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Perspective

Beyond Tumor Recapitulation: A Multidimensional Framework for Cancer Organoid Fidelity

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Abstract

Patient-derived cancer organoids (PDOs) are increasingly used to investigate tumor biology, treatment response, and precision oncology, yet their correspondence to the tumors from which they originate is often summarized using global terms such as "recapitulation" or "fidelity." Such terminology obscures the fact that tumor–organoid correspondence may differ across genomic, clonal, cellular, spatial, metabolic, organelle, temporal, and therapeutic dimensions. Here, cancer organoid fidelity is reframed as a reference-specific and dimension-specific property organized across three analytically distinguishable but biologically interdependent domains: composition, state, and function. We examine how spatial sampling, tissue processing, culture establishment, matrix and medium composition, oxygen and nutrient availability, passage, and multicellular reconstruction can preserve selected tumor features while reshaping others. This distinction is particularly important for metabolism, mitochondrial and lysosomal function, autophagy and mitophagy, ferroptosis, treatment adaptation, and acquired resistance, for which similar phenotypes may arise through different upstream mechanisms. We introduce Culture-Induced State Distortion (CISD) to describe reproducible culture-associated states that differ from the parental tumor reference and propose the Organoid Fidelity Mapping Framework (OFMF) to classify dimension-specific evidence and define the limits of biological inference. Applying the framework to published colorectal cancer and glioblastoma organoid studies illustrates how validated features can coexist with unassessed dimensions. We also consider how reference availability, experimental resources, and clinical feasibility constrain the assessment and interpretation of fidelity. This framework supports question-driven selection of the minimum sufficient model rather than equating increasing experimental complexity with increasing biological fidelity.

Keywords: patient-derived organoids; cancer organoids; tumor fidelity; tumor microenvironment; culture-induced state distortion; organoid fidelity mapping framework; biological fidelity; model validation

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1. Introduction: Beyond the Claim of Tumor Recapitulation

Patient-derived cancer organoids (PDOs) are three-dimensional ex vivo cultures established from patient tumor tissue under conditions that support the survival, self-organization, and expansion of malignant epithelial populations. Across these settings, organoid models can retain selected histological, molecular, and genomic features of their tumors of origin while supporting pharmacological screening and mechanistic studies [1–8].

This success has encouraged broad descriptions of cancer organoids as models that "recapitulate," "faithfully reproduce," or "preserve" the parental tumor. Yet histological resemblance, retention of tumor-associated genomic alterations, preservation of clonal heterogeneity, maintenance of tumor-associated cellular populations, and concordance with clinically relevant treatment responses represent distinct forms of evidence that may diverge within the same model [4,8–11]. This distinction is already apparent at the genomic level: genomic continuity and clonal fidelity are related, but they are not equivalent [7,8,11].

The same problem extends beyond malignant genotype. Cellular presence, biological state, patient-specific repertoire, and functional interaction should therefore be regarded as separate levels of evidence rather than interchangeable measures of microenvironmental fidelity [9]. Culture introduces an additional source of divergence [8,11,12]. Culture conditions are therefore not merely technical parameters; they can become determinants of the biology being measured [8,13,14]. Therapeutic response constitutes another distinct level of correspondence. Histological resemblance, genomic concordance, or retention of a molecular drug target cannot independently establish predictive treatment fidelity [10,15,16].

Taken together, the literature does not support treating cancer organoid fidelity as a global or binary attribute. The appropriate level of model complexity should depend on the biological processes required for the intended inference rather than on an expectation that every organoid reproduce the complete parental tumor. In this article, we examine these limitations across genomic and clonal composition, the tumor microenvironment, spatial and metabolic states, organelle and stress-response biology, temporal evolution, and therapeutic response, and propose a framework for interpreting the evidence supporting tumor–organoid correspondence.

2. A Multidimensional Framework for Cancer Organoid Fidelity

2.1. Composition, State, and Function Define Distinct Fidelity Domains

Evidence used to support cancer organoid fidelity spans different levels of biological organization. We define cancer organoid fidelity as a reference-specific and dimension-specific property organized across three analytically distinguishable but biologically interdependent domains: composition, state, and function (Figure 1). This formulation reflects observations that major tumor-associated genomic features can be retained despite incomplete clonal representation, endogenous immune populations can persist without proof that their original functional state has been preserved, and treatment-response fidelity requires direct comparison with an appropriate clinical reference [7–11].

Compositional fidelity describes what is represented in the model. Preservation of major drivers and broad genomic architecture can coexist with loss, enrichment, or altered representation of minor subclones, illustrating that tumor identity and clonal composition are related but distinct properties [7,8,11]. State fidelity describes the biological state occupied by those components. These measurements characterize the state of the organoid directly, but correspondence with the parental tumor requires an external reference.

Functional fidelity describes how the model behaves over time or under perturbation, including treatment response, adaptation, persistence, clonal selection, and acquired resistance. In metastatic gastrointestinal cancers, matched patient–organoid analyses provided direct evidence that organoid sensitivity and resistance could correspond to clinical treatment response [10]. Composition, state, and function are therefore complementary but not biologically independent domains. Genomic alterations and clonal composition can influence cellular metabolism and treatment response, while environmental conditions may alter the functional consequences of the same genotype [7,8,11,17]. Their analytical separation within the OFMF is intended to prevent unsupported inference, not to imply that these processes operate independently. For

example, metabolic measurements may establish pathway activity in an organoid, but a claim that the parental tumor's metabolic state has been preserved requires an appropriate tumor reference and sufficient evidence of the malignant population being compared. The strength of any fidelity claim must therefore reflect both its direct evidence and the unresolved dependencies relevant to its interpretation.

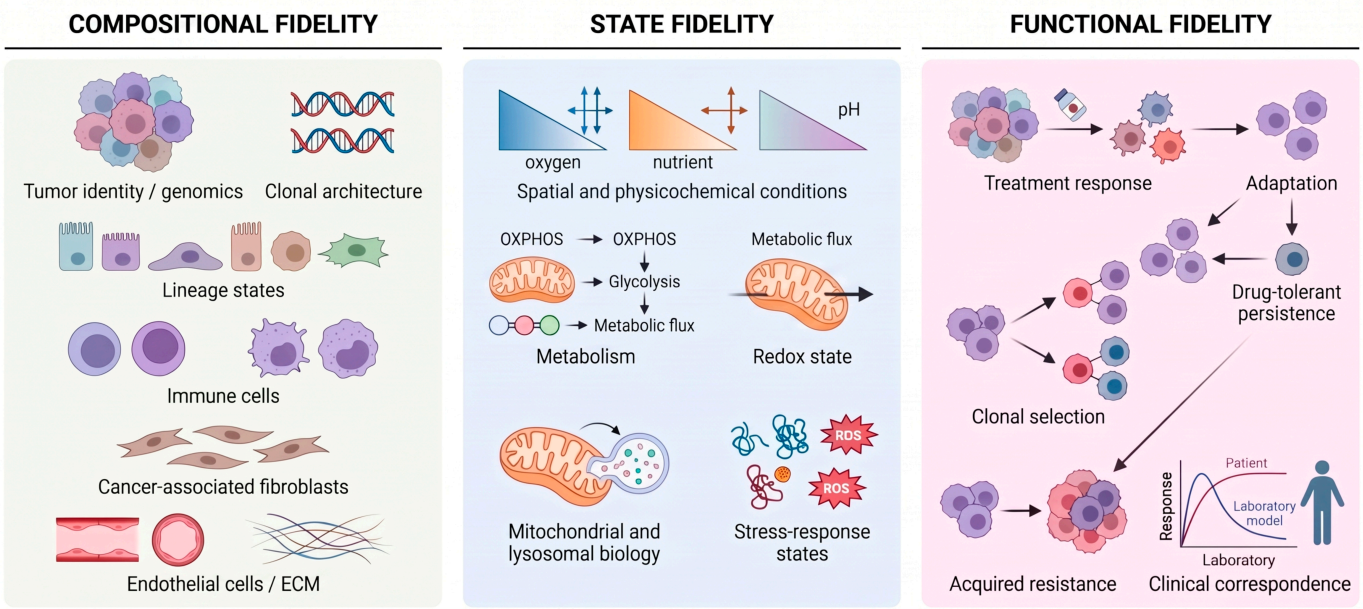


Figure 1. Multidimensional assessment of cancer organoid fidelity. Cancer organoid fidelity is conceptualized as a reference-specific and dimension-specific relationship between a patient tumor and its derived organoid rather than as a single global property. Compositional fidelity addresses which biological components are represented, including tumor-associated genomic alterations, malignant clones, lineage states, immune populations, cancer-associated fibroblasts, endothelial cells, and extracellular matrix components. State fidelity addresses the biological states occupied by these components, including spatial and physicochemical conditions, metabolic programs, redox state, mitochondrial and lysosomal biology, and stress-response states. Functional fidelity addresses dynamic behavior, including treatment response, adaptation, drug-tolerant persistence, clonal selection, acquired resistance, and correspondence with clinical behavior. The OFMF evidence states, validated, partially validated, discordant, not assessed, and not applicable, provide a dimension-specific vocabulary for reporting the strength and scope of available evidence. Validation in one dimension does not establish validation in another. The domains are analytically distinguished for evidence reporting but remain biologically interconnected. ECM, extracellular matrix; OFMF, Organoid Fidelity Mapping Framework; OXPHOS, oxidative phosphorylation. Arrows indicate directional relationships among biological states and processes, including metabolic transitions, clonal selection, and treatment adaptation. The background colors distinguish the three fidelity domains: compositional fidelity (pale green), state fidelity (pale blue), and functional fidelity (pale pink). Other colors are used to differentiate illustrative cellular and molecular elements and do not represent quantitative measurements or assigned evidence states. Created with BioRender.com.

2.2. Culture Can Preserve Tumor Biology While Also Reshaping It

Organoid culture is not biologically neutral, although matched tumor–organoid studies show that substantial genomic continuity can be maintained despite culture-associated selective pressures [8,11]. Protocol choice can also influence the biological state that emerges [14]. Three-dimensional growth adds another source of model-generated biology. Although spheroids and PDOs are distinct model classes, the methodological implication applies to both: three-dimensional architecture alone does not define the physicochemical state experienced by the cells.

We use Culture-Induced State Distortion (CISD) to describe a reproducible in vitro state that is absent, substantially weaker, or mechanistically different in the relevant biological reference. A culture-associated state may be reproducible and mechanistically informative while still differing from the state that existed in the parental tumor. This problem is particularly important for hypoxia-associated signaling, metabolism, mitochondrial function, redox balance, and stress-response pathways because each is highly sensitive to extracellular conditions [14,17–19]. Mechanistic correspondence therefore requires measurement of the relevant upstream state rather than inference from phenotype alone. Phenotypic similarity and mechanistic equivalence should consequently be treated as separate claims.

2.3. Fidelity Maps Define Evidence States and Inference Boundaries

A single label such as "high fidelity" obscures the fact that different properties of the same organoid model may show different degrees of correspondence with the biological reference [7–11]. Within the OFMF, each relevant dimension is assigned one of five evidence states. These classifications refer to individual biological dimensions rather than to the model as a whole.

The distinction between discordant and not assessed is especially important. An epithelial PDO may retain the genomic and clonal features of its parental tumor while its metabolic, redox, and vascular states remain uncharacterized [7,8,11,17,18]. In such cases, validated compositional fidelity must not be extended to unmeasured dimensions. Similarly, endothelial cell detection provides evidence of vascular composition but does not establish perfusion or functional vascular correspondence [20,21]. We therefore propose that each OFMF classification be accompanied by the biological reference, the measurements supporting the claim, and the relevant dimensions that remain unassessed. Not applicable should be reserved for properties outside the stated biological question, rather than for relevant features that were not examined. Table 1 summarizes the evidence required for dimension-specific claims and their appropriate comparators.

Table 1. Evidence requirements for dimension-specific claims of cancer organoid fidelity.

Fidelity Dimension	Fidelity Claim	Evidence Required to Support the Claim	Evidence That is Insufficient on Its Own	Appropriate Reference or Comparator	Representative References
Tumor identity	The organoid represents the malignant population from the sampled tumor	Histopathological characterization together with tumor-associated genomic, copy-number, structural, or other molecular features appropriate to the malignancy	Three-dimensional growth or morphology alone	Anatomically and temporally defined derivation specimen	[1,4,8]
Genomic fidelity	Major genomic features of the parental tumor are retained	Matched assessment of driver alterations, copy-number architecture, mutational burden, structural variants, or other relevant genomic features	Retention of a single driver alteration	Matched parental tumor specimen	[1,4,8,11]

Table 1. *Cont.*

Fidelity Dimension	Fidelity Claim	Evidence Required to Support the Claim	Evidence That is Insufficient on Its Own	Appropriate Reference or Comparator	Representative References
Clonal fidelity	Relevant malignant subclones and their representation are preserved	Clone-aware matched profiling using variant frequencies, single-cell analysis, clonal reconstruction, or other methods capable of resolving subclonal structure	Broad genomic similarity or preservation of dominant drivers	Spatially and temporally matched parental tumor	[7,8,11]
Microenvironmental composition	Relevant stromal or immune populations are represented	Direct characterization of relevant cell populations, ideally including their abundance and patient origin	Detection of a single lineage marker	Matched parental tumor microenvironment	[9]
Vascular microenvironmental fidelity	Relevant vascular composition, architecture, and function correspond to the defined tumor reference	Claim-specific assessment of endothelial and mural cell representation, vascular organization, and, where relevant, perfusion, permeability, or transport function	Endothelial marker expression, vessel-like morphology, or endothelial cell presence alone	Matched tumor vascular architecture and functional characteristics, or an explicitly defined physiological comparator for process-specific claims	[20–22]
Immune state and interaction	Immune populations retain biologically relevant state and tumor interaction	Functional assays of tumor reactivity, repertoire, activation, cytotoxicity, checkpoint response, or other question-specific interactions	Presence of immune cells alone	Matched tumor immune state or patient-specific functional reference	[9]
Spatial and physicochemical fidelity	Cells experience tumor-relevant oxygenation, transport, or related physicochemical conditions	Direct measurement of the relevant variable, including oxygen, pH, geometry, or transport where appropriate	Three-dimensional architecture, organoid diameter, or spatial position alone	Spatially resolved tumor or explicitly defined physiological reference	[19]
Metabolic fidelity	The organoid reproduces a tumor-relevant metabolic state	Metabolomics, extracellular-flux analysis, isotope tracing, metabolic imaging, or another direct pathway-level measurement together with an appropriate tumor comparator	Genotype, lineage identity, or expression of isolated metabolic markers alone	Matched tumor tissue or another clinically or biologically relevant metabolic reference	[17,18]
Organelle and stress-response fidelity	A mitochondrial, lysosomal, redox, or stress-response state corresponds to the parental tumor	Pathway-specific functional measurements together with an appropriate reference, including dynamic or flux measurements where required	Static marker abundance or morphology alone	Matched tumor state under the relevant condition	[17,18]

Table 1. *Cont.*

Fidelity Dimension	Fidelity Claim	Evidence Required to Support the Claim	Evidence That is Insufficient on Its Own	Appropriate Reference or Comparator	Representative References
Temporal or evolutionary fidelity	Changes observed in the organoid correspond to biologically relevant tumor evolution	Longitudinal profiling anchored to defined disease stages, treatment histories, or matched sequential patient material	Change occurring during passage alone	Temporally matched patient specimens or explicitly defined disease states	[7]
Therapeutic fidelity	Ex vivo treatment response corresponds to patient treatment behavior	Matched organoid–patient response analysis under a defined therapeutic context	Drug-induced organoid killing, target expression, or genomic target retention alone	Clinical treatment response	[10]
Culture-induced state distortion	A measured state is generated, amplified, or mechanistically altered by culture	Direct comparison with the relevant biological reference and, where possible, association with culture conditions or derivation parameters	Detection of an unusual or stress-associated phenotype in culture alone	Relevant parental tumor state	[14,19]

Table 1 footnote. Evidence requirements are dimension- and claim-specific and should be interpreted relative to an explicitly defined biological reference. The measurements listed are examples rather than mandatory assay combinations; the required evidentiary resolution depends on the biological inference being made. Evidence obtained in one fidelity dimension should not be extrapolated to another. "Insufficient on its own" indicates that a measurement may contribute useful information but cannot independently establish the stated fidelity claim. Absence of measurement should be classified as not assessed rather than interpreted as evidence of concordance or discordance. A dimension intentionally excluded from the biological question may be classified as not applicable. CISD, Culture-Induced State Distortion; PDO, patient-derived organoid. Note: Bold text in the first column identifies the fidelity dimensions evaluated in the framework.

3. Genomic and Clonal Fidelity

3.1. Genomic Continuity Does Not Guarantee Complete Clonal Representation

Genomic concordance between patient tumors and derived organoids provides strong evidence of biological continuity with the tumor of origin. Breast cancer organoid biobanks similarly preserved major histopathological features, hormone-receptor and HER2 status, copy-number alterations, and sequence variants during propagation, while colorectal cancer organoid collections retained major mutational and molecular features of their parental tumors [3,4].

Large-scale matched analyses confirm this continuity while also revealing its limits. These data support preservation of malignant genomic identity without implying complete equivalence at every level of clonal organization [8]. Disease-specific studies show the same pattern. Bladder cancer organoids likewise preserved multiclonal genetic landscapes across disease stages and reproduced aspects of longitudinal tumor evolution and treatment-associated change [7].

Genomic continuity and clonal fidelity should therefore be treated as related but distinct properties. Culture stability is another separate property: an organoid may remain genomically stable after establishment despite selective loss or enrichment of particular populations during sampling or derivation [8,11,23]. Genomic fidelity should be reported at the level actually assessed. Longitudinal changes in those populations constitute an evolutionary dimension considered separately in Section 7.

3.2. Sampling, Establishment, and Passage Impose Sequential Selection Bottlenecks

Tumor–organoid discordance can arise before culture begins. For genomic comparisons, the most appropriate reference is therefore the anatomically and temporally defined specimen used for organoid derivation rather than an unrestricted concept of "the patient tumor" [24]. Selection continues during organoid establishment. Culture conditions can therefore influence which populations ultimately establish and expand [8,14].

The resulting organoid reflects a series of biological filters: intratumoral heterogeneity, anatomical sampling, survival during tissue processing, adaptation to niche conditions, successful establishment, and continued propagation. Tissue dissociation may add a state-selection pressure by inducing transcriptional responses during processing, although this effect concerns cellular state more directly than clonal composition [13]. These sequential filters and the points at which Culture-Induced State Distortion may arise are summarized in Figure 2.

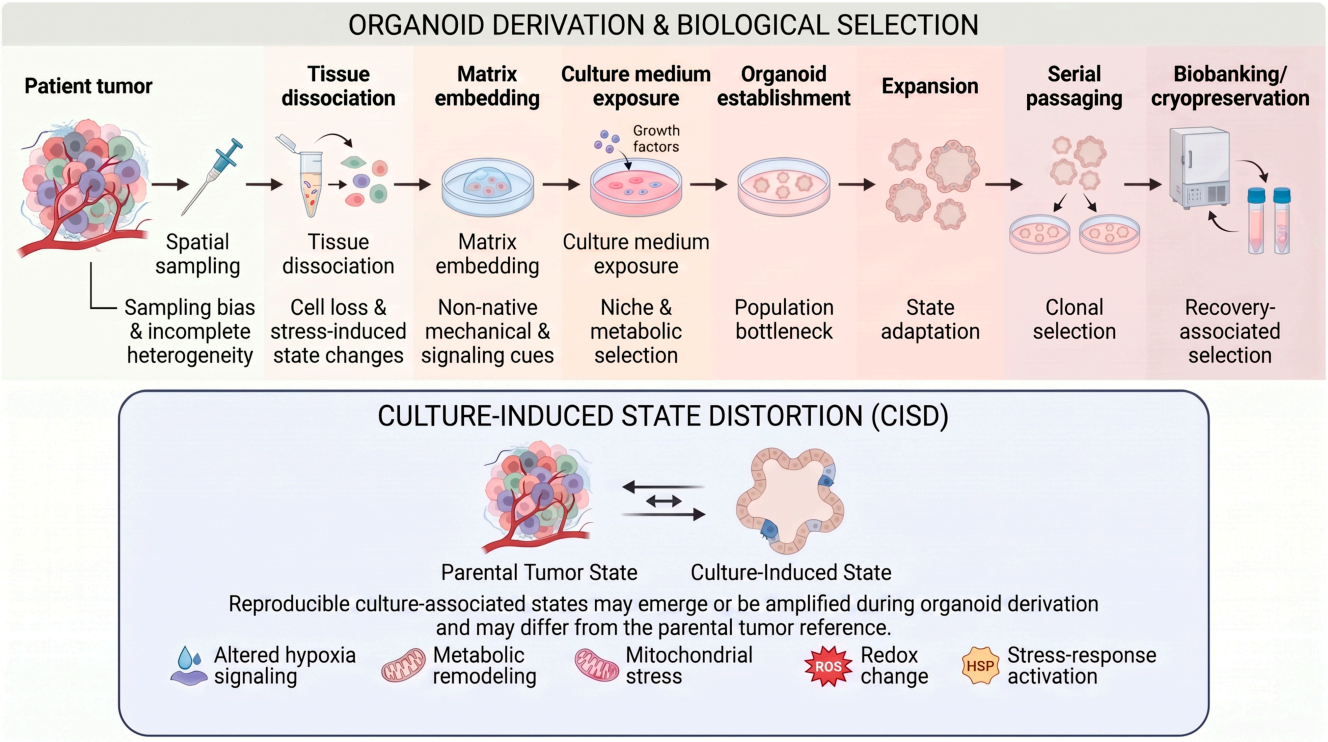


Figure 2. Sequential biological selection during cancer organoid derivation and the emergence of Culture-Induced State Distortion. Organoid derivation exposes patient tumor material to sequential biological and technical filters that can alter the representation and state of the resulting culture. Spatial sampling may incompletely capture intratumoral heterogeneity, whereas tissue dissociation can induce cell loss, stress responses, and selective survival. Matrix embedding and defined culture media introduce non-native mechanical, signaling, nutrient, and growth-factor conditions that can favor particular populations or cellular states. Organoid establishment constitutes an additional bottleneck, followed by expansion, serial passaging, and biobanking or cryopreservation, each of which may contribute to state adaptation, clonal selection, or recovery-associated selection. Culture-Induced State Distortion (CISD) denotes a reproducible culture-associated state that is absent, substantially weaker, or mechanistically different in the relevant parental tumor reference. Examples include altered hypoxia signaling, metabolic remodeling, mitochondrial stress, redox change, and stress-response activation. CISD does not imply technical failure; rather, it identifies culture-associated biology whose attribution to the parental tumor requires direct validation. Arrows in the upper panel indicate the sequential steps of organoid derivation, whereas the bidirectional arrows in the lower panel illustrate the conceptual relationship between the parental tumor state and the culture-induced state. Created with BioRender.com.

Selection also affects the interpretation of tumor purity. Greater analytical clarity therefore does not necessarily imply more complete preservation of the original tumor diversity [8]. Passage introduces another potential source of change. Passage number is therefore biologically relevant whenever the intended inference depends on clonal representation or evolutionary change.

3.3. Genomic Fidelity Should Be Claimed Only at the Level That Was Measured

Statements that an organoid "preserves the tumor genome" are too broad unless the level of genomic organization being assessed is specified. The resolution of the genomic-fidelity claim should match both the biological question and the measurement used to support it. Malignant identity should also be established before broader genomic-fidelity claims are made because a patient-derived three-dimensional culture is not necessarily dominated by malignant cells. Histological appearance alone may therefore be insufficient, and confirmation can require tumor-associated mutations, copy-number alterations, structural variants, or other tumor-specific molecular features [3].

Genomic-fidelity reporting should specify the anatomical and temporal origin of the parental specimen, passage at molecular characterization, tumor purity where available, genomic assay, alterations evaluated, and the level of concordance being claimed. Single-cell analysis is particularly informative when the intended inference concerns newly emerging copy-number states or lineage-specific genomic diversification that would be obscured in bulk measurements [25]. Genomic fidelity establishes molecular continuity with a defined tumor reference; it does not establish equivalence to the tumor as a whole. Those dimensions require independent evidence.

4. Cellular and Microenvironmental Fidelity

4.1. Preserving Cells Is Not the Same as Preserving an Ecosystem

Genomic continuity does not imply preservation of the multicellular environment in which malignant cells operate. An epithelial organoid may therefore preserve tumor identity while omitting processes that depend on non-malignant compartments [9,26,27]. Conventional epithelial PDO protocols predominantly expand tumor epithelial populations and generally do not maintain the full native stromal, immune, or vascular ecosystem [28–30]. Optimized lung-cancer ALI organoids have also been shown to retain immune and fibroblast populations and major features of the parental immune landscape, with viable immune populations maintained for approximately 30 days under the tested conditions [31].

Cellular presence, cellular state, and functional interaction should be evaluated separately. Functional heterogeneity may also exist between malignant clones from the same patient: single-cell-derived colorectal cancer organoids have shown substantial diversity in HLA class I peptide presentation, indicating that clonal representation can influence the antigenic landscape available for immune recognition [32]. Microenvironmental fidelity is also time dependent. Claims about the microenvironment should therefore refer to the populations and states present at the time the biological inference is made.

4.2. Native Preservation and Experimental Reconstitution Preserve Different Kinds of Information

Native preservation and experimental reconstitution provide different types of biological information. Similar cellular composition can coexist with different immune-state transitions and functional interactions [26,27,33]. Defined reconstitution offers greater experimental control over cell identity, source, ratio, activation state, and timing, but it also changes the biological context being modeled. Altering cellular composition can therefore modify both tumor-cell state and therapeutic response [26,27]. Patient matching further shapes the strength of the inference. Reconstitution can recover defined aspects of

patient-specific stromal biology without being equivalent to native preservation [26,27]. The key distinction is between preserved and reconstructed biological context.

4.3. Interaction Fidelity Requires Functional Validation, Not a Census of Cell Types

Increasing the number of cellular compartments does not automatically increase microenvironmental fidelity. Immune models illustrate this clearly: comparable cellular composition does not necessarily preserve the same immune-state transition or tumor–immune interaction [33].

Interaction fidelity should be assessed dynamically using measurements appropriate to the process under study. Marker-defined cellular presence is insufficient when the inference depends on what those populations actually do [9,33]. The same principle applies to vascular and transport reconstruction: restoring a process provides process-specific evidence, not proof of complete vascular fidelity.

4.4. Microenvironmental Fidelity Should Be Reported by Subdimension and Matched to the Question

The statement that a model "preserves the tumor microenvironment" is usually too broad. A culture may contain T cells without preserving tumor-reactive clonotypes, include CAFs at a non-physiological ratio, or reconstruct epithelial–stromal interactions without reproducing the complete parental microenvironment [9,26,27,33].

Model configuration also changes what a therapeutic-response experiment measures. These configurations therefore answer different biological questions. The appropriate model is the one that represents and validates the cellular relationships required for the intended inference, not the one with the greatest number of incorporated compartments. Preserving cells is therefore not enough. This distinction leads to the next question: whether those cells and interactions experience spatial, physicochemical, and metabolic conditions that correspond to the biological reference.

5. Spatial, Physicochemical and Metabolic Fidelity

5.1. Spatial and Physicochemical Gradients Are Model-Generated Variables

Three-dimensional growth changes the physical environment experienced by cells. Spatial heterogeneity may therefore be experimentally informative while also reflecting conditions generated or amplified by the culture system [19,34,35]. Oxygen provides a clear example. These experiments were performed in spheroids rather than PDOs, but the methodological implication is directly relevant to organoid studies: a three-dimensional center does not define a particular oxygen state [19].

Physicochemical conditions can also be imposed and measured experimentally. Extracellular matrix properties add both signaling and transport constraints [35–37]. This illustrates why matrix effects should be evaluated at the level of the specific biological readout rather than assumed to affect all fidelity dimensions equally.

In colorectal cancer PDOs, Han et al. generated embedded bioprinted organoid arrays within an engineered tumor microenvironment incorporating approximately 7.5-kPa stiffness and hypoxic conditions [35]. Such engineering can improve correspondence for selected biological dimensions without establishing global tumor fidelity [35]. No single culture condition can be assumed to be physiologically appropriate across tumor types or biological questions. Microfluidic and engineered platforms can restore selected aspects of oxygen delivery, metabolite clearance, matrix context, and drug transport, but those additions support fidelity claims only when the relevant process is measured against an appropriate reference [34,35].

5.2. Metabolic Fidelity Depends on Environmental Context

Metabolic state is especially sensitive to spatial and physicochemical context and cannot be inferred from genomic identity or lineage classification alone [17,38,39]. Patient-derived organoids can nevertheless retain biologically meaningful metabolic programs. In malignant rhabdoid tumor models, methotrexate and DHODH inhibition induced apoptosis in tumoroids, and methotrexate delayed tumor growth in patient-derived xenografts [39].

Different metabolic assays interrogate different levels of biology. Matched tissue–organoid transcriptional analysis combined with metabolomics, isotope tracing, and perturbational validation provides stronger evidence than a single surrogate readout [39]. Metabolic validation is particularly informative when analogous measurements are available in the patient and the derived organoid. In matched PET–PDO studies, differences between organoid exposure conditions and patient circulation were addressed by comparing kinetic uptake parameters rather than raw tracer concentrations, underscoring the need for a normalized and biologically appropriate comparator [38].

Subtype-associated metabolic programs can also remain detectable outside the native tissue environment. In high-grade serous ovarian cancer PDOs, low- and high-OXPHOS subtypes were distinguished by lactate dehydrogenase activity, MCT4 expression, and hyperpolarized [1-^{13}C]pyruvate-to-lactate conversion and showed corresponding differences in treatment response [40].

These findings argue against reducing metabolic fidelity to categorical labels such as glycolytic or OXPHOS-dependent. Neither alone establishes that the same pathway operates with the same magnitude or regulation in the patient tumor [17,40]. Culture medium is part of the metabolic experiment rather than neutral technical background [17,39,41]. More physiologically matched media, including human-plasma-like or tumor-interstitial-fluid-based formulations, may improve interpretation of metabolic phenotypes in organoid systems [39,41].

Cellular composition also shapes metabolism. Metabolic remodeling in immune-resistant organoids illustrates how adding or retaining an immune compartment can alter both metabolic state and the biological question being addressed [42]. Metabolic fidelity should therefore be evaluated relative to three elements: the biological reference, the culture environment, and the measurement itself. Wide-field optical redox imaging, for example, can quantify treatment-induced redox changes and metabolic heterogeneity within PDOs, but these measurements describe the model rather than tumor fidelity in the absence of matched patient-state validation [18].

5.3. Same Phenotype Does Not Guarantee the Same Mechanism

Spatial and metabolic fidelity converge on a common problem: similar downstream phenotypes can arise from different upstream conditions. In patient tumors, oxygen and nutrient exposure are shaped by vascular architecture, perfusion, tissue geometry, cellular consumption, stromal composition, and systemic substrate supply. In organoid culture, hypoxia itself can alter collective behavior; lung tumor organoids exposed to hypoxic conditions showed cluster migration involving cancer-stem-cell-associated populations [43]. Oxygenation is therefore an experimentally active determinant of phenotype rather than a passive background variable.

This distinction becomes particularly important when hypoxic or metabolic stress signatures are present in both the parental tumor and the organoid. Abnormal tumor vasculature can produce heterogeneous oxygen and nutrient availability in vivo, whereas similar responses may arise in vitro through diffusion limitations and culture conditions [20,22,44]. Accordingly, transcriptomic similarity alone cannot establish a shared mechanism. Where

matched tissue is available, spatial relationships between vascular structures and stress-associated markers should be compared with the corresponding organoid patterns. Controlled changes in oxygen tension, nutrient availability, or organoid size can help determine the contribution of culture-dependent factors, while quantitative assessment of oxygen consumption and diffusion may further inform the interpretation of spatial hypoxia [44]. Paired analyses of pathway activity and spatial distribution, accounting for patient-specific and technical variation, may further support this distinction. Importantly, an overlapping stress response may reflect both native tumor biology and culture-associated adaptation. Without suitable reference tissue or perturbational evidence, its origin should remain unresolved rather than being classified definitively as CISD.

The same limitation applies to metabolic readouts. Stronger metabolic-fidelity claims require alignment between the biological reference and the level of measurement, as shown by matched patient PET–PDO radiotracer analysis, matched tumor–organoid metabolic profiling, and multimodal functional characterization of PDO metabolism [17,38,39]. Figure 3 illustrates how similar downstream phenotypes in tumors and organoids may arise from different upstream spatial and metabolic conditions.

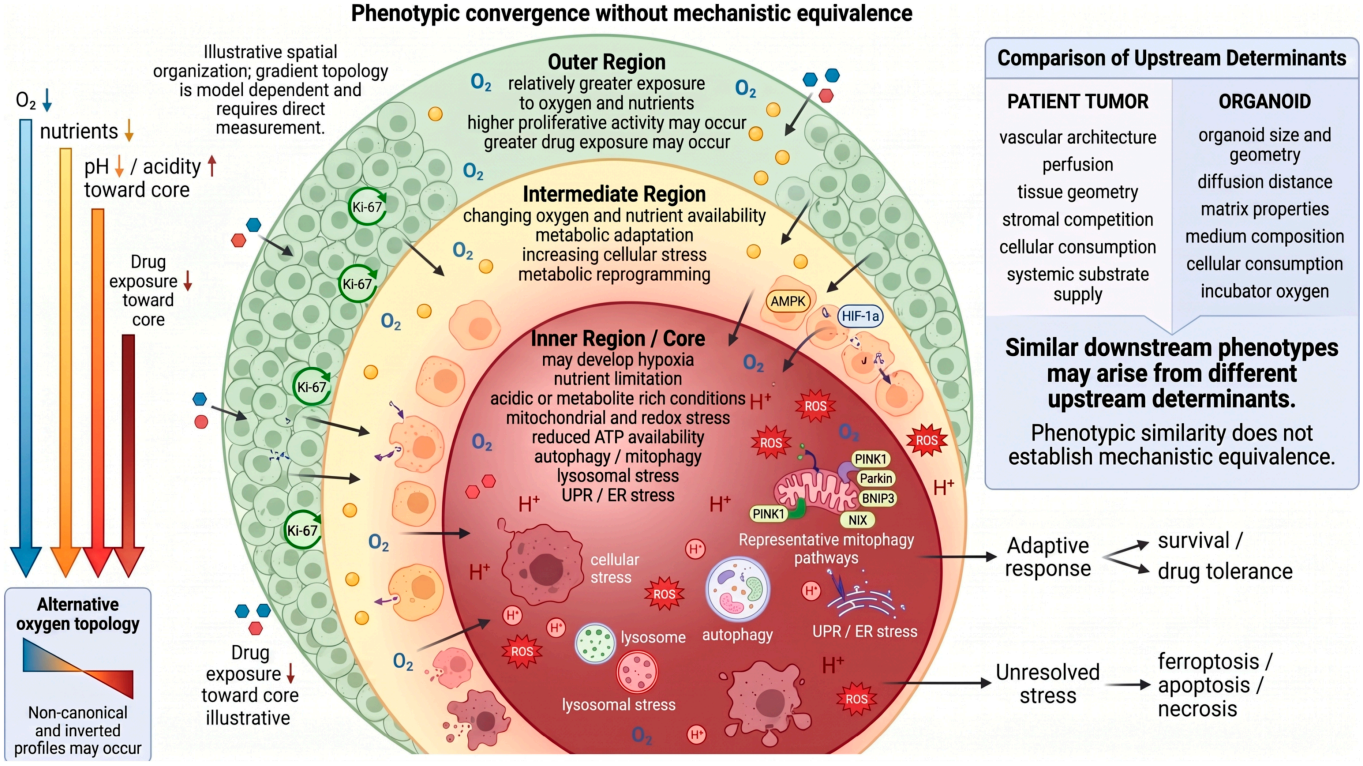


Figure 3. Phenotypic convergence without mechanistic equivalence in spatially heterogeneous cancer organoids. The radial organization shown is illustrative rather than universal: oxygen, nutrient, pH, and therapeutic gradients in three-dimensional cancer models are model-dependent and require direct measurement. Outer regions may experience relatively greater oxygen, nutrient, and drug exposure, whereas intermediate and inner regions may develop metabolic adaptation, nutrient limitation, acidic or metabolite-rich conditions, mitochondrial and redox stress, autophagy or mitophagy, lysosomal stress, unfolded-protein responses, and reduced ATP availability. Non-canonical or inverted oxygen profiles may also occur, emphasizing that spatial position alone does not define physicochemical state. PINK1, Parkin, BNIP3, and NIX are shown as representative mitophagy-related pathways rather than as an obligatory or exclusive mechanism. Similar downstream phenotypes may emerge in patient tumors and organoids through different upstream determinants. In tumors, oxygenation

and nutrient exposure are shaped by vascular architecture, perfusion, tissue geometry, stromal competition, cellular consumption, and systemic substrate supply, whereas in organoids they may be dominated by organoid size and geometry, diffusion distance, matrix properties, medium composition, cellular consumption, and incubator oxygen. Adaptive stress responses may promote survival and drug tolerance, whereas unresolved stress may contribute to ferroptosis, apoptosis, or necrosis. Phenotypic similarity therefore does not establish mechanistic equivalence, and spatial or metabolic fidelity requires validation of the relevant upstream conditions. AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; ER, endoplasmic reticulum; HIF-1α, hypoxia-inducible factor 1α; NIX, Nip3-like protein X; ROS, reactive oxygen species; UPR, unfolded protein response. Created with BioRender.com.

Phenotypic similarity may be sufficient when the aim is to examine responses to a defined hypoxic or metabolic perturbation, but stronger claims of tumor-state fidelity require additional evidence. A question-dependent, tiered approach may reduce this burden. Initial characterization can document culture conditions, organoid size, morphology, and relevant molecular markers, while selected functional measurements can establish whether the pathway of interest is active under the experimental conditions. Direct oxygen measurements, metabolic flux analysis, isotope tracing, or pathway-specific perturbations become particularly important when the conclusions depend on the underlying mechanism rather than on phenotype alone [17–19,38,39,44]. These approaches are not interchangeable, and their use should depend on the biological inference being made. Moreover, functional validation within an organoid does not establish correspondence with the parental tumor without an appropriate reference. Representative assays and their interpretative limitations are summarized in Table 2.

Table 2. Molecular and functional readouts for spatial, metabolic, organelle, and stress-response fidelity in cancer organoid models.

Biological Dimension	Representative Molecular or Functional Readouts	Informative Validation Approaches	What the Evidence Can Support	Insufficient on Its Own	Representative References
Oxygenation and hypoxia state	Local O_2 concentration, HIF-1α-associated signaling, hypoxia-responsive transcriptional programs	Direct oxygen probes or nanosensors, spatial oxygen imaging, controlled oxygen-gradient systems, spatial molecular profiling	Whether cells experience a defined oxygen state and whether hypoxia-associated responses occur under that condition	Organoid radius, central location, necrotic core, or HIF-1α expression alone	[19,34,45]
Spatial and transport state	pH, nutrient exposure, drug penetration, diffusion distance, local proliferative state	Direct pH or nutrient measurements, spatial drug-distribution assays, quantitative geometry, microfluidic transport measurements, controlled perfusion	Spatially resolved physicochemical and transport conditions within the model	Three-dimensional architecture or spatial position alone	[19,34,35,46]
Glycolytic and oxidative metabolism	ECAR, OCR, lactate production, ATP-related readouts, glycolytic reserve, oxidative capacity	Extracellular-flux analysis, metabolomics, isotope tracing, metabolic imaging, pathway perturbation	Metabolic activity and pathway dependence under defined culture conditions	Expression of glycolytic or mitochondrial markers alone	[17,18,38,40,41,47,48]

Table 2. *Cont.*

Biological Dimension	Representative Molecular or Functional Readouts	Informative Validation Approaches	What the Evidence Can Support	Insufficient on Its Own	Representative References
Substrate utilization and metabolic flux	ˆ13C incorporation, pathway-specific carbon utilization, metabolite turnover	Stable-isotope tracing, LC–MS metabolomics, flux modeling, matched tumor–organoid metabolic profiling	Which substrates contribute to specific metabolic pathways and whether pathway use is conserved relative to a defined reference	Metabolite abundance or transcript expression alone	[17,38,39,41]
Redox state	ROS, glutathione-related metabolites, optical redox ratio, oxidative damage	Optical redox imaging, ROS assays, metabolomics, antioxidant perturbation, oxidative-damage measurements	Redox balance and treatment-induced redox responses under defined conditions	ROS signal alone	[18,42,49,50]
Mitochondrial state	OCR, membrane potential, mitochondrial mass, morphology, cristae integrity, ATP-related function	Extracellular-flux assays, membrane-potential probes, quantitative imaging, ultrastructure, isotope tracing, functional perturbation	Respiratory function and selected aspects of mitochondrial state	Mitochondrial abundance or morphology alone	[17,18,47,51,52]
Autophagy	LC3-II turnover, p62/SQSTM1 turnover, autophagosome formation and clearance	Lysosomal inhibition controls, tandem fluorescent LC3 reporters, turnover assays, autophagosome–lysosome fusion assays	Dynamic autophagic flux and degradative pathway function	Increased LC3 signal or autophagosome accumulation alone	[53,54]
Mitophagy	PINK1, Parkin, BNIP3, NIX, mitochondrial cargo recognition and clearance	Mitophagy reporters, genetic or pharmacological perturbation, mitochondrial cargo-clearance assays, pathway-specific imaging	Functional mitochondrial quality control and contribution of mitophagy to phenotype or treatment response	Expression or accumulation of mitophagy-associated proteins alone	[52,53,55]
Lysosomal competence	Lysosomal pH, cathepsin activity, autophagosome–lysosome fusion, proteolytic capacity	pH-sensitive lysosomal probes, cathepsin assays, fusion assays, lysosomal inhibition, cargo-degradation measurements	Whether the lysosomal compartment is functionally capable of downstream degradation	Lysosome abundance or morphology alone	[53,54]
ER stress and unfolded-protein response	ATF3/CHOP/CHAC1-associated signaling, UPR-related transcriptional changes, ER-stress markers	Transcriptomics combined with RT-qPCR, immunoblotting, pathway perturbation, functional rescue where appropriate	Activation and functional involvement of an ER-stress/UPR program	Expression of a single UPR-associated marker alone	[49,56]

Table 2. *Cont.*

Biological Dimension	Representative Molecular or Functional Readouts	Informative Validation Approaches	What the Evidence Can Support	Insufficient on Its Own	Representative References
Ferroptosis	Lipid peroxidation, Fe^{2+} dependence, GPX4 activity or expression, glutathione depletion, system xc− dependence	Lipid-ROS assays, iron measurements, GPX4 or system xc− perturbation, ferroptosis-inducing agents, rescue with ferroptosis inhibitors	Mechanistic involvement of ferroptotic cell death	GPX4 expression, ROS elevation, or cell death alone	[49,50,57]
Drug-tolerant or persister state	Residual survival, reversible growth arrest, metabolic or stress remodeling, state-specific transcriptional programs	Longitudinal sampling, drug withdrawal and rechallenge, single-cell profiling, lineage-resolved analysis, repeated viability measurements	Reversible treatment adaptation and distinction from stable acquired resistance	Residual viability at a single post-treatment time point	[58–62]
Acquired resistance	Stable resistance phenotype, clonal expansion, persistent signaling adaptation, resistance-associated genomic or state changes	Longitudinal genomic and transcriptomic profiling, repeated treatment cycles, rechallenge, clonal reconstruction, pathway perturbation	Stable treatment resistance and its underlying adaptive or evolutionary mechanism	A single resistant endpoint without temporal characterization	[58,59,61,63,64]

Table 2 footnote. The listed readouts and assays are representative rather than mandatory combinations. Their evidentiary value depends on the biological question, experimental condition, and reference used for comparison. Marker abundance, morphology, or pathway-associated expression may support mechanistic interpretation but should not be equated automatically with pathway activity, flux, or fidelity. Functional validation establishes that a process operates within the organoid; correspondence with the parental tumor requires an appropriate external reference. ECAR, extracellular acidification rate; ER, endoplasmic reticulum; GPX4, glutathione peroxidase 4; LC3, microtubule-associated protein 1 light chain 3; OCR, oxygen-consumption rate; ROS, reactive oxygen species; UPR, unfolded protein response. Note: Bold text in the first column identifies the biological dimensions evaluated in the table.

6. Organelle and Stress-Response Fidelity

6.1. Mitochondrial and Organelle State Is Context Dependent

Mitochondria, lysosomes, the endoplasmic reticulum, and the autophagic machinery respond to oxygen availability, nutrient supply, redox balance, growth-factor signaling, matrix context, and therapeutic stress [17,53,57]. An organoid may preserve major tumor-associated genomic features while developing an intracellular stress landscape that differs from that of the parental tumor [17,18].

Organelle and stress-response fidelity cannot be inferred from pathway detection alone. Likewise, increased mitochondrial mass may reflect biogenesis, impaired clearance, or accumulation of damaged organelles [17,18]. In cell-line-derived Ishikawa and SH-SY5Y spheroids, lithium exposure produced mitochondrial swelling, cristae disruption, vacuolization, and double-membrane structures consistent with mitophagy-associated remodeling, although definitive LC3- or PINK1/Parkin-based pathway validation was not performed [51].

6.2. Autophagy, Mitophagy and Lysosomal Flux Require Functional Validation

Mitophagy is most informative when evaluated as a dynamic quality-control process rather than through static pathway-associated markers. Pharmacological or genetic inhibition of mitophagy enhanced statin-induced anticancer activity in PDOs, PDO-derived xenografts, and additional in vivo models, showing that mitochondrial quality control influenced therapeutic sensitivity [52].

A similar limitation applies to macroautophagy. Increased LC3 signal or accumulation of autophagic structures should therefore not be equated automatically with increased pathway activity. Autophagy and lysosomal competence are closely linked but separable processes. The key issue is whether initiation, trafficking, lysosomal degradation, and cargo clearance function in the biological state being claimed [53,54].

6.3. UPR, Oxidative Stress and Ferroptosis Span Multiple Fidelity Domains

Organelle stress responses are embedded within broader metabolic and environmental networks, so their interpretation depends on the conditions in which they arise. PDOs can provide mechanistically informative models when pathway identity is supported by functional evidence [49].

Ferroptosis can also shape therapeutic behavior. In patient-derived lung adenocarcinoma organoids, transcriptomic and functional analyses linked ATF3/CHOP/CHAC1 signaling to ferroptotic responses, providing mechanistic evidence beyond marker expression alone [49]. More generally, stress-pathway identity is strongest when established functionally. A PDO may reproduce a therapeutically relevant stress response without demonstrating that its untreated baseline state matches the parental tumor.

6.4. Tumor Stress Must Be Distinguished from Culture-Induced Stress

Three-dimensional culture can itself generate or amplify many of the stress programs under investigation [19,45,53]. Culture-Induced State Distortion (CISD) is therefore particularly relevant to organelle biology. A culture-associated state can remain reproducible and mechanistically informative; the limitation is whether that state can be attributed to the parental tumor.

Functional perturbation strengthens mechanistic interpretation but does not establish tumor fidelity on its own. Demonstrating that the same pathway operates with comparable magnitude and biological significance in the parental tumor requires an appropriate tumor-based comparator. Organelle and stress-response fidelity is therefore best reported by pathway, condition, and reference rather than summarized as a single global property. A stress pathway can be biologically real in an organoid without being demonstrably preserved from the tumor.

7. Temporal and Therapeutic Fidelity

7.1. From Baseline State to Tumor Evolution

Baseline fidelity captures only one point in time, whereas tumors change during progression, metastasis, treatment, relapse, and repeated selective pressure [7,23,24]. Temporal fidelity concerns whether biologically relevant change occurs within the model over time; evolutionary fidelity asks whether those changes correspond to evolution observed in the patient or another explicitly defined biological reference.

Temporal change in culture may reflect expansion of pre-existing clones, reversible state transitions, therapy-induced adaptation, acquisition of stable resistance, passage-associated selection, or culture-specific evolution [7,23,59]. The strongest evidence for evolutionary fidelity comes from temporally anchored patient material. A treatment-naïve PDO should not be interpreted as a model of recurrent disease simply because

resistance can be induced experimentally, whereas an organoid established after treatment already represents a biologically selected disease state. Evolutionary fidelity is best defined relative to a specified disease state, time point, and treatment history rather than to an undifferentiated concept of "the patient tumor."

Direct validation of patient-concordant evolution is often constrained by the availability of longitudinal tumor material. Repeated tissue sampling is not routinely justified solely for organoid research, and serial resections are generally limited to particular clinical circumstances. Where available, clinically indicated repeat biopsies, archived specimens, and longitudinal circulating tumor DNA measurements may provide information on treatment-associated or clonal changes, although these sources do not necessarily capture the spatial and cellular states represented in tissue samples [23,24,65]. Longitudinal organoid experiments can therefore characterize experimentally induced adaptation without establishing that the same evolutionary trajectory occurred in the patient. Stronger claims of patient-concordant evolution may require prospectively designed clinical studies with appropriate sampling and consent. When such comparisons are unavailable, evolutionary fidelity should remain not assessed, even if temporal changes within the organoid have been extensively characterized.

7.2. Drug Tolerance and Acquired Resistance Should Be Separated

Therapy resistance does not necessarily begin with stable genetic change. Reversible drug-tolerant states can precede more stable resistance and represent a distinct phase of treatment adaptation rather than established resistance [58,59]. Distinguishing these phases requires experimental designs that resolve reversibility and clonal change. Treatment response is therefore better viewed as a temporal trajectory spanning acute response, residual survival, reversible tolerance, continued selection, and more stable resistance rather than as a single category of therapeutic failure [58].

PDO models also show that adaptive resistance mechanisms can be induced and dissected experimentally [61,63,64]. Such experiments establish what the model can evolve under a defined selective pressure; patient-concordant evolution requires longitudinal evidence that the same trajectory occurs in the patient. Patient-concordant evolution, experimentally inducible evolution, and culture-autonomous evolution are therefore distinct concepts. Culture-autonomous evolution may represent a temporal form of CISD when adaptation produces states that resemble clinically recognized resistance biology without evidence that the same process occurred in the patient.

7.3. From Ex Vivo Response to Patient Prediction

A treatment that reduces organoid viability demonstrates experimental activity; therapeutic fidelity requires correspondence with an appropriate biological or clinical treatment reference. In metastatic gastrointestinal cancers, Vlachogiannis et al. reported 100% sensitivity, 93% specificity, 88% positive predictive value, and 100% negative predictive value across 21 evaluable patient–organoid treatment comparisons [10].

Treatment specificity is equally important. Therapeutic validation should therefore be assigned to a specific model, assay, treatment, and clinical setting rather than generalized to PDOs as a model class. In locally advanced rectal cancer, PDO-based prediction of chemoradiation response achieved 84.43% accuracy, 78.01% sensitivity, and 91.97% specificity in a co-clinical setting linked to a phase III trial [16].

Therapeutic fidelity also depends on whether the model contains the biological mechanism through which the treatment acts. This is particularly important for cellular therapies, for which tumor–immune interactions are absent from conventional epithelial drug-screening assays. In recurrent glioblastoma, patient-derived organoids exposed to the same

autologous dual-targeting CAR-T products administered clinically provided treatment-specific biological readouts, illustrating the value of retaining the mechanism relevant to the therapeutic question [66].

7.4. Predictive Validity Is Not the Same as Clinical Utility

Prospective patient–organoid concordance does not by itself establish clinical utility. Establishment success, assay failure, and turnaround time can all affect the performance of an organoid-guided workflow even though they are not dimensions of biological fidelity. Clinical utility requires evidence beyond analytical or predictive concordance, including demonstration that organoid results can be generated in a clinically actionable time frame and meaningfully inform treatment decisions [67]. Retrospective concordance alone cannot establish that organoid-guided treatment improves patient outcome.

Clinical performance should therefore be evaluated across the complete workflow rather than only among successfully established organoids. Therapeutic fidelity describes correspondence between model and treatment behavior. A model does not need maximal biological complexity to be therapeutically informative, but it must represent and validate the processes required for the intended treatment inference.

For studies intended to inform clinical decisions, we propose that the biological OFMF profile be accompanied by a separate assessment of operational feasibility. Relevant measures include organoid establishment rates, assay failure, time to result, and the proportion of enrolled patients for whom an evaluable result is available within the treatment decision window. These limitations are clinically consequential, as illustrated by the prospective SENSOR study, in which organoid-guided treatment proved difficult to implement and did not yield objective responses among the treated patients [67]. The relevance of vascular reconstruction should also be considered in relation to the treatment mechanism. Vascularized tumor-on-chip systems can capture tumor–vascular interactions that conventional epithelial cultures do not model, although this does not establish equivalence to the patient's native vasculature [21]. Operational performance and vascular-related readouts should therefore complement, rather than redefine, the three biological fidelity domains.

8. Fidelity-Guided Model Selection and the Organoid Fidelity Mapping Framework

8.1. Question-First Model Selection

No single cancer organoid platform is optimal for every biological question. Model suitability depends on whether the biological processes required for the intended inference are represented and adequately validated. For tumor-cell-intrinsic questions, a reductionist epithelial PDO may provide strong mechanistic interpretability if malignant identity, the relevant genomic context, and an appropriate functional readout are established. Studies of acquired resistance additionally require temporal design and longitudinal sampling because resistance cannot be inferred from a single baseline state.

Existing platforms illustrate this principle. Controlled oxygen-gradient systems provide stronger evidence for physicochemical variables when those variables are imposed, quantified, and experimentally manipulated rather than inferred from spontaneous three-dimensional heterogeneity [34]. Model selection should begin with the biological question, followed by identification of the processes required to answer it, determination of the fidelity dimensions that must be represented and validated, and only then selection of the experimental platform. This question-first logic is summarized in Figure 4.

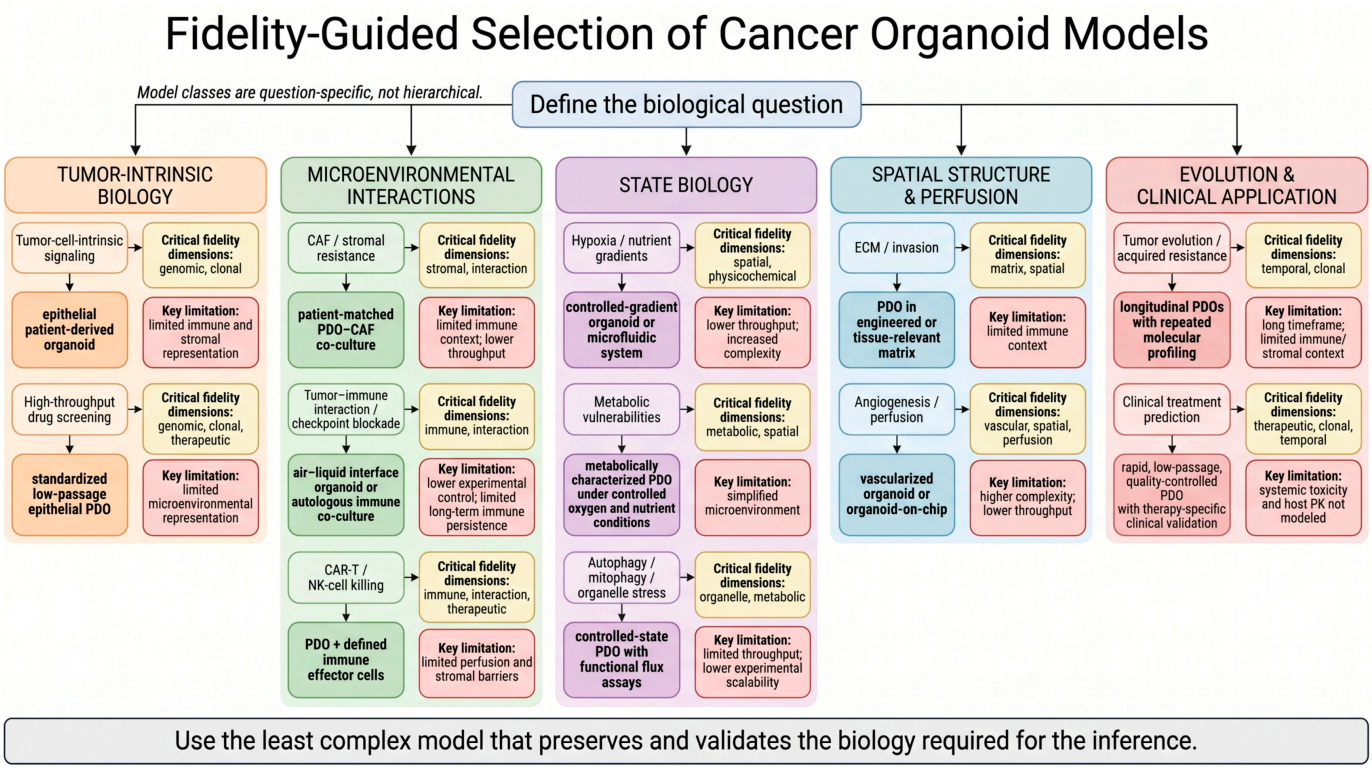


Figure 4. Fidelity-guided selection of cancer organoid models. Model selection should begin with the biological question rather than with platform complexity. Tumor-cell-intrinsic signaling and high-throughput drug-screening questions may be addressed using relatively reductionist epithelial patient-derived organoids when malignant identity and the relevant genomic, clonal, or therapeutic dimensions are validated. Questions involving stromal resistance, tumor–immune interaction, checkpoint blockade, or cellular therapy require incorporation or preservation of the corresponding microenvironmental components. State-biology questions involving hypoxia, nutrient gradients, metabolism, autophagy, mitophagy, or organelle stress benefit from controlled environmental conditions and pathway-specific functional assays. Questions involving extracellular matrix, invasion, angiogenesis, transport, or perfusion may require engineered matrices, vascularized systems, or organoid-on-chip platforms. Longitudinal and clinical applications additionally require temporal, clonal, and therapy-specific validation. Model classes are therefore question-specific rather than hierarchical. The guiding principle is to use the least complex model that preserves and validates the biology required for the intended inference. CAF, cancer-associated fibroblast; ECM, extracellular matrix; NK, natural killer; PDO, patient-derived organoid. Created with BioRender.com.

8.2. The Minimum Sufficient Model

We define the minimum sufficient model as the least complex experimental system that preserves and validates the biological dimensions required to answer a specified question. A tumor-cell-intrinsic signaling study may require confirmed malignant identity, the relevant genomic context, an appropriate lineage state, and a functional readout without requiring an immune or vascular compartment. In recurrent glioblastoma, patient-derived glioblastoma organoids treated with the same autologous dual-targeting CAR-T products administered clinically showed organoid cytolysis, target-antigen reduction, and cytokine responses that correlated with patient-associated biological readouts [66].

The same principle applies to spatial, metabolic, and organelle biology. Engineered colorectal cancer PDO arrays, for example, incorporated defined matrix stiffness and hypoxic conditions to recover selected extrinsic tumor features and improve correspondence with tissue-level readouts [35]. Model complexity should be increased only when a missing biological dimension must be restored and the resulting gain can be demonstrated exper-

imentally. Each added component also introduces new variables that require validation. Model quality depends on whether the validated dimensions match the intended inference, not on the number of incorporated compartments.

This principle also applies to multipurpose drug-screening platforms. Epithelial PDOs may be sufficient for initial tumor-cell-intrinsic sensitivity testing, whereas questions involving vascular drug delivery, tumor–endothelial interactions, or invasion require additional biological components and functional assessment. Recent vascularized lung tumoroid-on-chip models illustrate how incorporating a tumor–vascular interface can reveal processes that are not captured by conventional spheroid systems [21]. For high-throughput workflows, a reasonable strategy is therefore to use simpler models for initial screening and introduce vascularized or perfused systems when the mechanism under investigation warrants greater complexity. Model selection should remain linked to the intended inference rather than to a general expectation that vascularization improves every experimental endpoint.

The financial implications of fidelity assessment must also be considered. Comprehensive molecular profiling, spatial imaging, isotope tracing, and vascularized culture systems differ substantially in cost, technical requirements, and sample consumption. Requiring every laboratory to perform all of these analyses would be neither practical nor consistent with the minimum sufficient model principle. Instead, resources should be directed toward validating the dimensions essential to the primary hypothesis, with additional characterization pursued where it can resolve a specific biological uncertainty. Existing matched datasets and shared core facilities may reduce some of this burden, although access remains uneven. Importantly, limited resources do not justify broader fidelity claims: when a relevant dimension cannot be assessed, this limitation should be reported rather than treated as evidence of correspondence.

8.3. OFMF Defines the Inference Boundary

Question-specific model selection creates a reporting problem because studies may describe organoids as "high fidelity" despite having validated different biological properties. A global descriptor obscures these differences and encourages extrapolation beyond the available evidence.

The Organoid Fidelity Mapping Framework (OFMF) is intended as a complementary biological reporting layer rather than a replacement for technical quality control, authentication, biobanking, manufacturing standards, or assay validation. Biological fidelity asks which properties of a defined reference have been preserved, altered, reconstructed, or left unmeasured. This distinction becomes increasingly relevant as organoid-specific technical standards are developed. These initiatives strengthen standardization, reproducibility, and traceability, but they do not establish correspondence between an organoid and its biological tumor reference [68]. The OFMF begins with explicit definition of that reference. The resolution of a fidelity claim cannot exceed the resolution of the reference used to validate it.

The evidence states used by the OFMF are defined in Section 2.3. The distinction between discordant and not assessed remains essential because absence of measurement is not evidence of either preservation or failure. Culture-associated divergence is recorded separately through Culture-Induced State Distortion (CISD). The designation does not imply technical error; it identifies a state whose attribution to the patient tumor has not been established.

Retrospective organoid biobanks present a particular challenge when matched tumor specimens are unavailable or insufficiently characterized. Archived histopathology, vascular staining, clinical imaging, and previously generated molecular data may provide

useful contextual information, but they do not offer equivalent evidence for every fidelity dimension. For example, endothelial or perivascular markers in archived sections may inform vascular composition and organization, whereas perfusion and local oxygen delivery cannot be inferred reliably from these findings alone [20,22]. Reference datasets from comparable tumors may help place organoid findings within a broader biological context but cannot demonstrate patient-specific vascular concordance. Under these circumstances, the OFMF remains useful for documenting the available evidence and its limitations, while vascular properties lacking a suitable comparator should be designated not assessed.

The output of the OFMF is an inference boundary, not a model ranking. These profiles are not contradictory; they describe what each model can and cannot support. Conclusions should not extend beyond the biological dimensions that have been represented and sufficiently validated. A 14-domain reporting checklist for applying the OFMF across provenance, tumor identity, genomic and clonal fidelity, lineage state, microenvironmental composition, biological interactions, spatial and physicochemical conditions, metabolism, organelle and stress-response states, temporal evolution, therapeutic response, and culture-induced state distortion is provided in Supplementary Table S1.

The proposed checklist may also assist reviewers and editors in evaluating whether organoid fidelity claims are adequately supported. Such an assessment should consider the definition of the parental tumor reference, the methods used to validate each claimed dimension, and the explicit reporting of discordant or unassessed properties. For microenvironmental fidelity, cellular composition, biological state, and functional interactions should be distinguished, particularly when vascularization is invoked to explain treatment response. Recent community-informed reporting recommendations for gastrointestinal organoid research have emphasized the importance of standardized experimental documentation and transparent reporting [68]. The OFMF is intended to complement these efforts by specifying the biological inferences supported by the available evidence. Its adoption will nevertheless depend on access to reference material, laboratory resources, and the feasibility of specialized measurements. The checklist should therefore guide the interpretation and reporting of evidence without making extensive multimodal characterization a universal requirement.

8.4. Applying the OFMF to Published Patient-Derived Organoid Models

The practical use of the OFMF can be illustrated by applying its evidence categories to two established patient-derived organoid studies. In colorectal cancer, van de Wetering et al. established a living biobank that retained major genomic features, represented established colorectal cancer molecular subtypes across the biobank, and supported pharmacological screening [3]. These findings provide evidence for selected compositional features and experimental drug sensitivity, but they do not independently establish preservation of the native stromal or vascular environment, metabolic state, or clinical treatment response. In glioblastoma, Jacob et al. developed patient-derived organoids that retained histological features, cellular heterogeneity, gene-expression patterns, and tumor-associated mutations, and demonstrated experimentally relevant infiltration and treatment responses [6]. These findings support several compositional and functional properties, while leaving other aspects of patient-specific microenvironmental and therapeutic fidelity unresolved. Table 3 illustrates the application of OFMF classifications to selected dimensions of these two published models.

Table 3. Illustrative application of the Organoid Fidelity Mapping Framework to published colorectal cancer and glioblastoma organoid studies.

OFMF Dimension	Colorectal PDOs—van de Wetering et al. [3]	Glioblastoma Organoids—Jacob et al. [6]
Tumor identity	Validated: matched histological and tumor-associated molecular features	Validated: matched histological and tumor-associated molecular features
Genomic fidelity	Validated for assessed features: matched mutation and copy-number analyses in available patient pairs	Validated for assessed features: matched somatic variants and allele frequencies
Clonal fidelity	Partially validated: shared driver-associated subclones identified in matched samples, but preservation of their relative representation was incomplete	Not assessed for parental clonal-proportion fidelity: mutational diversity and cellular heterogeneity retained, but matched quantitative representation of malignant subclones not established
Lineage and differentiation-state fidelity	Not assessed for comprehensive patient-matched differentiation-state fidelity: major CRC molecular subtypes represented across the biobank, without demonstration of complete parental cellular-state preservation	Partially validated: matched cellular identities and transcriptional states
Microenvironmental compositional fidelity	Not assessed: preservation of the complete native stromal and immune compartments	Partially validated: selected macrophage/microglial, T-cell, and stromal populations retained
Vascular microenvironmental fidelity	Not assessed: native vascular composition and function	Not assessed for functional vascular fidelity: CD31-positive structures reported, but patient-specific perfusion and transport correspondence not established
Metabolic fidelity	Not assessed: matched tumor–organoid metabolic state	Not assessed: matched tumor–organoid metabolic state
Experimental functional evidence (not a fidelity classification)	Drug-response screening and genotype–drug associations demonstrated	Drug/CAR-T responses and xenograft infiltration demonstrated
Clinical therapeutic fidelity	Not assessed: patient treatment-response concordance	Not assessed: patient treatment-response concordance
Temporal and evolutionary fidelity	Not assessed: patient-concordant longitudinal evolution	Not assessed: patient-concordant evolution, despite culture-time characterization

Table 3 Footnote. The classifications are restricted to the evidence reported in the original studies [3,6]. "Validated" and "partially validated" refer to the specified features rather than complete fidelity across an entire domain. "Not assessed" indicates that the corresponding patient-specific fidelity claim was not established; it does not imply absence of the biological property. Experimental functional characterization is distinguished from demonstrated fidelity to patient outcomes. The examples illustrate inference boundaries rather than comparative model quality. Note: Bold text in the first column indicates the OFMF dimensions evaluated in this comparative analysis.

9. Conclusions and Future Perspectives

Cancer organoids have become powerful experimental systems by combining patient-derived tumor material with renewable culture, functional perturbation, and increasing compatibility with multicellular and engineered model configurations. Evidence obtained in one domain should not be extrapolated automatically to another. A major challenge

for future organoid studies is to distinguish tumor-derived biology from states generated or amplified by the culture system. Future fidelity studies should therefore define the biological reference with sufficient spatial, temporal, and molecular resolution to support the claim being made.

Advances in synthetic matrices, bioprinting, immune co-culture, assembloids, vascularized systems, microfluidics, and organoid-on-chip technologies will continue to expand the processes that can be modeled experimentally [26,27,31,33–35,46]. This principle underlies the minimum sufficient model: the least complex system that preserves and validates the biology required to answer a defined question. Clinical translation requires the same level of precision. High-dimensional imaging, molecular profiling, and computational analysis may improve characterization and response classification, but greater analytical precision does not by itself establish biological fidelity [18,69].

The broader goal is not to construct organoids that reproduce every feature of a tumor, but to establish which features are retained, altered, or remain unexamined in relation to a defined biological reference. The application of OFMF will necessarily depend on access to matched patient material, experimental resources, and the feasibility of longitudinal and functional validation. These constraints should limit the scope of fidelity claims rather than discourage the use of simpler or incompletely characterized models. By making such boundaries explicit, the framework offers a way to assess organoid studies according to the biological questions they can reliably address, without equating model complexity with overall fidelity.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/organoids5040036/s1, Table S1: Organoid Fidelity Mapping Framework (OFMF): 14-domain reporting checklist for cancer organoid studies.

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Abbreviations

The following abbreviations are used in this manuscript:

ALI	Air–liquid interface
CAF	Cancer-associated fibroblast
CD8	Cluster of Differentiation 8
cGAS	Cyclic GMP-AMP synthase
CISD	Culture-Induced State Distortion
CRC	Colorectal cancer
ECAR	Extracellular acidification rate
ECM	Extracellular matrix
ER	Endoplasmic reticulum
FAO	Fatty acid oxidation
HCC	Hepatocellular carcinoma

ICB	Immune checkpoint blockade
IFN-γ	Interferon gamma
MDSCs	Myeloid-derived suppressor cells
MHC-I	Major histocompatibility complex class I
MRT	Malignant rhabdoid tumor
mtDNA	Mitochondrial DNA
mtROS	Mitochondrial reactive oxygen species
OCR	Oxygen-consumption rate
OFMF	Organoid Fidelity Mapping Framework
OXPHOS	Oxidative phosphorylation
PD-1	Programmed cell death protein 1
PD-L1	Programmed death-ligand 1
PDAC	Pancreatic ductal adenocarcinoma
PDO	Patient-derived organoid
PDOX	Patient-derived organoid xenograft
PERK	Protein kinase RNA-like endoplasmic reticulum kinase
ROS	Reactive oxygen species
STING	Stimulator of interferon genes
TCA	Tricarboxylic acid cycle
TCR	T-cell receptor
TILs	Tumor-infiltrating lymphocytes
TME	Tumor microenvironment
UPR	Unfolded protein response

References

1. Gao, D.; Vela, I.; Sboner, A.; Iaquinta, P.J.; Karthaus, W.R.; Gopalan, A.; Dowling, C.; Wanjala, J.N.; Undvall, E.A.; Arora, V.K.; et al. Organoid cultures derived from patients with advanced prostate cancer. *Cell* **2014**, *159*, 176–187. [CrossRef] [PubMed]
2. Boj, S.F.; Hwang, C.-I.; Baker, L.A.; Chio, I.I.C.; Engle, D.D.; Corbo, V.; Jager, M.; Ponz-Sarvise, M.; Tiriac, H.; Spector, M.S.; et al. Organoid models of human and mouse ductal pancreatic cancer. *Cell* **2015**, *160*, 324–338. [CrossRef] [PubMed]
3. van de Wetering, M.; Francies, H.E.; Francis, J.M.; Bounova, G.; Iorio, F.; Pronk, A.; van Houdt, W.; van Gorp, J.; Taylor-Weiner, A.; Kester, L.; et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell* **2015**, *161*, 933–945. [CrossRef] [PubMed]
4. Sachs, N.; de Ligt, J.; Kopper, O.; Gogola, E.; Bounova, G.; Weeber, F.; Balgobind, A.V.; Wind, K.; Gracanin, A.; Begthel, H.; et al. A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity. *Cell* **2018**, *172*, 373–386.e10. [CrossRef] [PubMed]
5. Kopper, O.; de Witte, C.J.; Lõhmussaar, K.; Valle-Inclan, J.E.; Hami, N.; Kester, L.; Balgobind, A.V.; Korving, J.; Proost, N.; Begthel, H.; et al. An organoid platform for ovarian cancer captures intra- and interpatient heterogeneity. *Nat. Med.* **2019**, *25*, 838–849. [CrossRef] [PubMed]
6. Jacob, F.; Salinas, R.D.; Zhang, D.Y.; Nguyen, P.T.; Schnoll, J.G.; Wong, S.Z.H.; Thokala, R.; Sheikh, S.; Saxena, D.; Prokop, S.; et al. A Patient-Derived Glioblastoma Organoid Model and Biobank Recapitulates Inter- and Intra-tumoral Heterogeneity. *Cell* **2020**, *180*, 188–204.e22. [CrossRef] [PubMed]
7. Minoli, M.; Cantore, T.; Hanhart, D.; Kiener, M.; Fedrizzi, T.; La Manna, F.; Karkampouna, S.; Chouvardas, P.; Genitsch, V.; Rodriguez-Calero, A.; et al. Bladder cancer organoids as a functional system to model different disease stages and therapy response. *Nat. Commun.* **2023**, *14*, 2214. [CrossRef] [PubMed]
8. Herranz-Ors, C.; Bhosle, S.G.; Beck, A.E.; Gilbert, J.G.R.; Picco, G.; Valle-Inclan, J.E.; Muyas, F.; Valentini, S.; Andres, A.E.; Ansari, R.; et al. A tumour-derived organoid biobank maps cancer gene dependencies. *Nature* **2026**, *657*, 765–774. [CrossRef] [PubMed]
9. Neal, J.T.; Li, X.; Zhu, J.; Giangarra, V.; Grzeskowiak, C.L.; Ju, J.; Liu, I.H.; Chiou, S.-H.; Salahudeen, A.A.; Smith, A.R.; et al. Organoid Modeling of the Tumor Immune Microenvironment. *Cell* **2018**, *175*, 1972–1988.e16. [CrossRef] [PubMed]
10. Vlachogiannis, G.; Hedayat, S.; Vatsiou, A.; Jamin, Y.; Fernández-Mateos, J.; Khan, K.; Lampis, A.; Eason, K.; Huntingford, I.; Burke, R.; et al. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science* **2018**, *359*, 920–926. [CrossRef] [PubMed]
11. Verduin, M.; Hoosemans, L.; Vanmechelen, M.; van Heumen, M.; Piepers, J.A.F.; Astuti, G.; Ackermans, L.; Schijns, O.E.M.G.; Kampen, K.R.; Tjan-Heijnen, V.C.G.; et al. Patient-derived glioblastoma organoids reflect tumor heterogeneity and treatment sensitivity. *Neurooncol. Adv.* **2023**, *5*, vdad152. [CrossRef] [PubMed]
12. Drost, J.; Clevers, H. Organoids in cancer research. *Nat. Rev. Cancer* **2018**, *18*, 407–418. [CrossRef] [PubMed]

13. van den Brink, S.C.; Sage, F.; Vértesy, Á.; Spanjaard, B.; Peterson-Maduro, J.; Baron, C.S.; Robin, C.; van Oudenaarden, A. Single-cell sequencing reveals dissociation-induced gene expression in tissue subpopulations. *Nat. Methods* **2017**, *14*, 935–936. [CrossRef] [PubMed]
14. Slovackova, J.; Bernatik, O.; Cimborova, K.; Barak, M.; Hendrych, M.; Kocourkova, K.; Sulcova, M.; Olha, J.; Cerna, K.A.; Hodny, Z.; et al. From patient to tumor organoid: Culture protocol choice controls glioblastoma tumor architecture and identity. *Brain Pathol.* **2026**, e70125. [CrossRef] [PubMed]
15. Ooft, S.N.; Weeber, F.; Dijkstra, K.K.; McLean, C.M.; Kaing, S.; van Werkhoven, E.; Schipper, L.; Hoes, L.; Vis, D.J.; van de Haar, J.; et al. Patient-derived organoids can predict response to chemotherapy in metastatic colorectal cancer patients. *Sci. Transl. Med.* **2019**, *11*, eaay2574. [CrossRef] [PubMed]
16. Yao, Y.; Xu, X.; Yang, L.; Zhu, J.; Wan, J.; Shen, L.; Xia, F.; Fu, G.; Deng, Y.; Pan, M.; et al. Patient-Derived Organoids Predict Chemoradiation Responses of Locally Advanced Rectal Cancer. *Cell Stem Cell* **2020**, *26*, 17–26.e6. [CrossRef] [PubMed]
17. Ali, H.A.; Karasinska, J.M.; Topham, J.T.; Johal, D.; Kalloger, S.; Metcalfe, A.; Warren, C.S.; Miyagi, A.; Tao, L.V.; Kevorkova, M.; et al. Pancreatic cancer tumor organoids exhibit subtype-specific differences in metabolic profiles. *Cancer Metab.* **2024**, *12*, 28. [CrossRef] [PubMed]
18. Gillette, A.; Udgata, S.; Schmitz, A.E.; Stoecker, J.N.; Kratz, J.D.; Deming, D.A.; Skala, M.C. Wide-Field Optical Redox Imaging with Leading-Edge Detection Enables Assessment of Treatment Response and Heterogeneity in Patient-Derived Cancer Organoids. *Cancer Res.* **2025**, *85*, 4329–4340. [CrossRef] [PubMed]
19. Debruyne, A.C.; Okkelman, I.A.; Heymans, N.; Pinheiro, C.; Hendrix, A.; Nobis, M.; Borisov, S.M.; Dmitriev, R.I. Live Microscopy of Multicellular Spheroids with the Multimodal Near-Infrared Nanoparticles Reveals Differences in Oxygenation Gradients. *ACS Nano* **2024**, *18*, 12168–12186. [CrossRef] [PubMed]
20. Jain, R.K. Normalization of tumor vasculature: An emerging concept in antiangiogenic therapy. *Science* **2005**, *307*, 58–62. [CrossRef] [PubMed]
21. Wang, X.; Zhu, G.; Lu, J.; Hu, X.; Zheng, W.; Yang, K.; Zhang, W.; Huang, Y.; Hu, S.; Zhao, B.; et al. A Vascularized Lung Tumoroid-on-Chip Model for the Accurate Assessment of Anti-Invasion Agents. *Adv. Funct. Mater.* **2026**, *36*, e26981. [CrossRef]
22. Martin, J.D.; Seano, G.; Jain, R.K. Normalizing Function of Tumor Vessels: Progress, Opportunities, and Challenges. *Annu. Rev. Physiol.* **2019**, *81*, 505–534. [CrossRef] [PubMed]
23. Lee, S.H.; Hu, W.; Matulay, J.T.; Silva, M.V.; Owczarek, T.B.; Kim, K.; Chua, C.W.; Barlow, L.J.; Kandoth, C.; Williams, A.B.; et al. Tumor Evolution and Drug Response in Patient-Derived Organoid Models of Bladder Cancer. *Cell* **2018**, *173*, 515–528.e17. [CrossRef] [PubMed]
24. Gerlinger, M.; Rowan, A.J.; Horswell, S.; Larkin, J.; Endesfelder, D.; Gronroos, E.; Martinez, P.; Matthews, N.; Stewart, A.; Tarpey, P.; et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N. Engl. J. Med.* **2012**, *366*, 883–892. [CrossRef] [PubMed]
25. Bollen, Y.; Stelloo, E.; van Leenen, P.; van den Bos, M.; Ponsioen, B.; Lu, B.; van Roosmalen, M.J.; Bolhaqueiro, A.C.F.; Kimberley, C.; Mossner, M.; et al. Reconstructing single-cell karyotype alterations in colorectal cancer identifies punctuated and gradual diversification patterns. *Nat. Genet.* **2021**, *53*, 1187–1195. [CrossRef] [PubMed]
26. Schuth, S.; Le Blanc, S.; Krieger, T.G.; Jabs, J.; Schenk, M.; Giese, N.A.; Büchler, M.W.; Eils, R.; Conrad, C.; Strobel, O.; et al. Patient-specific modeling of stroma-mediated chemoresistance of pancreatic cancer using a three-dimensional organoid-fibroblast co-culture system. *J. Exp. Clin. Cancer Res.* **2022**, *41*, 312. [CrossRef] [PubMed]
27. Sharpe, B.P.; Nazlamova, L.A.; Tse, C.; Johnston, D.A.; Thomas, J.; Blyth, R.; Pickering, O.J.; Grace, B.; Harrington, J.; Rajak, R.; et al. Patient-derived tumor organoid and fibroblast assembloid models for interrogation of the tumor microenvironment in esophageal adenocarcinoma. *Cell Rep. Methods* **2024**, *4*, 100909. [CrossRef] [PubMed]
28. Cattaneo, C.M.; Dijkstra, K.K.; Fanchi, L.F.; Kelderman, S.; Kaing, S.; van Rooij, N.; van den Brink, S.; Schumacher, T.N.; Voest, E.E. Tumor organoid-T-cell coculture systems. *Nat. Protoc.* **2020**, *15*, 15–39. [CrossRef] [PubMed]
29. Yuan, J.; Li, X.; Yu, S. Cancer organoid co-culture model system: Novel approach to guide precision medicine. *Front. Immunol.* **2023**, *13*, 1061388. [CrossRef] [PubMed]
30. Gu, Z.; Wu, Q.; Shang, B.; Zhang, K.; Zhang, W. Organoid co-culture models of the tumor microenvironment promote precision medicine. *Cancer Innov.* **2024**, *3*, e101. [CrossRef] [PubMed]
31. Xing, H.; Liu, R.; Chen, Y.; Chen, X.; Gao, X.; Shen, C.; Zhang, S.; Tang, Y.; Wu, L.; You, M.; et al. Optimized patient-derived lung cancer organoids recapitulating the immune landscape for precision therapy evaluation. *Cell. Oncol.* **2026**, *49*, 15. [CrossRef] [PubMed]
32. Demmers, L.C.; Kretzschmar, K.; Van Hoeck, A.; Bar-Epraïm, Y.E.; van den Toorn, H.W.P.; Koomen, M.; van Son, G.; van Gorp, J.; Pronk, A.; Smakman, N.; et al. Single-cell derived tumor organoids display diversity in HLA class I peptide presentation. *Nat. Commun.* **2020**, *11*, 5338. [CrossRef] [PubMed]

33. Córdoba, L.; Cueto, F.J.; Cantero-Cid, R.; Abad-Moret, R.; Díaz, E.; Álvarez-Benayas, J.; Fernández-Felipe, J.; Jiménez-Rodríguez, J.; Arvelo-Rosario, D.; Mata-Martínez, P.; et al. Colorectal Air–Liquid Interface Organoids Preserve Tumour-Immune Architecture and Reveal Local Treg Expansion After PD-1 Blockade. *Cancers* **2026**, *18*, 132. [CrossRef] [PubMed]
34. Baek, S.; Ha, H.-S.; Park, J.S.; Cho, M.J.; Kim, H.-S.; Yu, S.E.; Chung, S.; Kim, C.; Kim, J.; Lee, J.Y.; et al. Chip collection of hepatocellular carcinoma based on O_2 heterogeneity from patient tissue. *Nat. Commun.* **2024**, *15*, 5117. [CrossRef] [PubMed]
35. Han, J.; Jeong, H.; Choi, J.; Kim, H.; Kwon, T.; Myung, K.; Park, K.; Park, J.I.; Sánchez, S.; Jung, D.; et al. Bioprinted Patient-Derived Organoid Arrays Capture Intrinsic and Extrinsic Tumor Features for Advanced Personalized Medicine. *Adv. Sci.* **2025**, *12*, e2407871. [CrossRef] [PubMed]
36. Aisenbrey, E.A.; Murphy, W.L. Synthetic alternatives to Matrigel. *Nat. Rev. Mater.* **2020**, *5*, 539–551. [CrossRef] [PubMed]
37. Lumibao, J.C.; Okhovat, S.R.; Peck, K.L.; Lin, X.; Lande, K.; Yomtoubian, S.; Ng, I.; Tiriac, H.; Lowy, A.M.; Zou, J.; et al. The effect of extracellular matrix on the precision medicine utility of pancreatic cancer patient-derived organoids. *JCI Insight* **2024**, *9*, e172419. [CrossRef] [PubMed]
38. Khan, S.; Shin, J.H.; Ferri, V.; Cheng, N.; Noel, J.E.; Kuo, C.; Sunwoo, J.B.; Pratx, G. High-resolution positron emission microscopy of patient-derived tumor organoids. *Nat. Commun.* **2021**, *12*, 5883. [CrossRef] [PubMed]
39. Kes, M.M.G.; Morales-Rodriguez, F.; Zaal, E.A.; de Souza, T.; Proost, N.; van de Ven, M.; van den Heuvel-Eibrink, M.M.; Jansen, J.W.; Berkers, C.R.; Drost, J. Metabolic profiling of patient-derived organoids reveals nucleotide synthesis as a metabolic vulnerability in malignant rhabdoid tumors. *Cell Rep. Med.* **2025**, *6*, 101878. [CrossRef] [PubMed]
40. Chia, M.L.; Bulat, F.; Gaunt, A.; Ros, S.; Wright, A.J.; Sawle, A.; Porcu, L.; Vias, M.; Brenton, J.D.; Brindle, K.M. Metabolic imaging distinguishes ovarian cancer subtypes and detects their early and variable responses to treatment. *Oncogene* **2025**, *44*, 563–574. [CrossRef] [PubMed]
41. Vande Voorde, J.; Ackermann, T.; Pfetzer, N.; Sumpton, D.; Mackay, G.; Kalna, G.; Nixon, C.; Blyth, K.; Gottlieb, E.; Tardito, S. Improving the metabolic fidelity of cancer models with a physiological cell culture medium. *Sci. Adv.* **2019**, *5*, eaau7314. [CrossRef] [PubMed]
42. Chen, S.; Lv, C.; Li, Z.; Ma, B.; Ma, J.; Li, M.; Zhang, X.; Li, M.; Zhao, H.; Liu, W.; et al. Integrating Mass Spectrometry Imaging with Tumor Organoid-Immunity Platform to Identify Metabolic Adaptations in Tumor Immune Resistance. *Anal. Chem.* **2026**, *98*, 10728–10737. [CrossRef] [PubMed]
43. Li, Y.; Zou, J.; Fang, Y.; Zuo, J.; Wang, R.; Liang, S. Lung tumor organoids migrate as cell clusters containing cancer stem cells under hypoxic condition. *Biol. Cell* **2025**, *117*, e2400081. [CrossRef] [PubMed]
44. Grimes, D.R.; Kelly, C.; Bloch, K.; Partridge, M. A method for estimating the oxygen consumption rate in multicellular tumour spheroids. *J. R. Soc. Interface* **2014**, *11*, 20131124. [CrossRef] [PubMed]
45. Hubert, C.G.; Rivera, M.; Spangler, L.C.; Wu, Q.; Mack, S.C.; Prager, B.C.; Couce, M.; McLendon, R.E.; Sloan, A.E.; Rich, J.N. A Three-Dimensional Organoid Culture System Derived from Human Glioblastomas Recapitulates the Hypoxic Gradients and Cancer Stem Cell Heterogeneity of Tumors Found In Vivo. *Cancer Res.* **2016**, *76*, 2465–2477. [CrossRef] [PubMed]
46. Pal, S.; Shimshoni, E.; Torres, S.F.; Kong, M.; Tai, K.; Sangwan, V.; Bertos, N.; Bailey, S.D.; Bérubé, J.; Ingber, D.E.; et al. Patient-derived esophageal adenocarcinoma organ chip: A physiologically relevant platform for functional precision oncology. *J. Transl. Med.* **2025**, *23*, 577. [CrossRef] [PubMed]
47. Brand, M.D.; Nicholls, D.G. Assessing mitochondrial dysfunction in cells. *Biochem. J.* **2011**, *435*, 297–312. [CrossRef] [PubMed]
48. Divakaruni, A.S.; Paradyse, A.; Ferrick, D.A.; Murphy, A.N.; Jastroch, M. Analysis and interpretation of microplate-based oxygen consumption and pH data. *Methods Enzymol.* **2014**, *547*, 309–354. [CrossRef] [PubMed]
49. Li, J.; Ma, Q.; Huang, C.; Zeng, X.; He, J.; You, L.; Zhuang, X.; Xiao, P.; You, F.; Ren, Y.; et al. Mechanism of Saikosaponin D in regulating ferroptosis in patient-derived lung adenocarcinoma organoids via upregulation of ATF3/CHOP/CHAC1 signaling. *Sci. Rep.* **2026**, *16*, 2513. [CrossRef] [PubMed]
50. Chen, Q.; Jin, Y.; Liao, L.; Shen, R.; Ge, X.; Jiang, Q.; Huang, Y.; Sun, Q.; Liu, D.; Liu, L.; et al. Patients-Derived Organoids Sequencing-based FOXP4 Facilitates Radioresistance by Transcriptionally Modifying GPX4 to Regulate ferroptosis in Colorectal Cancer. *Adv. Sci.* **2025**, *12*, e07080. [CrossRef] [PubMed]
51. Yıldırım, B.; Archibong-Omon, A.A.; Bilir, A. Ultrastructural evaluation of lithium-induced autophagic and mitochondrial stress in 3D endometrial and neuroblastoma spheroids. *Sci. Rep.* **2025**, *15*, 37652. [CrossRef] [PubMed]
52. Tao, Z.-H.; Han, J.-X.; Xu, J.; Zhao, E.; Wang, M.; Wang, Z.; Lin, X.-L.; Xiao, X.-Y.; Hong, J.; Chen, H.; et al. Screening of patient-derived organoids identifies mitophagy as a cell-intrinsic vulnerability in colorectal cancer during statin treatment. *Cell Rep. Med.* **2025**, *6*, 102039. [CrossRef] [PubMed]
53. Klionsky, D.J.; Abdel-Aziz, A.K.; Abdelfatah, S.; Abdellatif, M.; Abdoli, A.; Abel, S.; Abeliovich, H.; Abildgaard, M.H.; Abudu, Y.P.; Acevedo-Arozena, A.; et al. Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition). *Autophagy* **2021**, *17*, 1–382. [CrossRef] [PubMed]

54. Weng, N.; Qin, S.; Liu, J.; Huang, X.; Jiang, J.; Zhou, L.; Zhang, Z.; Xie, N.; Wang, K.; Jin, P.; et al. Repurposing econazole as a pharmacological autophagy inhibitor to treat pancreatic ductal adenocarcinoma. *Acta Pharm. Sin. B* **2022**, *12*, 3085–3102. [CrossRef] [PubMed]
55. McWilliams, T.G.; Prescott, A.R.; Allen, G.F.; Tamjar, J.; Munson, M.J.; Thomson, C.; Muqit, M.M.; Ganley, I.G. Mito-QC illuminates mitophagy and mitochondrial architecture in vivo. *J. Cell Biol.* **2016**, *214*, 333–345. [CrossRef] [PubMed]
56. Hetz, C.; Zhang, K.; Kaufman, R.J. Mechanisms, regulation and functions of the unfolded protein response. *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 421–438. [CrossRef] [PubMed]
57. Stockwell, B.R.; Angeli, J.P.F.; Bayir, H.; Bush, A.I.; Conrad, M.; Dixon, S.J.; Fulda, S.; Gascón, S.; Hatzios, S.K.; Kagan, V.E.; et al. Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. *Cell* **2017**, *171*, 273–285. [CrossRef] [PubMed]
58. El Laithy, Y.; De Oliveira, W.A.A.; Pabba, A.; Qualizza, A.; Cosemans, G.; Garcia-Diaz, P.; Richard, F.; Athanasouli, P.; Luci, C.R.; De Wispelaere, W.; et al. Chemotherapy-Treated Breast Cancer Cells Activate the WNT Signaling Pathway to Enter a Diapause-Like Early Persister State. *Cancer Res.* **2026**, *86*, 310–330. [CrossRef] [PubMed]
59. Sharma, S.V.; Lee, D.Y.; Li, B.; Quinlan, M.P.; Takahashi, F.; Maheswaran, S.; McDermott, U.; Azizian, N.; Zou, L.; Fischbach, M.A.; et al. A Chromatin-Mediated Reversible Drug-Tolerant State in Cancer Cell Subpopulations. *Cell* **2010**, *141*, 69–80. [CrossRef] [PubMed]
60. Hangauer, M.J.; Viswanathan, V.S.; Ryan, M.J.; Bole, D.; Eaton, J.K.; Matov, A.; Galeas, J.; Dhruv, H.D.; Berens, M.E.; Schreiber, S.L.; et al. Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition. *Nature* **2017**, *551*, 247–250. [CrossRef] [PubMed]
61. Chen, G.; Gong, T.; Wang, Z.; Wang, Z.; Lin, X.; Chen, S.; Sun, C.; Zhao, W.; Kong, Y.; Ai, H.; et al. Colorectal cancer organoid models uncover oxaliplatin-resistant mechanisms at single cell resolution. *Cell. Oncol.* **2022**, *45*, 1155–1167. [CrossRef] [PubMed]
62. Álvarez-Varela, A.; Novellasdemunt, L.; Barriga, F.M.; Hernando-Momblona, X.; Cañellas-Socias, A.; Cano-Crespo, S.; Sevillano, M.; Cortina, C.; Stork, D.; Morral, C.; et al. Mex3a marks drug-tolerant persister colorectal cancer cells that mediate relapse after chemotherapy. *Nat. Cancer* **2022**, *3*, 1052–1070. [CrossRef] [PubMed]
63. Gulay, K.C.M.; Zhang, X.; Pantazopoulou, V.; Patel, J.; Esparza, E.; Babu, D.S.P.; Ogawa, S.; Weitz, J.; Ng, I.; Mose, E.S.; et al. Dual Inhibition of KRASG12D and Pan-ERBB Is Synergistic in Pancreatic Ductal Adenocarcinoma. *Cancer Res.* **2023**, *83*, 3001–3012. [CrossRef] [PubMed]
64. Boos, S.L.; Loevenich, L.P.; Vosberg, S.; Engleitner, T.; Öllinger, R.; Kumbrink, J.; Rokavec, M.; Michl, M.; Greif, P.A.; Jung, A.; et al. Disease Modeling on Tumor Organoids Implicates AURKA as a Therapeutic Target in Liver Metastatic Colorectal Cancer. *Cell. Mol. Gastroenterol. Hepatol.* **2022**, *13*, 517–540. [CrossRef] [PubMed]
65. Siravegna, G.; Mussolin, B.; Buscarino, M.; Corti, G.; Cassingena, A.; Crisafulli, G.; Ponzetti, A.; Cremolini, C.; Amatu, A.; Lauricella, C.; et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. *Nat. Med.* **2015**, *21*, 795–801. [CrossRef] [PubMed]
66. Logun, M.; Wang, X.; Sun, Y.; Bagley, S.J.; Li, N.; Desai, A.; Zhang, D.Y.; Nasrallah, M.P.; Pai, E.L.-L.; Oner, B.S.; et al. Patient-derived glioblastoma organoids as real-time avatars for assessing responses to clinical CAR-T cell therapy. *Cell Stem Cell* **2025**, *32*, 181–190.e4. [CrossRef] [PubMed]
67. Ooft, S.N.; Weeber, F.; Schipper, L.; Dijkstra, K.; McLean, C.; Kaing, S.; van de Haar, J.; Prevoo, W.; van Werkhoven, E.; Snaebjornsson, P.; et al. Prospective experimental treatment of colorectal cancer patients based on organoid drug responses. *ESMO Open* **2021**, *6*, 100103. [CrossRef] [PubMed]
68. VanDussen, K.L.; Karakasheva, T.A.; Mahe, M.M.; Burclaff, J.; Arrieta-Viana, L.F.; Williamson, I.; McCauley, H.A.; García, A.J.; Magness, S.T.; Hamilton, K.E. Guiding Principles: Reporting Elements for Gastrointestinal Organoid Research. *Cell. Mol. Gastroenterol. Hepatol.* **2026**, *20*, 101772. [CrossRef] [PubMed]
69. Doerfler, R.; Chen, J.; Kim, C.; Smith, J.D.; Harris, M.; Singh, K.B.; Isett, B.; Dadey, R.E.; Brown, D.D.; Lee, A.V.; et al. Integrating artificial intelligence-driven digital pathology and genomics to establish patient-derived organoids as new approach methodologies for drug response in head and neck cancer. *Oral Oncol.* **2025**, *171*, 107742. [CrossRef] [PubMed]

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