv 2023;5(1).
[38] Zhao Y, et al. *Personalized drug screening using patient-derived organoid and its clinical relevance in gastric cancer*. Cell Rep Med 2024;5(7):101627.
[39] de Witte CJ, et al. *Patient-derived ovarian cancer organoids mimic clinical response and exhibit heterogeneous inter- and intrapatient drug responses*. Cell Rep 2020;31(11):107762.
[40] Ding S, et al. *Patient-derived micro-organospheres enable clinical precision oncology*. Cell Stem Cell 2022;29(6):905–917 e6.
[41] He X, et al. *Patient-derived organoids as a platform for drug screening in metastatic colorectal cancer*. Front Bioeng Biotechnol 2023;11:1190637.
[42] Mertens S, et al. *Drug-repurposing screen on patient-derived organoids identifies therapy-induced vulnerability in KRAS-mutant colon cancer*. Cell Rep 2023;42(4):112324.
[43] Narasimhan V, et al. *Medium-throughput drug screening of patient-derived organoids from colorectal peritoneal metastases to direct personalized therapy*. Clin Cancer Res 2020;26(14):3662–70.
[44] Ooft SN, et al. *Prospective experimental treatment of colorectal cancer patients based on organoid drug responses*. ESMO Open 2021;6(3):100103.
[45] Ratliff M, et al. *Patient-Derived Tumor Organoids for Guidance of Personalized Drug Therapies in Recurrent Glioblastoma*. Int J Mol Sci 2022;23(12).
[46] Tan T, et al. *Unified framework for patient-derived, tumor-organoid-based predictive testing of standard-of-care therapies in metastatic colorectal cancer*. Cell Rep Med 2023;4(12):101335.
[47] Lampis S, et al. *Organoids as a new approach for improving pediatric cancer research*. Front Oncol 2024;14:1414311.
[48] Pauli C, et al. *Personalized In Vitro and In Vivo Cancer Models to Guide Precision Medicine*. Cancer Discov 2017;7(5):462–77.
[49] Takahashi N, et al. *An in vitro system for evaluating molecular targeted drugs using lung patient-derived tumor organoids*. Cells 2019;8(5).
[50] Yi HG, et al. *A bioprinted human-glioblastoma-on-a-chip for the identification of patient-specific responses to chemoradiotherapy*. Nat Biomed Eng 2019;3(7):509–19.
[51] Zhu L, et al. *A clinically compatible drug-screening platform based on organotypic cultures identifies vulnerabilities to prevent and treat brain metastasis*. EMBO Mol Med 2022;14(3):e14552.
[52] Almstedt E, et al. *Real-time evaluation of glioblastoma growth in patient-specific zebrafish xenografts*. Neuro Oncol 2022;24(5):726–38.
[53] Charbonneau M, et al. *The development of a rapid patient-derived xenograft model to predict chemotherapeutic drug sensitivity/resistance in malignant glial tumors*. Neuro Oncol 2023;25(9):1605–16.
[54] Gatzweiler C, et al. *Functional Therapeutic Target Validation using Pediatric Zebrafish Xenograft Models*. Cancers (Basel) 2022;14(3).
[55] Florenes VA, et al. *A three-dimensional ex vivo viability assay reveals a strong correlation between response to targeted inhibitors and mutation status in melanoma lymph node metastases*. Transl Oncol 2019;12(7):951–8.
[56] Lau LMS, et al. *In vitro and in vivo drug screens of tumor cells identify novel therapies for high-risk child cancer*. EMBO Mol Med 2022;14(4):e14608.
[57] Kornauth C, et al. *Functional Precision Medicine Provides Clinical Benefit in Advanced Aggressive Hematologic Cancers and Identifies Exceptional Responders*. Cancer Discov 2022;12(2):372–87.
[58] Snijder B, et al. *Image-based ex-vivo drug screening for patients with aggressive haematological malignancies: interim results from a single-arm, open-label, pilot study*. Lancet Haematol 2017;4(12):e595–606.
[59] Haladik B, et al. *Image-based drug screening combined with molecular profiling identifies signatures and drivers of therapy resistance in pediatric AML*. Cell Rep Med 2025:102304.
[60] Heinemann T, et al. *Deep morphology learning enhances ex vivo drug profiling-based precision medicine*. Blood Cancer Discov 2022;3(6):502–15.
[61] Kazianka L, et al. *Comparing functional and genomic-based precision medicine in blood cancer patients*. Hemasphere 2025;9(4):e70129.
[62] Lee S, et al. *High-throughput identification of repurposable neuroactive drugs with potent anti-glioblastoma activity*. Nat Med 2024.
[63] Schmid JA, et al. *Efficacy and feasibility of pharmacoscopy-guided treatment for acute myeloid leukemia patients who have exhausted all registered therapeutic options*. Haematologica 2024;109(2):617–21.
[64] Wegmann R, et al. *Molecular and functional landscape of malignant serous effusions for precision oncology*. Nat Commun 2024;15(1):8544.
[65] Ntafoulis I, et al. *A repurposed drug selection pipeline to identify CNS-penetrant drug candidates for glioblastoma*. Pharmaceuticals (Basel) 2024;17(12).
[66] Nykanen N, et al. *Ex vivo drug screening informed targeted therapy for metastatic parotid squamous cell carcinoma*. Front Oncol 2021;11:735820.
[67] Pishas KI, et al. *High-throughput drug screening identifies novel therapeutics for Low Grade Serous Ovarian Carcinoma*. Sci Data 2024;11(1):1024.
[68] Hongisto V, et al. *High-throughput 3D screening reveals differences in drug sensitivities between culture models of JIMT1 breast cancer cells*. PLoS One 2013;8(10):e77232.
[69] Muguruma M, et al. *Differences in drug sensitivity between two-dimensional and three-dimensional culture systems in triple-negative breast cancer cell lines*. Biochem Biophys Res Commun 2020;533(3):268–74.
[70] Napoli GC, Figg WD, Chau CH. *Functional drug screening in the era of precision medicine*. Front Med (Lausanne) 2022;9:912641.
[71] Genta S, et al. *Patient-derived cancer models: valuable platforms for anticancer drug testing*. Front Oncol 2022;12:976065.
[72] Letai A, Bhola P, Welm AL. *Functional precision oncology: Testing tumors with drugs to identify vulnerabilities and novel combinations*. Cancer Cell 2022;40(1):26–35.
[73] Taverna JA, et al. *Ex vivo drug testing of patient-derived lung organoids to predict treatment responses for personalized medicine*. Lung Cancer 2024;190:107533.