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RADIATION BIOLOGY (C PORADA AND P WILSON, SECTION EDITORS)

Creation and Development of Patient-Derived Organoids for Therapeutic Screening in Solid Cancer

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Abstract

Purpose of Review Clinical implementation of personalized cancer therapy necessitates translational cancer models that faithfully represent the molecular and cellular features of human cancer. Current patient-derived preclinical models, including cell line and xenograft models, are limited by incomplete recapitulation of parental tumor heterogeneity and long induction times, impeding their ability to directly inform clinical decision-making. Newly emerging patient-derived organoids (PDOs) of solid tumors retain the intra-tumoral heterogeneity lost in many preclinical models and mirror the therapeutic responsiveness of their parent tumors. Herein, we explore the origins and rationale for organoid cancer modeling, the creation of PDO models through an illustrative example of glioma organoids, and their downstream use in comprehensive drug screens to guide oncologic therapy selection.

Recent Findings Cancer organoid models have been generated through numerous techniques, producing PDOs of brain, pancreatic, breast, and gastrointestinal cancer, among others. Recent evidence supports the creation of PDOs using a minimally processed approach, whereby manually parcellated tissue can produce viable organoids in the absence of tissue dissociation, an artificial extracellular matrix, and exogenous growth factors. Refinement of these models thus allows PDOs to serve as patient avatars, and early evidence demonstrates similar responses to chemotherapy and radiotherapy as the parent tumor.

Summary The retention of key molecular, histopathologic, and phenotypic features of numerous human cancers offers compelling support for the use of PDOs as translational cancer models. Given the ability to rapidly create these models following tumor resection, PDOs can be used as platforms for personalized drug screens to guide the selection of oncologic therapies.

Keywords Organoid · Personalized oncology · Glioma · Glioblastoma · Cancer · Patient-derived organoid

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Introduction

Solid cancers are often treated with maximal surgical resection, systemic therapy, and/or adjuvant radiotherapy aimed at disrupting tumor maintenance and proliferation [1]. While this paradigm remains standard, modern oncology has placed a growing emphasis on the development of targeted therapies and patient-specific treatment regimens. The advent of advanced genomic technology, such as next-generation sequencing (NGS) and transcriptomic analysis, allows for the rapid identification of the molecular characteristics of individual tumors [2]. Furthermore, increased appreciation for the heterogeneity of solid cancers has resulted in a push towards personalized oncology — treatment based on a tumor's molecular, metabolic, physiologic, and environmental factors [3]. In this regard, several genomic tools have emerged that can aid in personalizing cancer treatment decisions, including use of chemotherapy (ex. Oncotype DX in breast cancer) and radiation therapy (ex. GARD) [4–7]. However, these predictive tools were developed from aggregated data and thus may not fully capture the unique characteristics of any single tumor. As further technological advances have increased the feasibility of developing faithful ex vivo models, the ability to test treatment strategies on individual patients' tumor models is an exciting advance in facilitating personalized cancer treatment. A critical component of this approach is the need for translational models that reliably demonstrate the array of molecular phenotypes appreciated in complex human cancers [8].

Traditional laboratory cancer models have provided tremendous value in cancer research. The high throughput capacity of two-dimensional cell cultures facilitates rapid testing of novel therapeutics and provides the rationale for in vivo experimentation [9–11]. Also, patient-derived xenografts in mice have been utilized to study the biology of human cancer tissue and have guided many aspects of preclinical therapeutic development [12, 13]. Further, the breadth of tools to create genetically engineered mouse models (GEMMs) allows for the temporal and spatial control of tumor formation in vivo [13–15]. However, these models carry several limitations that may, in part, contribute to their shortcomings in establishing cancer therapy efficacy [16]. The selective pressures of serial cell culture often manifest in oligoclonal cell populations that lose the genotypic and phenotypic heterogeneity of the parent tissue and ultimately diverge from the primary sample and parental tumor [17–19]. For cancers with diverse intratumoral complexity, such as malignant glioma, breast cancer, and pancreatic cancer, the absence of tumor-tumor, tumor-parenchyma, and tumor-stroma interaction limits modeling of diverse cellular states and hierarchies [20–23]. Additionally, the requisite use of immunocompromised mice in patient-derived xenograft models limits study of the tumor-immune interface [15, 24]. While GEMMs enable cancer formation to occur in immunocompetent mice, species-specific differences may pose challenges in modeling some cancers, as seen with differential phenotypes in mice following aberrations in classical driver mutations of human cancer, such as *APC*, *BRCA1/2*, and *RB* [14, 25]. Similarly, tumors in GEMMs trend towards a homogeneous state compared to the heterogeneity seen with the progressive accumulation of genetic alterations in human tumors [26, 27]. Finally, murine models are time- and resource-intense and are relatively low-throughput models for preclinical drug screens. Thus, new modeling approaches, particularly in the realm of translational research and personalized oncology, may identify promising therapeutics more efficiently and accurately.

Cancer organoid models have emerged as preclinical and translational models. Organoids are heterogeneous, three-dimensional, self-organizing structures that can model the architecture and function of native organs and neoplasms [28]. In 2009, Sato et al. created the first organoids by developing crypt-villous structures from Lgr5 + intestinal stem cells [29•]. By embedding cells in Matrigel, an artificial extracellular matrix, and culturing in media enriched with epidermal growth factor (EGF), r-spondin 1, and Noggin, organoids resembling crypt and villous domains of freshly isolated small intestine crypts were produced and persisted for greater than 8 months [29•]. To date, numerous organoid models of human organs have been created, including brain, blood vessels, endometrium, fallopian tubes, intestine, kidney, lung, ovaries, pancreas, retina, stomach, taste buds, and testicles [29•, 30•, 31–40]. Following generation of many of these primary tissue models, cancer organoid models were produced, with an emphasis on their creation from primary patient tissue samples [22]. The resulting patient-derived organoids (PDOs) faithfully demonstrated many genotypic and phenotypic features of their native tissue. PDOs have been developed for bladder, breast, colorectal, gastric, metastatic gastrointestinal, liver, pancreatic cancer, and glioblastoma, among others [41, 42•, 43–51]. The translational implications for PDOs are most apparent in tumors characterized by inter-tumoral heterogeneity, diverse tumor cell states, and complex tumor microenvironments integral to tumor behavior and response to treatment. As such, PDOs may complement classical cancer models to support personalized predictions of human tumor responses to specific treatments.

Glioma, the most common primary malignant tumor of the central nervous system (CNS), exists along a histopathologic spectrum from WHO grade 1 to grade 4 [52, 53••]. The most aggressive of these is glioblastoma (GBM, IDH-WT, WHO grade 4), characterized by its infiltration of the brain parenchyma, cellular heterogeneity, and dismal clinical

prognosis [54]. While GBM cell lines have provided insight into the cellular hierarchy of glioma cell populations, these models are time intensive and challenging to establish [10, 50, 55–57]. Furthermore, the overrepresentation of GBM cell lines has limited the study of lower-grade lesions that frequently progress to higher-grade tumors [58, 59]. Given these challenges in utilizing two-dimensional in vitro models to recapitulate the characteristics of gliomas of various grades, the development of PDOs for glioma modeling has already improved the ability to study tumor biology and develop new therapies for these tumors.

PDOs of cancer recapitulate the architectural, cellular, and molecular features of a diverse array of tumors with the potential to take personalized and translational oncology from *bench-to-bedside* to a *bedside-to-bench-and-back* model [60]. Herein, we review the creation and applications of PDOs of cancer through the lens of glioma, followed by a discussion of the impact of PDOs on clinical oncology.

Creation and Utilization of Patient-Derived Organoids of Glioma

A critical advance in the development of glioma organoids came from Lancaster et al. with the creation of cerebral organoids [30•]. To generate cerebral organoids, human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs) were exposed to a series of stepwise media conditions and nutrient supplements [61]. The hESCs or iPSCs were cultured with basic fibroblast growth factor (bFGF) and rho-associated protein kinase (ROCK) inhibitor to induce the formation of embryoid bodies (EB). These EB were kept in suspension and subsequently cultured in neural induction media — composed of DMEM/F12, N2 supplement, GlutaMax, non-essential amino acids (NEAAs), and heparin — to induce the formation of neuroepithelial tissue. The primitive neuroepithelial tissue is then embedded in Matrigel droplets and cultured in a differentiation medium — composed of DMEM/F12, Neurobasal, N2 supplement, insulin, GlutaMax, NEAAs, antibiotics, 2-mercaptoethanol, and B27 supplement — for 4 days, and finally, they were transferred to a spinning bioreactor and differentiation medium containing vitamin A. The newly generated cerebral organoids developed primitive regions analogous to ventricles, choroid plexus, and retina, as well as functional and structural cortical organization [30•]. In addition, the cerebral organoids displayed regionalization of discrete primitive brain regions [30•]. Utilization of the in vitro cerebral organoid model, effectively mini-brains, stimulated interest using similar approaches to model brain cancer, leading to an expansion of glioma organoid models (Table 1). These models can be developed via co-culture of glioma stem-like cells (GSCs) and cerebral organoids, genetic engineering of cerebral organoids, or direct culture of minimally processed glioma tissue samples (Fig. 1) [62••, 63•, 64••, 65, 66].

Several studies have genetically manipulated cerebral organoids to model glioma [63•, 65, 66]. Using sleeping beauty transposon-mediated gene insertion for oncogene amplification and CRISPR/Cas9-mediated mutagenesis, Bian et al. introduced combinations of 15 clinically relevant genomic alterations seen in GBM, among other CNS tumors, to generate neoplastic cerebral organoids (NeoCOR) [65]. Three combinations of genetic alterations, including those affecting *CDKN2A/B*, *NF1*, *PTEN*, *TP53*, and epidermal growth factor receptor variant III (*EGFRvIII*), were then identified and classified as GBM-1, GBM-2, and GBM-3. These NeoCOR of GBM demonstrated similar cellular identities and transcriptomic signatures to analogous human GBMs [65]. Similarly, Ogawa et al. introduced the oncogene $HRas^{G12V}$ at the *TP53* locus to simultaneously knock out the tumor suppressor gene and introduce an oncogene [66]. Both models demonstrated invasive phenotypes on xenograft transplantation in mice as well as a propensity to undergo epithelial-mesenchymal transition, a transition seen in GBM tissue that is associated with a more aggressive and infiltrative phenotype [65, 66, 74, 75].

Adapting the approach to generate cerebral organoids, Hubert et al. developed the first PDOs of GBM utilizing GSCs from surgically excised human GBM tissue [62••]. Tissue samples were either finely minced and plated in a Matrigel suspension or dissociated into single-cell suspensions [62••]. Isolated cells were then maintained as tumorspheres and sorted for CD133, a marker of the GSC population [56, 76, 77]. CD133 + cells were plated in a complete medium — containing EGF, bFGF, B27 supplement, glutamine, sodium pyruvate, and antibiotics — and resultant tumorspheres were cultured in a Matrigel matrix and allowed to form three-dimensional organoids [62••]. Their GSC-based PDO model recapitulated hallmark features of GBM, including radio-resistance of GSCs, radio-sensitivity of non-GSC populations, single-cell infiltration that is lost in many xenograft models, and gradients of GSC density and hypoxia, all hallmarks of in vivo human GBMs [62••].

Similarly, Linkous et al. developed a PDO model of GBM by co-culturing GSCs with cerebral organoids termed glioma cerebral organoids (GLICO) [63•]. The GSCs are isolated from the primary tissue and maintained in serum-free media supplemented with EGF and bFGF as previously described [78, 79]. Mature GSCs are then placed in culture with cerebral organoids and are rapidly and efficiently engrafted into the organoid. Considerable bulk tumor growth was observed after 1 week, and at 2 weeks the GLICO demonstrated histopathologic features of human disease and generated a network of microtubes associated with invasion and proliferation [63•, 80]. Similarly, Krieger et al. utilized a GBM cerebral organoid model and transcriptomic analysis

Table 1 Organoid models of glioma

Source of glioma tissue	Method to generate organoids	Key findings	Reference
Patient-derived glioma stem-like cells (GSCs)	Finely minced primary tissue or single cell dissociation and isolation of CD133 + GSCs embedded in a Matrigel scaffold	Recapitulation of cell density and hypoxia gradients and selective radio-susceptibility of non-GSCs compared to radio-resistant GSCs	Hubert et al. [62••]
		Glioblastoma (GBM) organoids demonstrated increased resistance to standard of care and targeted therapies compared to two-dimensional spheroid cultures	Sundar et al. [67]
	Glioma cerebral organoid (GLICO) model: co-culture of GSCs with cerebral organoids	Generation of histopathologic features of glioma, phenocopying primary tissue samples, and differential chemotherapeutic response of GLICOs compared to two-dimensional cell culture	Linkous et al. [63•]
		Three-dimensional GBM organoids provide the necessary tumor microenvironment to model the distribution of cellular states found in primary GBM samples	Pine et al. [68]
	Co-culture of GSCs and iPSCs; addition of dispersed GSCs to cerebral organoids; fusion of GSCs tumorspheres to cerebral organoids	Evaluation of three different assays of GSC based organoids to model invasion	Goranci-Buzhala et al. [69]
	Co-culture of mouse cerebral organoids with GBM stem cell tumorspheres	Infiltration of the cerebral organoid core and expression of GBM stem cell markers	Da Silva et al. [70]
	Co-culture of GBM cells with cerebral organoids	Modeling of GBM invasion along microtubes and associated transcriptional analysis revealing that receptor-ligand pairs drive invasion	Krieger et al. [71]
Primary human glioma tissue	Primary patient tissue parcellated into ~ 1 mm^3 samples, cultured in the absence of an artificial extracellular matrix or exogenous growth factors	Creation of minimally processed patient-derived GBM organoids that retain histologic and molecular signatures of their parent tumors	Jacob et al. [64••, 72]
		Generation of minimally processed patient-derived lower-grade glioma organoids that recapitulate histologic and molecular features of the parent tumor	Abdullah et al. [73••]
Human embryonic stem cells or induced-pluripotent stem cells (iPSCs)	Sleeping beauty transposon and CRISPR/Cas9-mediated mutagenesis of cerebral organoids to form neoplastic cerebral organoids (NeoCOR)	Combination-based mutagenesis of cerebral organoids generated GBM-like organoids that carried similar transcriptional profiles and invasive phenotypes compared to human GBM	Bian et al. [65]
	CRISPR/Cas9-mediated mutagenesis of cerebral organoids and co-culture of patient-derived tumor cells with cerebral organoids	Generation of malignant cerebral organoids demonstrating an aggressive infiltrative phenotype when xenografted into mouse brains	Ogawa et al. [66]

GSC glioma stem-like cell, *GBM* glioblastoma, *GLICO* glioma cerebral organoid, *iPSCs* induced pluripotent stem cells, *NeoCOR* neoplastic cerebral organoid

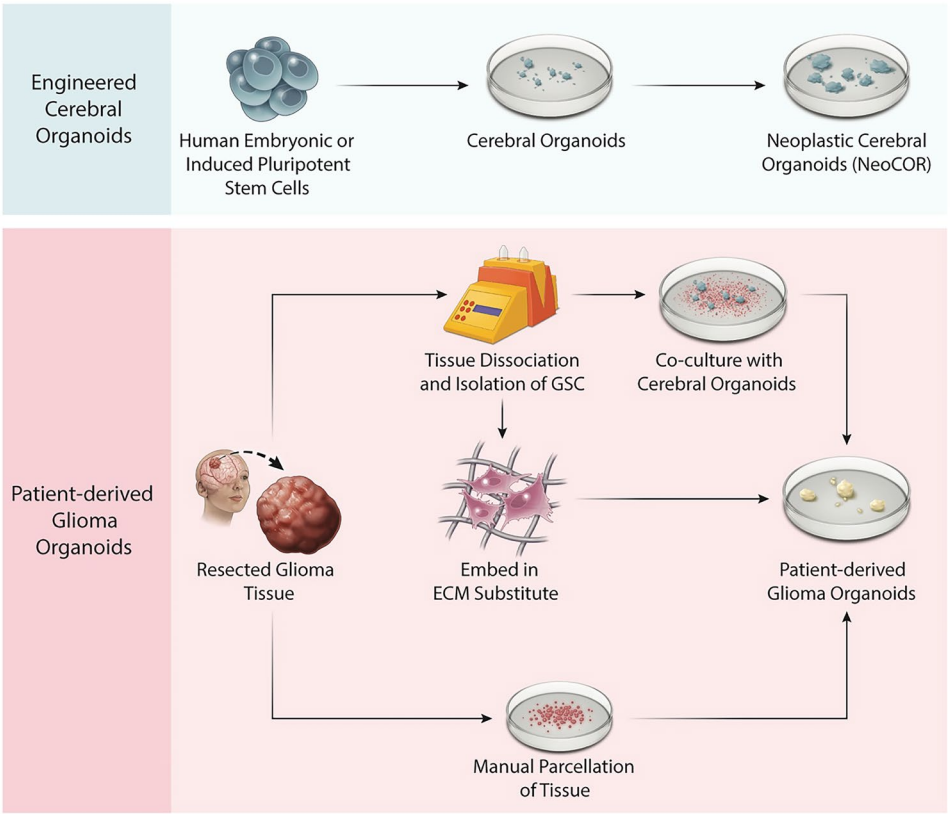

Fig. 1 Workflow for the creation of glioma organoid models

of receptor-ligand pairing to highlight the importance of the tumor microenvironment in microtube formation and GBM invasion [71].

In defining the inter- and intra-tumoral heterogeneity associated with GBM, single-cell RNA sequencing of GBM identified the presence of four cellular states, neural progenitor-like cells (NPC), oligodendrocyte progenitor-like cells (OPC), astrocyte-like cells (AC), and mesenchymal-like cells (MES) [81••]. Analysis of the patient-derived GLICO model developed by Linkous et al. revealed an enrichment in the NPC/OPC signature [68]. Furthermore, the enriched NPC cellular state was lost following organoid dissociation and analysis of the two-dimensional culture, providing more evidence as to the value of the tumor microenvironment in maintaining the diverse cellular states of glioma [68]. Despite the value of GSC-based organoid models, the dependence of the models on exogenous growth factors (EGF and bFGF), and the use of an artificial extracellular matrix rather than native extracellular matrix introduces laboratory conditions that could lead to drift in the cellular and molecular features of these cultures.

To maintain the native cellular hierarchy and tumor architecture, Jacob et al. successfully generated GBM organoids from minimally processed primary tissue samples [64••, 72]. The tissue is acquired from the operating room and taken directly to the laboratory, where it is parcellated into approximately 1-mm-diameter pieces [64••]. A critical advantage over prior techniques is the absence of single-cell dissociation, added growth factors, such as EGF and bFGF, or artificial Matrigel extracellular matrix. After 1 week in culture, the tissue formed well-rounded spheres, and within 2–4 weeks a mature and cell-rich organoid is achieved [64••, 72]. Importantly, the GBM organoids retained histopathologic features of their parent tumor including nuclear atypia, hypoxia gradients, and high Ki67 indices [64••]. Similarly, organoids retained their intracellular heterogeneity, maintaining CD31+ vascular cell populations, markers for glial cells such as GFAP and S100B, and markers of neural progenitor cells and GSCs such as DCX, NESTIN, SOX2, and OLIG2 [64••]. Furthermore, the GBM organoids retained features at the 4-week time point, as well as after freezing and reanimation, indicating the ability to create clinically relevant biobanks [64••, 72]. Given the profound inter-cellular heterogeneity seen in GBM, RNA, and exome sequencing analyses were performed on the PDOs and revealed similarities in gene expression, copy number variants, and somatic variants [64••]. The GBM PDOs also retained tumor subregion features, including mutations in *PTEN* and *EGFR* from different regions of the same primary tumor, indicating their ability to maintain intra-tumoral heterogeneity [64••, 72].

Recently, our group successfully generated the first PDO models of lower-grade glioma (LGG), including CNS WHO

grade 1–3 disease [73••]. With modifications to the minimally processed glioma sample approach, namely organoid culture at intracranial physiologic oxygen tension, we demonstrated a 91% success rate in all grades of glioma organoid formation and an 87% success rate for grade 1–3 tumors [73••]. As such, the high fidelity of the workflow is unlikely to systematically exclude subsets of tumors from being made into organoids. The basis for these modifications was the extracellular environment of the intracranial vault, wherein oxygen tension is shown to be less than ambient room air [82–84]. These findings are consistent with the role of hypoxia in promoting GSC expansion [85]. While grade 4 tumors have likely reached a mutational burden that limits their sensitivity to perturbations of environmental conditions, we hypothesized that lower-grade tumors require culture conditions that better mirror physiologic conditions in the human brain [73••]. Additionally, in vitro culture conditions that better recapitulate physiologic conditions may produce more biologically relevant results. Similar to prior PDO models of GBM, LGG organoids maintained markers of stemness, proliferation, and vascularity. Genomic and metabolomic analyses of the organoids demonstrated maintenance of common mutations in LGG, such as those in *IDH1/2*, *TP53*, *NOTCH1/2*, *CIC*, and *ATRX*, retention of copy number variations, and similar levels of the IDH1-R132H oncoprotein and the oncometabolite 2-hydroxyglutarate [73••]. There is emerging data, particularly in glioma, that tumors harbor significant differences in biological features at the time of recurrence following treatment than at diagnosis [86, 87]. However, most existing models are derived from end-stage disease, given that these highly aggressive tumor cells are most likely to grow in vitro or in xenografts. Thus, treatment-naïve models of LGG can be used to study glioma biology independent of the effects of prior therapy.

While recent advances in GBM organoid models are promising, questions regarding the applicability of these models to studying some key aspects of glioma biology remain. For example, Jacob et al. reported an organoid success rate for IDH-wildtype tumors > 90% while IDH1-mutant tumors were generated at a much lower success rate of 66.7% [64••]. Furthermore, detailed characterization of the vascular and immune cell compartments in minimally processed samples is not yet available. While markers of vascular endothelial and immune cells are maintained in minimally processed glioma PDOs, it is possible that these non-glioma cell populations are not maintained as efficiently in long-term cultures relative to glioma cells. Contrary to cell-line models or GSC-derived organoids, minimally processed patient-derived LGG organoids cannot be expanded over time, thereby limiting the scale of experiments that can be performed with each model. Thus, while they present an excellent model for personalized oncology screening, they may not support high-throughput experimental approaches. Despite these limitations, the remarkable fidelity of PDOs underscores their utility for studying glioma biology in the laboratory setting.

Applications to Personalized Oncology and Research

Ideal patient-derived cancer models are reliable, moderate- to high-throughput, and able to replicate the cellular and molecular phenotypes of their parent tumors. Current in vitro models are limited in their clinical utility due to the homogeneity of the tumor cell populations and long induction times. Conversely, the relatively rapid rate at which PDO models can be generated for many cancers enables experimentation within a clinically relevant timeline. Three-dimensional organoid architecture permits tumor-tumor and tumor-stroma interactions that are critical for the development of cellular hierarchies and tumor cell proliferation. The ability of PDOs to retain the genetic and transcriptomic signatures of their parent tumors allows them to act as patient avatars and may provide value for preclinical drugs screens [88, 89]. Growing evidence for the reliability and efficiency of PDOs as preclinical translational models supports potential uses of these models as avatars in oncology trials. If successfully implemented, one could use tissue from a patient's biopsy or resection specimen to create a faithful PDO, screen this PDO across multiple treatments including systemic therapies and/or radiation, and use the information from this screen to guide personalized treatment recommendations for the patient. Together with established tumor characterization methods and treatment pipelines, a comprehensive report of histopathologic features, genetic alterations, and responsiveness of PDOs to various therapies has the potential to guide evidence-based and patient-specific oncology care (Fig. 2).

Critical to PDO use in personalized clinical drug screens are reliable, efficient, and timely protocols for organoid generation. Given that the primary tissue sample is a limiting resource, any PDO model must prioritize high success rates for organoid creation. While there is variability between models and cancer types, success rates for producing organoid models are generally higher than for producing cell lines, ranging from 60 to 95% [42•, 50, 64••, 73••, 90–92, 93•, 94]. Additionally, to generate the appropriate number of replicates for organoid-based drug screens, successful splitting and expansion of organoids from a single sample expand their use and longevity [95]. Compared with the time to generate and assay patient-derived xenografts and genetically engineered mice, most organoid models can be established in less than four weeks [96, 97]. Prior studies have completed rapid comprehensive drug screens, with Yan

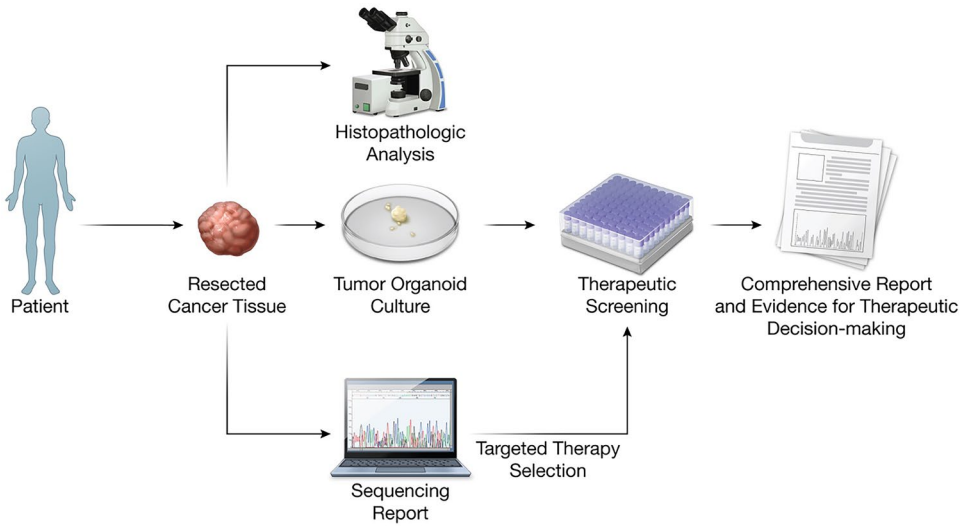

Fig. 2 Integrative model of histopathology, advanced genomic sequencing, and therapeutic response of patient-derived organoid models for precision oncology care

et al. screening responses of PDOs of gastric cancer to 37 drugs in under 2 weeks, ideal for timely prediction of patient responses to treatment [45, 98]. Tiriac et al. outline a similar timeline for drug screening utilizing PDOs of pancreatic cancer, with organoid creation to screening being completed in around 6 weeks [99].

Several studies have examined the feasibility of PDOs in conducting patient-specific drug screens [42•, 47, 48, 64••, 93•, 94, 100–104]. Wetering et al. utilized colorectal cancer organoids to screen 83 therapeutics, including chemotherapies and targeted therapies for treatment response [102]. Jacob et al. targeted tumor-specific genetic mutations affecting EGFR, NF1, PI3K, and EGFRvIII using gefitinib, trametinib, and everolimus, respectively, and used EGFRvIII-specific CAR-T cells to target organoids carrying this mutation [64••]. Sachs et al. screened 6 drugs targeting EGFR and HER2 signaling pathways, including downstream inhibitors of PI3K, AKT, and mTORC1/2, in their breast cancer PDOs [42•]. Furthermore, cancer organoids are shown to respond to treatment based on their genetic alterations, as seen with BRCA2-mutant organoids responding to olaparib, EGFR-mutant organoids to erlotinib, and EGFR-mutant/MET-amplified organoids to crizotinib [94]. Additional PDO-based drug screens have been conducted in bladder, ovarian, endometrial, pancreatic, lung, and other gastrointestinal cancers, among others [44, 45, 47, 93•, 94, 99, 105, 106]. Similar to parent tissue, PDOs retained features of chemoresistance and radioresistance, providing faithful representation of the parent tumor phenotype [42•, 67]. Furthermore, many studies cite the ability of their PDO models to be biobanked and later reanimated, thus generating stored clinical databases for future screening and analysis [42•, 45, 64••, 102, 107]. From a clinical standpoint, the ability to create and screen organoids in less than 6 weeks is optimal as it allows PDO formation and screening to occur in the period between surgical resection and initiation of subsequent therapy [108]. In the case of GBM, the drug screening protocol can overlap with the receipt of the standard of care temozolomide chemotherapy and radiotherapy and guide additional therapeutic considerations upon recurrence or progression [109].

Conclusions

Recapitulation of the three-dimensional native tissue structure, maintenance of molecular and cellular heterogeneity, and rapid generation of patient-derived models present significant advantages for organoids relative to classical cancer models. The advent of organoid development from human cancer samples, either by isolation of cancer stem cells or minimally processed primary tissue specimens, has paved the way for PDO models. Relative to other cancer models, the rapid speed of generation and more faithful representation of human cancer offer new opportunities for personalized oncology. Over the course of the last decade, cancer organoid models have been shown to retain histopathologic and molecular features of their primary tumors, as well as demonstrating treatment responses akin to those of the parent tumor. As such, PDOs are a potential model for high-throughput drug screens to guide the selection of oncologic therapies. The translational implications for PDOs are most apparent in cancers with limited therapeutic options and those characterized by patient-to-patient heterogeneity, diverse intra-tumoral cellular states, and complex tumor microenvironments integral to tumor behavior, as illustrated here in our discussion of malignant glioma. Despite the promising prospects of current studies, the use of PDOs in clinical oncology care is not without drawbacks. Variability in organoid generating methodologies, wide ranges of

organoid creation success rates, and incomplete characterization of non-tumor cell populations in organoid models are key limitations. While there is a need for standardization of PDO models, current data suggest their continued expansion and utility as translational cancer models. PDOs are poised to support the development and implementation of personalized oncology treatment programs.

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Declarations

Conflict of Interest The authors declare no competing interests.

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• Of importance
•• Of major importance

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