

REVIEW ARTICLE

Integrating patient-derived organoids and multi-omics to decode spatiotemporal therapeutic resistance

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Abstract

Intratumoral heterogeneity, alongside the dynamic evolution of cancer cells, continues to pose principal obstacles to efficacious cancer therapies. Although patient-derived organoids (PDOs) represent the gold standard for recapitulating patient-specific histopathology, their full utility emerges only through integration with high-resolution molecular profiling. This review synthesizes recent progress in combining PDOs with multi-omics approaches—including genomics, single-cell transcriptomics, and spatial omics—to elucidate the underpinnings of therapeutic resistance. We initially explore how organoid genomic profiling can monitor clonal dynamics and subclonal selection under therapeutic pressure. Next, we underscore the contributions of single-cell RNA sequencing in delineating transcriptional plasticity, detecting infrequent drug-tolerant persister populations, and charting non-genetic adaptive pathways. Of particular importance, we address the nascent domain of spatial transcriptomics, which preserves the structural integrity of organoids to uncover how proximate cell-cell interactions and niche elements shield tumor cells from therapeutic insult. Through the fusion of these multifaceted datasets, we advance a novel paradigm that frames resistance not as a fixed binary state, but as a spatiotemporally evolving adaptive phenomenon. The review culminates in delineating the translational promise of this synergistic paradigm for devising combination regimens that concurrently address genetic aberrations and adaptive microenvironments.

Keywords: Patient-derived organoids; Multi-omics profiling; Therapeutic resistance; Spatiotemporal heterogeneity; Clonal evolution; Single-cell RNA sequencing

1. Introduction

Drug resistance remains a principal factor leading to the failure of various cancer treatments, thereby limiting the long-term efficacy of targeted therapy, chemotherapy, and immunotherapy.^{1,2} Intratumoral heterogeneity is a key driver of this phenomenon, enabling tumors to dynamically adapt to the therapeutic selective pressure through genetic evolution and non-genetic plasticity.³ These adaptive mechanisms evolve over time and within the spatially

heterogeneous tumor microenvironment, promoting the emergence, survival, and ultimately the progression of drug-resistant subpopulations.⁴ Consequently, there is an increasing focus on viewing drug resistance as a dynamic, spatiotemporal process amenable to clinical intervention, rather than as a static molecular phenotype or state.⁵

Although precision oncology has been widely applied in clinical practice, most current drug resistance-related biomarkers are established primarily through overall tumor

analysis at a single pretreatment time point. This strategy, which relies on static, population-average signals, is insufficient to accurately depict the mechanisms underlying the emergence and maintenance of drug resistance and therefore has significant limitations in predicting long-term therapeutic responses and acquired drug resistance.^{6,7} In contrast, drug resistance in tumors is not a fixed molecular event but a dynamic process driven by the continuous evolution of resistant clones, reversible cellular state transitions, and spatially constrained microenvironments.⁸ Primary resistance arises from genetic or epigenetic heterogeneity present before treatment, while acquired resistance gradually develops through clonal selection and adaptive reprogramming under therapeutic pressure; together, these processes shape the spatiotemporal evolution of the resistant phenotype.⁹ Moreover, traditional preclinical models, such as two-dimensional *in vitro* cell line systems and genetically engineered mouse models, often lack patient-specific fidelity and scalability, thereby limiting their translational value in personalized treatment selection and drug resistance prediction.¹⁰

Patient-derived organoids (PDOs) have emerged as a highly clinically relevant three-dimensional (3D) *in vitro* model system that largely preserves the histopathological features, key genomic alterations, and treatment response profiles of the primary tumor.¹¹ Importantly, the establishment timeline of PDOs can be aligned with the clinical decision-making window, rendering them useful not only for basic research on drug-resistance mechanisms but also for applications with substantial clinical translational potential.¹² Moreover, this system supports standardized, scalable drug-sensitivity and drug-resistance screening, providing a robust experimental platform for optimizing individualized treatment strategies and functionally validating drug-related molecular events.¹³ When combined with multi-omics technologies, including genomic analysis, single-cell transcriptomics, and spatial-resolution analysis methods, PDOs enable systematic and functional interrogation of drug resistance mechanisms and evolution trajectories that are often inaccessible in routine clinical samples due to limited sampling or insufficient temporal and spatial resolution.^{14–16}

In this review, we focus on the potential applications of PDO-based platforms integrated with multi-omics analyses to address clinically relevant challenges, including the discovery of predictive and adaptive biomarkers,

patient stratification based on tumor evolution risk, and the design of rational combined treatment strategies to prevent or overcome treatment resistance. We further discuss recent evidence supporting the use of longitudinal tracking and spatial resolution analysis of PDOs to simulate the tumor evolution trajectory under treatment pressure and the formation of therapy-protective microenvironments. Finally, we highlight the unique advantages of this integrated framework in bridging static molecular profiling and functional precision oncology, thereby providing a theoretical and technical foundation for the development of more durable and individualized cancer treatment strategies.

2. Patient-derived organoids for modeling tumor evolution

2.1. Establishment and high-fidelity features of patient-derived organoids

Patient-derived organoids are typically constructed from primary or metastatic tumor tissues obtained through surgical resection or biopsy, thereby establishing a direct and clear correspondence between *in vitro* models and the specific patient.¹¹ With the standardization of tissue dissociation procedures, continuous optimization of culture media, and systematic definition of key growth factor combinations, the success rate and reproducibility of PDO establishment across various cancer types have significantly improved.^{17,18}

Notably, the efficiency and biological fidelity of PDO establishment are strongly influenced by the histopathological characteristics of the originating tumor (Table 1). In general, epithelial-derived malignancies are particularly well suited for organoid modeling because PDO cultures effectively preserve epithelial architecture and lineage-specific differentiation programs.¹⁹ Gastrointestinal malignancies, including colorectal, gastric, pancreatic ductal adenocarcinoma, and hepatobiliary tumors, represent some of the most successfully modeled cancer types, largely due to their glandular or ductal epithelial organization and well-defined stem cell hierarchies.^{20,21} Similarly, epithelial tumors such as lung adenocarcinoma and several breast cancer subtypes, including hormone receptor-positive and human epidermal growth factor receptor 2-positive tumors, have demonstrated high organoid establishment success rates while maintaining histopathological and genomic fidelity.^{22–24}

Table 1. Histopathology-dependent suitability of tumor types for patient-derived organoid (PDO) modeling

Tumor type	Histopathological characteristics	PDO establishment efficiency	Key advantages of organoid modeling	Major challenges	Typical research applications	Ref
Colorectal cancer	Glandular epithelial architecture with well-defined intestinal stem cell hierarchy	High	Strong preservation of tumor heterogeneity and differentiation gradients	Moderate stromal and immune cell loss during culture	Drug sensitivity testing, clonal evolution, and resistance mechanism studies	25,26
Gastric cancer	Mixed glandular and diffuse epithelial subtypes	High-moderate	Maintains subtype-specific differentiation programs and genomic features	Diffuse-type tumors may show variable growth stability	Molecular subtype stratification, targeted therapy screening	27,28
Pancreatic ductal adenocarcinoma	Ductal epithelial origin with dense desmoplastic stroma	High	Faithfully reproduces genetic drivers and chemoresistance phenotypes	Limited representation of stromal and immune microenvironment	Chemotherapy resistance modeling, metabolic dependency studies	29,30
Hepatobiliary tumors (hepatocellular carcinoma, cholangiocarcinoma)	Hepatic epithelial or biliary ductal differentiation	Moderate-high	Retains metabolic heterogeneity and signaling pathway diversity	Variable growth efficiency depending on differentiation status	Precision drug screening, immunotherapy response prediction	31-33
Lung adenocarcinoma	Glandular epithelial differentiation with diverse oncogenic drivers	High	Preserves oncogenic mutations and signaling pathway heterogeneity	Limited immune and vascular compartment representation	Targeted therapy resistance modeling, genomic-driven treatment selection	34
Breast cancer (hormone receptor-positive/human epidermal growth factor receptor 2 [HER2]-positive)	Epithelial lineage with hormone-dependent differentiation	Moderate-high	Maintains receptor signaling and treatment response characteristics	Hormonal microenvironment may be incompletely reproduced	Endocrine therapy testing, HER2-targeted therapy evaluation	35
Triple-negative breast cancer	High intratumoral heterogeneity with variable differentiation states	Moderate	Captures aggressive tumor subpopulations and plasticity states	Requires co-culture systems to preserve immune and stromal interactions	Drug tolerance and tumor plasticity research	36,37
Melanoma	Neural crest-derived lineage with strong immune interaction	Moderate	Captures tumor-immune interaction potential when co-cultured	Requires immune cell incorporation for functional relevance	Immunotherapy response modeling	38
Sarcomas	Mesenchymal origin with low epithelial content	Low	Can model specific lineage differentiation programs in selected subtypes	Difficult long-term expansion and structural maintenance	Rare tumor biology studies, lineage differentiation research	39
Hematological malignancies	Lacks three-dimensional epithelial architecture	Limited	Emerging organoid-like systems allow partial microenvironment modeling	Traditional PDO systems are poorly applicable	Bone marrow niche modeling, immune microenvironment studies	40

In contrast, tumors characterized by extensive stromal composition or mesenchymal differentiation, such as sarcomas and highly desmoplastic tumors, often present greater challenges for stable organoid generation due to reduced epithelial tumor cell fractions and strong dependence on microenvironmental support.^{41,42} Hematological malignancies are also less amenable to conventional organoid culture systems because they lack the 3D epithelial architecture found in these systems.^{43,44} Furthermore, histopathological subtypes characterized by pronounced immune infiltration or extreme intratumoral heterogeneity, such as triple-negative breast cancer or certain melanoma subtypes, can be modeled using PDOs but often require advanced co-culture strategies to preserve microenvironmental complexity and enhance biological relevance.^{45–47} These histopathology-dependent differences highlight the importance of tumor-specific optimization of organoid culture conditions and provide a biological rationale for interpreting PDO-based therapeutic studies.

Importantly, despite these differences in establishment efficiency, PDOs generally retain the genetic landscape, clonal architecture, and phenotypic heterogeneity of the original tumor, making them powerful platforms for investigating tumor evolution and therapeutic resistance.^{48,49} Preservation of these key biological characteristics enables PDOs to serve as reliable preclinical models for functional drug screening and multi-omics profiling, thereby

facilitating translational research and precision oncology applications.

Numerous clinically relevant validation studies have shown that the drug sensitivity and resistance profiles of PDOs closely mirror patients' treatment responses and prognosis, supporting their feasibility and reliability as a platform for predicting treatment outcomes and optimizing treatment plans.^{48,50} These features make PDOs an ideal system for integrating multi-omics technologies, including genomics, transcriptomics, and spatially resolved analyses, especially for studies of tumor evolution and drug resistance mechanisms that require high-fidelity patient-matched materials (Figure 1).

Compared with traditional tumor cell lines, PDOs more completely preserve key features of tumor heterogeneity, including coexisting genetic subclonal structures and stratified cell-state lineages. This retention of clonal complexity provides a necessary foundation for genomic analyses of mutation accumulation, clonal expansion, and subclonal selection under therapeutic selection pressure.⁵¹ At the same time, the coexistence of multiple phenotypes and transcriptional states in the organoid system makes it an ideal model for single-cell transcriptomic studies to analyze cell plasticity and state transitions.⁵² Moreover, the 3D organizational structure of PDOs provides a natural basis for spatially resolved analyses, enabling spatial omics techniques to systematically depict cell–cell interactions

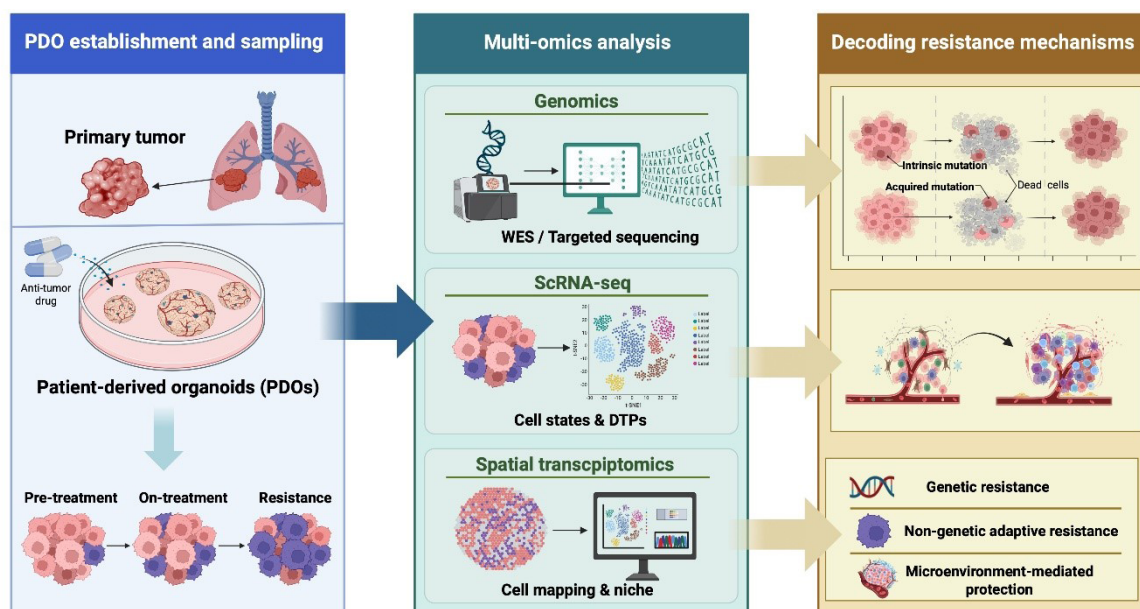


Figure 1. Overview of a PDO-based multi-omics framework for investigating therapeutic resistance. Created with Biorender.com. Gao, C. (2026) <https://app.biorender.com/illustrations/694f8ab3fee8f9608e499616>.

Abbreviations: DTPs: Drug-tolerant persister cells; PDO: Patient-derived organoid; scRNA-seq: Single-cell RNA sequencing; WES: Whole-exome sequencing.

and the tumor microenvironment's role in shaping treatment responses and drug resistance.⁵³

Patient-derived tumor tissues are used to generate organoids that preserve tumor heterogeneity and treatment response. Longitudinal drug exposure enables temporal sampling. Genomic profiling tracks clonal dynamics, single-cell RNA sequencing (scRNA-seq) resolves transcriptional states, and spatial transcriptomics preserves tissue architecture and microenvironmental context. Integration of these datasets enables spatiotemporal characterization of resistance evolution.

2.2. Longitudinal drug exposure and evolutionary modeling in patient-derived organoids

Patient-derived organoids can be exposed to clinically relevant treatment regimens in controlled *in vitro* conditions, enabling longitudinal simulation of therapeutic exposure. This provides a highly controlled experimental system for modeling the evolution of tumor resistance driven by therapeutic stress. By longitudinal sampling of organoids during drug intervention, researchers can obtain well-defined, time-resolved materials. Combined with population-level and single-cell genomic analyses, these samples can be used to reconstruct clonal dynamics, the selection process, and the trajectory of resistance evolution^{54,55} (Figure 2). At the same time, longitudinal scRNA-seq captures transient adaptive transcriptional programs that precede the establishment of stable resistance phenotypes, as well as the subsequent persistent tolerance state that maintains resistance.⁵⁶ Further, when longitudinal

experiments are integrated with spatial transcriptomics analyses, the way in which therapeutic stress reshapes the spatial organization, cell–cell interactions, and therapy-protective microenvironments within organoids over time can be systematically analyzed.⁵⁷

Multiple tumor subclones coexist at baseline. Drug treatment suppresses sensitive clones while enriching resistant subclones, followed by clonal fixation through secondary genetic alterations. Longitudinal sequencing of PDOs recapitulates clinically relevant patterns of intrinsic and acquired resistance.

3. Genomic and single-cell profiling of organoid evolution

3.1. Genomic tracking of clonal selection under therapy

Systematic genomic analysis of PDOs enables longitudinal tracking of pressure-driven clonal selection and the evolutionary processes under controlled conditions. By conducting continuous whole-exome sequencing or targeted deep sequencing on PDOs exposed to clinically relevant drug treatments, researchers have observed a range of typical evolutionary events, including selective amplification of pre-existing drug-resistant subclones, the emergence of treatment-induced secondary mutations, and reproducible evolutionary trajectories in different patient samples (Figure 2). For example, in studies on targeted therapy and chemotherapy, the PDO model successfully reproduced the enrichment of common drug resistance-

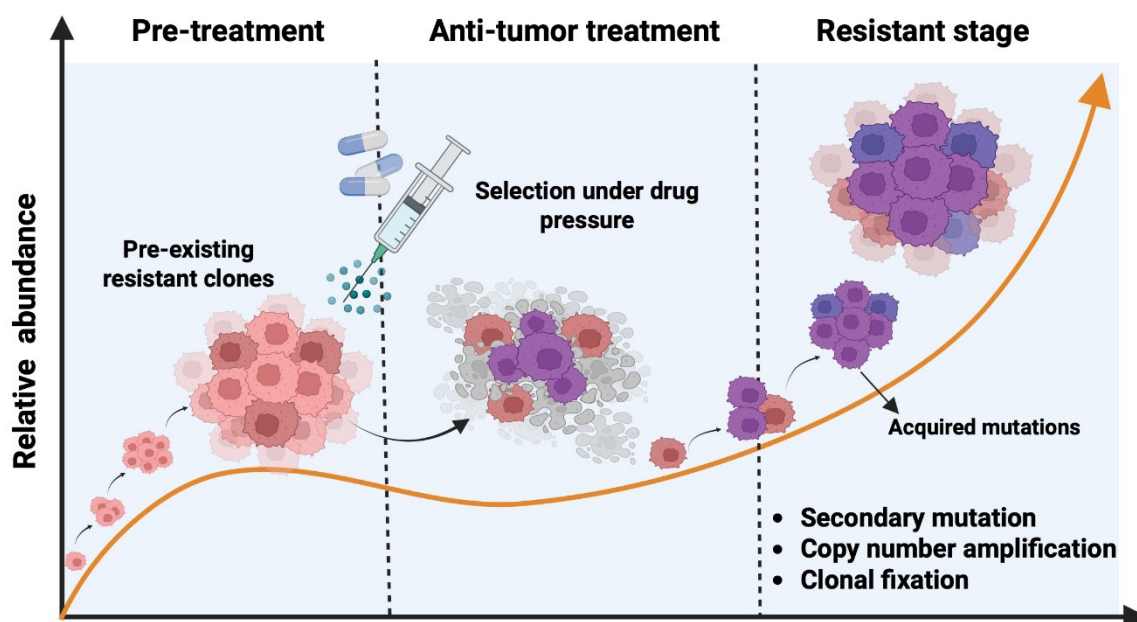


Figure 2. Genomic tracking of clonal selection in drug-treated patient-derived organoids. Created with Biorender.com. Gao, C. (2026) <https://app.biorender.com/illustrations/694f9c04fee8f9608e5b9bc2>.

driving mutations observed in clinical settings and revealed dynamic changes in which low-frequency subclones rapidly dominated the population under treatment pressure.^{52,58} Additionally, longitudinal PDO sequencing also identified drug resistance mutations that were undetectable before treatment but became dominant in recurrent samples, providing direct evidence for “hidden drug-resistant clones.”^{59,60}

This type of genomic tracking analysis provides actionable clues for clinical decision-making, enabling researchers to distinguish intrinsic drug resistance mediated by baseline genetic variations from acquired drug resistance gradually formed during treatment.^{61,62} More importantly, integrating genomic-level clonal evolution information with functional drug response data from the PDOs has been used to explore and validate adaptive treatment strategies. Relevant studies have shown that this integrated analysis can support early treatment plan adjustments or provide a basis for jointly blocking potential drug-resistant clones before clinical recurrence occurs,

thereby providing experimental and theoretical support for delaying or avoiding the occurrence of drug resistance.⁶³

3.2. Single-cell RNA sequencing to resolve transcriptional plasticity and drug-tolerant persister cells

Recent studies have shown that although genomic alterations play a central role in some resistance mechanisms, non-genetic transcriptional plasticity is equally crucial in tumor adaptation during treatment.⁶⁴ Drug-tolerant persister (DTP) cells represent a subgroup that enters a reversible tolerance state under drug stress. Their survival does not rely on stable genetic mutations but is primarily mediated by transcriptional reprogramming and epigenetic regulation, thereby providing a transitional “reservoir” for the subsequent emergence of acquired resistance.^{65,66} scRNA-seq enables the identification of rare DTP cells in PDOs or clinical samples.⁶⁷ These cells survive drug stress through reversible and adaptive transcriptional programs, and their emergence typically precedes the formation of stable

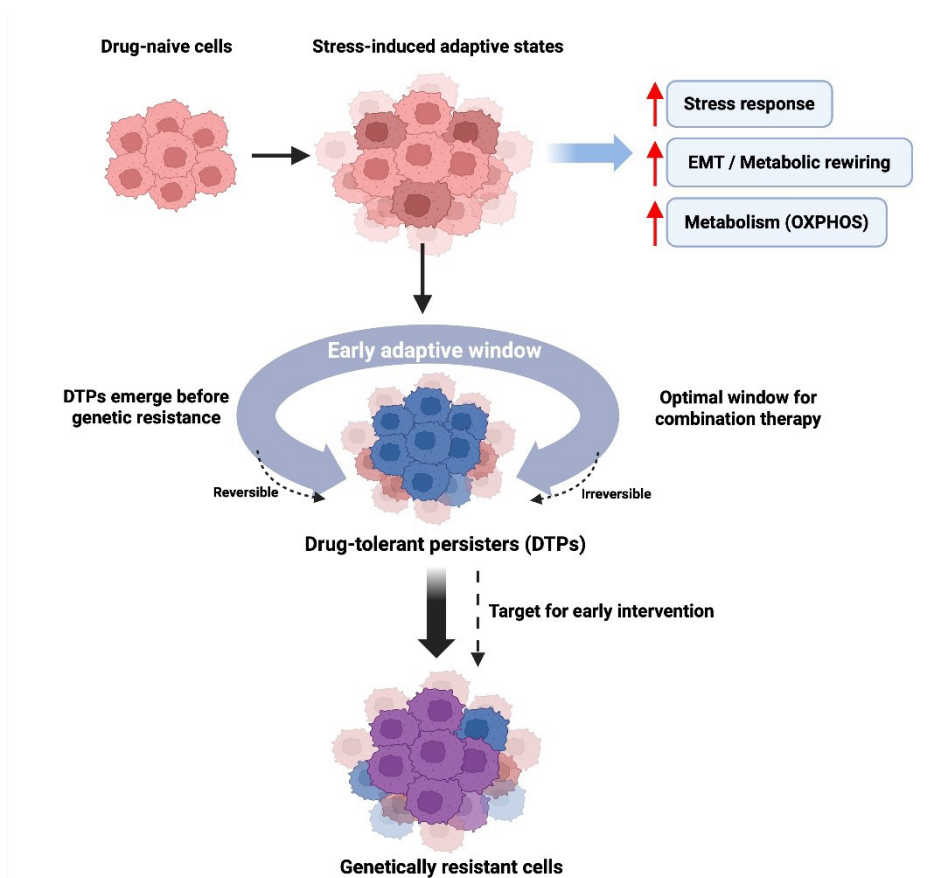


Figure 3. Single-cell analysis reveals adaptive transcriptional states preceding genetic resistance. Time-resolved scRNA-seq identifies stress-induced adaptive states and DTP cells in treated PDOs. These transient states support survival under therapy and may transition into stable genetically resistant populations, highlighting early adaptive phases of resistance. Created with Biorender.com. Gao, C. (2026) <https://app.biorender.com/illustrations/694fa35d234c7f0d7c1aedc7>.

Abbreviations: DTP: Drug-tolerant persister; EMT: Epithelial–mesenchymal transition; OXPHOS: Oxidative phosphorylation; PDO: Patient-derived organoid; scRNA-seq: Single-cell RNA sequencing.

genetic resistance phenotypes. As such, they are regarded as important potential targets for early intervention and resistance blockade^{65,68} (Figure 3). Based on the PDO-based drug resistance model, the identification and functional analysis of DTP cells have become a research hotspot. For instance, in colorectal cancer PDOs, scRNA-seq combined with machine-learning strategies successfully defined the DTP cell subpopulations and was subsequently used to screen candidate drugs for combination with targeted therapy, providing experimental evidence for inhibiting tolerant cells and enhancing treatment responses.

The identification and characterization of DTP cells not only contribute to understanding the early non-genetic transcriptional plasticity mechanisms of drug resistance but also provide a crucial opportunity for early intervention before the complete formation of drug resistance in clinical settings. Early monitoring of DTP cells or their transcriptional characteristics may serve as biomarkers to predict minimal residual disease and the future risk of drug resistance, thereby guiding strategies such as drug combination or timing optimization to delay or prevent the progression of drug resistance, and improve treatment durability and patient prognosis.⁶⁶

3.3. Temporal trajectories of adaptive states

One of the major advantages of PDOs is their ability to recreate the dynamic adaptation and drug-resistance evolution trajectory of tumors under therapeutic stress in a controllable environment. Through longitudinal sampling, time-resolved scRNA-seq, and the integration of clonal lineage information, researchers were able to systematically depict the continuous evolutionary process from the initial untreated cells through DTP cells to the formation of a stable genetically resistant population.⁶⁹

Previous studies have used scRNA-seq to reconstruct the lineage evolution of drug-resistant cells in gastric cancer, analyze dynamic changes in key driver genes during treatment, and demonstrate that targeting these genes can enhance sensitivity to docetaxel.⁷⁰ Similarly, in colorectal cancer PDOs, scRNA-seq identified drug-resistant cell populations associated with enhanced oxidative phosphorylation and adenosine triphosphate metabolism, as well as potential drug-resistant genes and transcription factors, revealing characteristic transcriptional states related to drug resistance.⁷¹

Overall, these studies demonstrate that integrating the PDOs platform with high-resolution omics technologies not only enables the reconstruction of the temporal trajectory and cellular state transitions of treatment adaptation across various tumor types but also provides important insights for clinical translation. By identifying the critical window periods for the emergence of drug resistance, combined

or sequential interventions targeting the metabolic or signal-dependent vulnerabilities of early DTP cells can be implemented before resistance genes become fixed, thereby delaying or even preventing the development of drug resistance.

4. Architectural determinants of resistance development in spatial omics

4.1. Application of spatial transcriptomics in patient-derived organoids

The rapid development of spatial transcriptomics technology enables researchers to obtain spatial information at the whole-transcriptome level while preserving the 3D organizational structure of PDOs, thereby enabling *in situ* analysis of the relationship between tumor cell states and their physical location.^{72,73} By retaining the spatial coordinates of RNA in tissue sections, spatial transcriptomics achieves the joint mapping of gene expression and tissue structure, overcoming the limitation of scRNA-seq that loses spatial information during tissue dissociation, and revealing the regional distribution of different cell states and molecular programs in the local microenvironment of tumors, such as tumor invasion frontiers, immune infiltration areas, or hypoxic zones, which have clear biological significance.^{74,75}

Further integrating spatial transcriptomic data with scRNA-seq enables not only precise localization of key drug-resistant subpopulations within PDOs but also analysis of *in situ* interactions and signaling among tumor cells, stroma, and immune cells (Figure 4). This provides a new perspective and experimental evidence for understanding the spatial heterogeneity and formation of drug resistance under therapeutic stress.^{74,76} With the application of high-resolution spatial platforms such as Visium and Stereo-seq, spatial omics offers unique advantages for analyzing the spatiotemporal correlations between the architecture and molecular functions of the tumor microenvironment, and is gradually becoming an important tool in precision oncology for studying drug resistance mechanisms and guiding treatment strategy design.^{77,78}

Spatial transcriptomics maps transcriptionally distinct tumor cell populations to specific microenvironmental regions, including hypoxic and matrix-rich niches. Local cell-cell interactions and paracrine signaling contribute to the maintenance of drug-resistant states.

4.2. Spatial localization of plasticity and resistant cell states

Spatial transcriptomics indicates that cell states exhibiting transcriptional plasticity or drug-resistance phenotypes after treatment are not randomly distributed within the tumor tissue, but tend to be enriched in specific microenvironmental regions. Previous studies have shown

that in melanoma models, spatial transcriptomics research has revealed that different drug resistance states (such as stress state, lipid metabolism dominant state, or phosphoinositide 3-kinase (PI3K) signaling pathway activation state) exhibit significant spatial autocorrelation, forming specific enriched regions within the tissue and accompanied by different signaling pathway communication patterns. This indicates that the maintenance of drug resistance states is closely related to local microenvironmental signals.⁷⁹ Further, in human tumor samples and *in vivo* models, the combined analysis of spatial transcriptomics and scRNA-seq revealed the coexistence of drug-resistant cells with specific immune microenvironment characteristics (such as the degree of immune infiltration or the distribution of necrotic areas), indicating that the composition of the tumor microenvironment can directly affect the fate choice of drug-resistant cells.^{80,81}

Overall, these studies emphasize that the “hotspots” of drug resistance within tumors are often closely coupled with the local tissue structure and interactions with adjacent cells. For instance, hypoxic areas, matrix-rich regions, or immune exclusion zones frequently converge on specific populations of resistant cells. This understanding goes beyond the single-cell transcriptomic analysis paradigm that focuses solely on the cellular state, highlighting the significant role of spatial location in the formation of treatment sensitivity and protective microenvironments. From a translational medicine perspective, it suggests that targeting local drug-resistant microenvironments or intervening in cell interactions within specific spatial domains may become potential strategies for enhancing therapeutic efficacy (Figure 4).

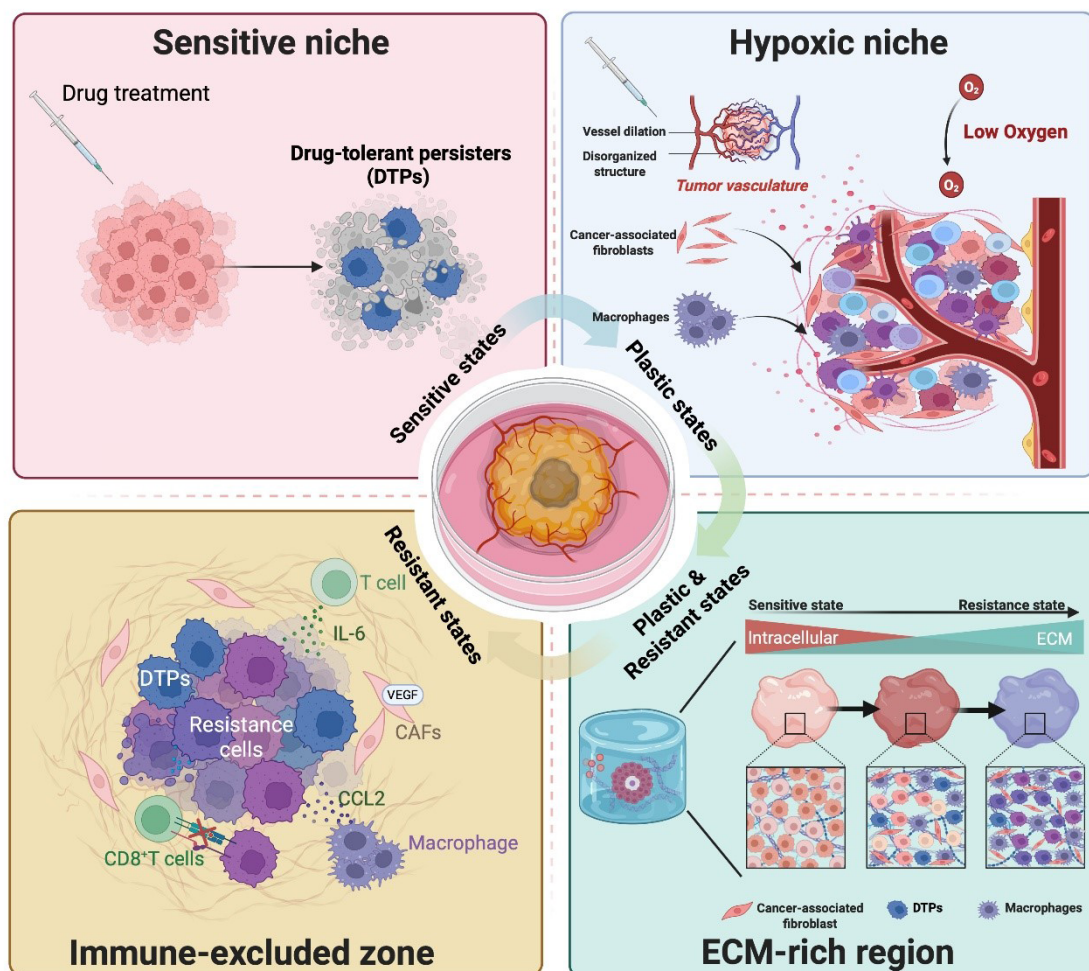


Figure 4. Spatial organization of resistant and plastic cell states within PDOs. Created with Biorender.com. Gao, C. (2026) <https://app.biorender.com/illustrations/6950ca3876e730d41eb73df9>.

Abbreviations: CAFs: Cancer-associated fibroblasts; CCL2: C–C motif chemokine ligand 2; ECM: Extracellular matrix; IL-6: Interleukin-6; PDO: Patient-derived organoid; VEGF: Vascular endothelial growth factor.

4.3. Niche effects, local interactions, and protection from therapy

Spatial omics studies have shown that drug resistance is not only influenced by the inherent program of tumor cells, but is also regulated by local microenvironmental signals and cell-to-cell interactions. Spatial transcriptomics and spatial multi-omics analyses have revealed that paracrine signals, metabolic gradients, and stress-response pathways form unique microenvironment ligand-receptor networks in specific tumor regions, which can promote the survival of drug-resistant cells. A study found that in spatial multi-omics research on pancreatic cancer, the abnormal co-expression of ligands and receptors between tumor cells and macrophages, as well as fibroblasts, was associated with malignant progression areas in the tumor and was significantly correlated with poor prognosis in patients. This indicates that the microenvironment signals synergistically promote drug resistance and disease progression.⁸²

In PDOs or clinical samples, these microenvironmental characteristics can temporarily protect tumor cells from drug action or promote adaptive transcriptional programs under therapeutic stress, which further explains why seemingly effective therapies cannot completely eliminate all cell populations in the overall analysis.^{83,84} Spatial multi-omics technologies not only depict the *in situ* interactions between tumor cells and their surrounding immune and stromal components, but also provide a novel framework for identifying localized signaling hubs within the microenvironment that facilitate drug resistance.^{74,85} These approaches offer important mechanistic insights that support the rational design of combination therapeutic strategies targeting protective microenvironmental niches.

However, despite these technological advances, current PDO systems still do not fully recapitulate key immune and vascular compartments of the tumor ecosystem. This limitation limits their capacity to fully model immune cell infiltration, vascular-mediated signaling, and dynamic nutrient or oxygen gradients, which are critical for shaping therapeutic responses and resistance evolution.^{86–88} Therefore, there is an increasing need to develop advanced multicellular co-culture systems and integrate PDOs with organ-on-a-chip and microfluidic technologies to more comprehensively reconstruct the architecture of the tumor microenvironment. Such platforms enable spatially organized multicellular interactions and provide controlled experimental conditions that more closely mimic *in vivo* tumor ecosystems.^{89,90} Continued refinement of these approaches is expected to enhance the physiological relevance and translational potential of PDO-based models for investigating therapeutic resistance and guiding precision oncology strategies.

5. Integrative multi-omics frameworks

5.1. Converging genomic, single-cell, and spatial datasets

Although genomics and single-cell sequencing offer advantages for analyzing tumor cell diversity and transcriptional plasticity, tissue dissociation inevitably loses the *in situ* spatial information of cells.⁹¹ Integrating genomics, single-cell transcriptomics, and spatial omics to apply them to PDOs can reconstruct tumor evolution across multiple dimensions, better meeting the needs of clinical translational research (Figure 5).

Genomic analysis reveals patterns of drug resistance mutations and clonal structure, single-cell transcriptomics interprets the dynamic plasticity of cell states, and spatial omics anchors these molecular states to the *in situ* tissue structure and microenvironment background.⁹² This multimodal strategy not only enhances the ability to analyze tumor heterogeneity but also distinguishes gene-fixed resistance, transcriptional adaptive tolerance, and microenvironment-mediated protective mechanisms.⁹³ Spatial multi-omics integration provides comprehensive data to understand the interactions between local cell states and the microenvironment, helping to reveal the mechanisms of drug resistance formation and supporting the development of comprehensive biomarkers, providing a basis for the design of precision treatment strategies.⁹⁴

Pre-existing tumor heterogeneity, therapy-induced transcriptional plasticity, and spatial niche-mediated stabilization collectively drive the emergence and fixation of resistant tumor populations. The model highlights key stages amenable to therapeutic intervention.

5.2. Computational strategies for multimodal integration

The successful integration and clinical application of genomic, single-cell, and spatial data rely on powerful computational frameworks capable of handling heterogeneous, multi-scale, and high-dimensional data.

Current strategies mainly focus on three directions: First, using joint embedding and alignment methods to map data from different modalities into a shared latent variable space, thereby identifying common structural and cell-state representations across omics within the same framework. Such methods include variational autoencoder (VAE) and deep generative models, graph embedding and graph neural network (GNN) models, and multimodal dimensionality reduction and co-embedding algorithms.^{95–97}

Second, developing trajectory inference and clonal evolution models to integrate longitudinal genomic data with cell-state transitions, allowing reconstruction of the temporal sequences and evolutionary paths under

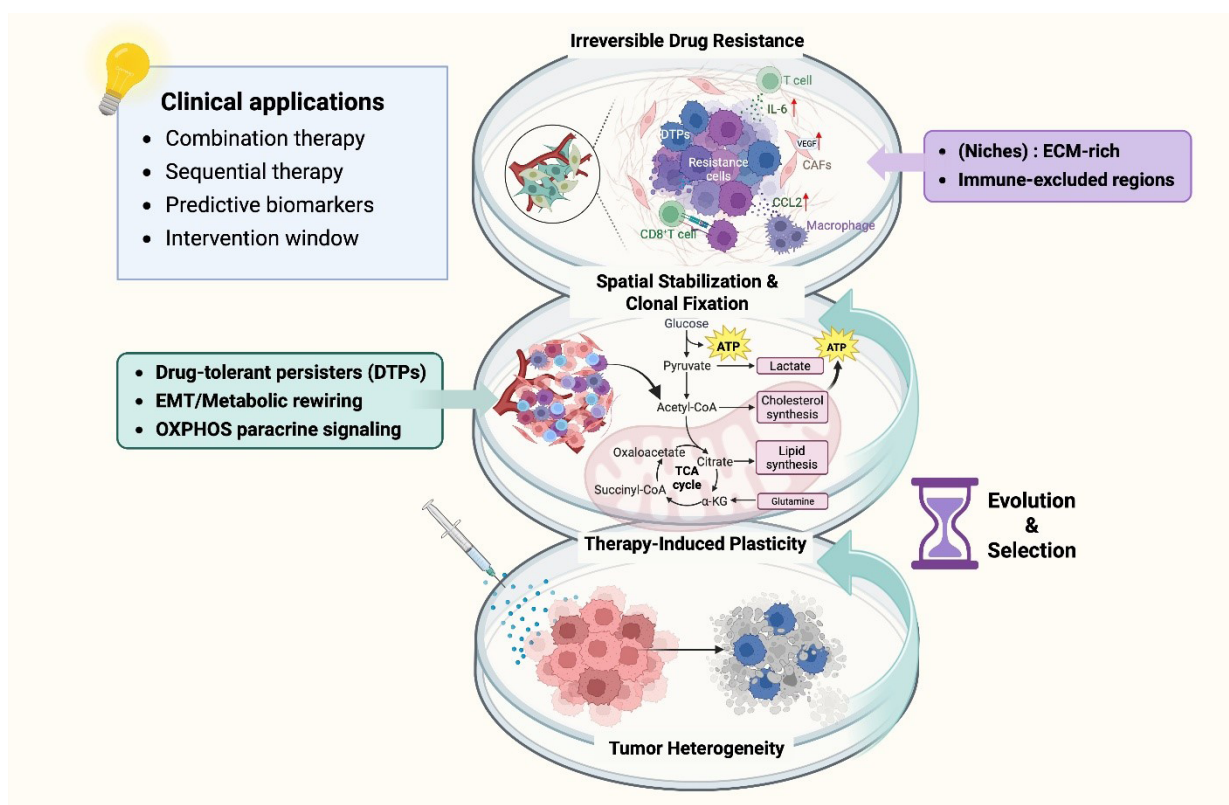


Figure 5. Conceptual model of therapeutic resistance as a spatiotemporally evolving process. Created with Biorender.com. Gao, C. (2026) <https://app.biorender.com/illustrations/695112528a2c3313a2629904>.

Abbreviations: ATP: Adenosine triphosphate; CAFs: Cancer-associated fibroblasts; CCL2: C–C motif chemokine ligand 2; ECM: Extracellular matrix; EMT: Epithelial–mesenchymal transition; IL-6: Interleukin-6; OXPHOS: Oxidative phosphorylation; TCA: Tricarboxylic acid cycle; VEGF: Vascular endothelial growth factor.

treatment responses.^{14,98,99}

Third, leveraging machine learning and deep learning frameworks (such as VAEs, GNNs, etc.) to address batch effects, modality differences, and high-dimensional sparsity issues, thereby making the connection between molecular features and drug response phenotypes more reliable and predictable.^{100,101}

Furthermore, emerging methods such as spatially integrated multi-omics integrate spatial and multiple single-cell modalities through probabilistic alignment strategies, enabling the depiction of multi-omics spatial patterns in complex tissues.¹⁰² Future research could focus on developing more reproducible multi-omics integration frameworks to more effectively convert these complex datasets into clinically applicable biomarkers and drug-resistance prediction models, while also addressing the data processing requirements of large-scale clinical samples.

5.3. Conceptual models emerging from patient-derived organoid-based studies

The integrative multi-omics analyses based on the PDO platform have led to a new conceptual model of drug

resistance. Drug resistance is not an isolated event resulting from a single mutation, but rather the gradual formation of an adaptive system that is interrelated in both time and space. In this model, the inherent heterogeneity of tumors provides the substrate; treatment pressure induces cellular transcriptional plasticity and multiple temporary adaptive states, which are subsequently stabilized within a specific spatial microenvironment, ultimately forming an irreversible drug-resistant population through clonal selection and genetic fixation.⁵² This model not only expands our understanding of the nature of drug resistance but also provides a mechanistic basis for explaining the common phenomenon of an initial therapeutic response followed by recurrence in clinical settings, thereby challenging the traditional strategies based on static single markers.¹⁰³ Research conducted on the PDOs model indicates that such 3D *in vitro* systems can better preserve the internal heterogeneity and microenvironmental characteristics of tumors, enabling them to reproduce the complex process of drug resistance evolution and to be used for systematic analysis of drug responses and elucidation of mechanisms. This provides an important experimental platform for precise anti-cancer drug screening, design of combination

therapies, and identification of the critical window of drug resistance.^{51,104,105}

Furthermore, the integration of multi-omics analysis based on the PDO platforms not only retains the heterogeneity and 3D structure of the primary tumor *in vitro*, but also reveals the molecular network and dynamic cellular state of the emergence of drug resistance through the combination of multiple levels of data, such as genomics, transcriptomics, epigenomics, and even proteomics. This enables a more comprehensive depiction of the evolution of drug resistance and the identification of potential intervention nodes. Compared with traditional *in vitro* cell lines or *in vivo* mouse models, this platform has greater clinical relevance, enabling the prediction of individual patient drug responses, optimization of combination therapy regimens, and providing a robust experimental foundation with strong translational potential for designing precise anti-tumor treatment strategies and implementing early intervention during the drug resistance window.⁵²

6. Translational implications

6.1. Informing rational combination and sequential therapies

Integrated multi-omics analysis of PDOs provides a functional framework for the rational design of combination and sequential therapeutic strategies. Multi-dimensional omics approaches enable simultaneous characterization of genetic determinants of drug resistance and treatment-induced adaptive cellular state transitions, thereby facilitating the identification of early vulnerability windows during therapeutic intervention. By integrating PDO-based drug screening with transcriptomic and clonal evolution analyses, researchers can identify persistent cell states that evade single-agent therapy and uncover survival programs driven by tumor microenvironmental cues. Because PDO models preserve the genetic heterogeneity of primary tumors, they are particularly valuable for uncovering reversible drug tolerance mechanisms and predicting resistance trajectories.⁶¹ For example, pancreatic cancer PDOs exhibit clone-specific chemotherapy sensitivity patterns that closely mirror the evolution of clinical resistance.¹⁰⁶ Similarly, single-cell transcriptomic analyses of colorectal cancer PDOs have revealed enrichment of resistant subpopulations characterized by adaptive transcriptional programs.¹⁰⁷ Furthermore, co-culture experiments incorporating fibroblasts have shown that microenvironmental signaling can directly influence drug sensitivity, highlighting the importance of targeting adaptive phenotypes, which often serve as early indicators of resistance development.^{108,109} Therapeutically targeting these early adaptive mechanisms or co-inhibiting them alongside primary oncogenic signaling pathways may prevent the establishment of irreversible resistance, thereby

improving treatment durability and clinical outcomes.^{110,111}

Longitudinal PDO modeling further enables dynamic evaluation of therapeutic response trajectories under sequential treatment regimens. By monitoring the time-dependent responses of organoids to different drug combinations, researchers can identify synergistic interactions and potential cross-resistance risks associated with specific treatment sequences. Such analyses provide critical data to optimize treatment order and to avoid therapeutic regimens that inadvertently promote the evolution of resistance in clinical settings.^{112,113} In parallel, emerging multi-omics-guided functional screening strategies are being used to construct personalized drug combination profiles by integrating PDO drug sensitivity data with genomic, transcriptional, and microenvironmental features, thereby offering quantitative guidance for individualized treatment optimization.^{114,115}

Importantly, recent studies have highlighted the translational value of PDO-based multi-omics integration in guiding the design of combination therapies across diverse cancer types. In pancreatic ductal adenocarcinoma, integrative transcriptomic and pharmacological profiling of PDOs has revealed distinct metabolic and signaling dependencies associated with chemotherapy resistance. These findings support combination strategies that pair standard chemotherapy with inhibitors targeting adaptive survival pathways, such as PI3K/protein kinase B signaling and oxidative stress response programs. These approaches have demonstrated enhanced therapeutic efficacy in preclinical validation models.^{116–118} Similarly, in colorectal cancer, single-cell transcriptomic profiling of PDOs has identified drug-tolerant subpopulations enriched for oxidative phosphorylation and stress-response signaling. This knowledge guides combination strategies that integrate cytotoxic chemotherapy with metabolic inhibitors or epigenetic modulators to eliminate tolerant cellular states and delay the emergence of resistance.^{24,119}

In non-small cell lung cancer, PDO-based genomic and transcriptomic analyses have uncovered co-occurring oncogenic drivers and adaptive signaling rewiring following targeted therapy exposure. These insights enable rational co-targeting strategies such as combining epidermal growth factor receptor inhibitors with downstream pathway inhibitors or microenvironment-modulating agents to suppress resistant subclonal expansion.^{34,58} In hepatocellular carcinoma, pharmaco-proteogenomic characterization of PDO biobanks has further facilitated the identification of patient-specific metabolic vulnerabilities and immune-related signaling pathways, supporting personalized combination regimens that integrate targeted therapies with immunomodulatory strategies.^{120–122}

Collectively, these findings highlight how PDO-based

multi-omics integration bridges mechanistic discovery and functional drug-response evaluation. By enabling the identification of cancer-type-specific vulnerabilities and adaptive resistance programs, PDO platforms provide a robust translational framework for optimizing combination therapy design and advancing precision oncology.

6.2. Identifying biomarkers of adaptive resistance

An important research direction in translational studies is the identification of biomarkers that can predict adaptive drug resistance before clinical recurrence. Traditional single markers often fail to accurately capture the complex process of drug resistance evolution. In contrast, multi-omics analyses can integrate clonal structure, transcriptional plasticity, and spatial microenvironment characteristics to identify more predictive composite biomarkers across multiple dimensions. By combining genomic, transcriptomic, proteomic, and even spatial omics analyses of tumor tissues or PDOs, candidate biomarkers related to drug-tolerance status, stress-response pathways, or protective local microenvironments can be identified—features that cannot be detected individually by traditional large-scale genomic analysis.¹²³ In particular, multi-omics strategies have been used to construct spatial “cell state signatures” related to immune treatment responses. These signatures integrate relevant cell types with their tissue locations, improving the prediction of efficacy and potential risk of immune checkpoint inhibitor therapies.^{124,125}

Furthermore, candidate biomarkers based on PDOs can be cross-validated and dynamically monitored using patient clinical samples (such as biopsies or liquid biopsy circulating tumor DNA [ctDNA]/circulating tumor cells), thereby supporting their use in monitoring treatment responses and guiding adaptive therapy adjustment.¹²⁶ For instance, combining proteogenomic analysis of a large-scale liver cancer PDOs biomaterial library has identified specific metabolic pathways and expression features that can precisely predict drug responses and inform combination therapy strategies.¹²⁰ The discovery and validation of these multimodal biomarkers provide comprehensive, clinically valuable tools for precisely predicting the evolution of drug resistance, optimizing treatment plans, and implementing early interventions.

6.3. Predictive potential and limitations in clinical settings

Although the multi-omics platform based on PDOs has shown significant potential for predicting treatment responses and analyzing resistance mechanisms, its current clinical application is limited by several factors. Firstly, the success rate of establishing PDOs varies significantly across tumor types, being strongly influenced by factors such as tumor cell content, stromal components, and sampling

quality, which poses challenges for the model’s usability and predictive consistency.^{119,120,127–129}

Secondly, conventional PDOs lack a complete tumor microenvironment (including immune and stromal cells) and vascular structure, which limits their ability to accurately predict therapies that rely on cell interactions (such as immunotherapy).^{130,131} This limitation is particularly critical when studying resistance to immunotherapeutic strategies, as immune checkpoint blockade and other immune-based treatments depend heavily on dynamic interactions between tumor cells and immune populations. Conventional PDO cultures are predominantly composed of epithelial tumor cells and therefore fail to fully recapitulate key biological processes, including immune cell infiltration, antigen presentation, cytokine-mediated communication, and vascular-mediated immune trafficking.^{87,88,90} The absence of these components restricts the ability of PDO models to capture immune-mediated mechanisms of adaptive resistance and reduces their predictive accuracy for immunotherapy response.

Recent technological advancements are beginning to address these challenges. Multicellular co-culture systems incorporating tumor-infiltrating lymphocytes, cancer-associated fibroblasts, macrophages, or endothelial cells have shown increasing capability in preserving tumor–immune and tumor–stroma interactions, thereby providing more physiologically relevant platforms for evaluating therapeutic responses.^{45,132} Furthermore, the integration of PDOs with organ-on-a-chip platforms and microfluidic technologies offers promising opportunities to reconstruct vascular perfusion, nutrient and oxygen gradients, and immune cell migration dynamics within controlled *in vitro* environments. These platforms enable spatially organized multicellular interactions and facilitate longitudinal monitoring of treatment responses under conditions that more closely resemble *in vivo* tumor ecosystems.^{86,133,134} Future research should focus on optimizing standardized multicellular co-culture strategies, improving the stability and functional maintenance of immune and vascular compartments, and integrating high-resolution multi-omics profiling with these advanced platforms. Such developments are expected to substantially enhance the translational relevance of PDO-based models for investigating immunotherapy resistance and guiding personalized therapeutic strategies.

Currently, the 3D *in vitro* culture system of organoids lacks sufficient standardization and a controllable microenvironment, resulting in limited nutrients and oxygen diffusion, poor stability, and low reproducibility during passage, making it difficult to achieve multi-generation expansion.^{135,136} This limits the ability to determine long-term drug sensitivity and to conduct functional studies. Standardized organoid culture usually

cannot maintain complex microenvironment components such as immune cells, making it difficult to maintain long-term retention of immune and stromal signals and limiting the precise simulation of *in vivo* immune responses and tumor microenvironments.^{137,138} Therefore, future research needs to develop more stable immortalization strategies and long-term microenvironment reconstruction systems, including blood vessels, immunity, and stroma, such as multi-cell co-culture methods combined with microfluidic chips and synthetic matrices, to enhance the long-term stability and physiological relevance of organoids.^{139,140}

In addition, the PDO establishment and culture remain labor-intensive and time-consuming, and they lack sufficient standardization.^{141,142} Differences in procedures across laboratories will affect reproducibility and cross-center comparability. The longitudinal multi-omics analysis itself imposes high requirements on time, cost, and data processing capabilities, which pose practical limitations for routine clinical applications. To address these issues, it is necessary to standardize PDO modeling workflows and multi-omics data-analysis pipelines, enhance automation, and integrate them closely with clinical data, such as biopsies and liquid biopsies (e.g., ctDNA), to improve the models' predictive accuracy and clinical usability.¹⁴³

With advances in technology and the development of strategies such as co-culture and organ-on-a-chip, PDO platforms and microenvironment reconstruction are gradually addressing the shortcomings of traditional models. Their roles in guiding precise treatment strategies, drug screening, and drug resistance prediction are expected to be more widely verified and applied in future clinical practice.

7. Conclusion

The combination of PDOs with multi-omics technologies has fundamentally changed our understanding of the spatiotemporal evolution of tumor resistance. Within the framework of integrating genomics, single-cell, and spatial omics, the PDO platforms simultaneously capture the genetic heterogeneity of tumors, the plasticity of cell states, and microenvironment signals, thereby transcending the limitations of traditional static biomarkers and reconstructing the dynamic process of resistance evolution. "PDOs + multi-omics" not only enables systematic analysis of temporary adaptive states and transcriptional plasticity related to treatment, but also provides a framework to elucidate how resistance stabilizes in different spatial ecological niches. Based on the dynamic multi-omics data from this platform, it is expected to drive the rational design of combined or sequential therapies, patient stratification, and the discovery of biomarkers to predict adaptive resistance, thereby enabling more clinically relevant drug screening and treatment optimization. With the maturity

of PDO cultivation methods and high-throughput omics technologies, this integrated framework is expected to facilitate the transformation of mechanistic understanding into clinical applications in precision oncology, thereby improving the accuracy of predicting treatment responses for individual patients and extending the duration of therapeutic efficacy.

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Conflict of interest

The authors declare that they have no competing interests.

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