The animal era is waning — NAMs reshaping cancer drug discovery

in vitro 3D models are changing translational cancer research

Regulatory pressure and scientific opportunity are accelerating a fundamental shift in translational research and preclinical development. In 2025, leading agencies and funders signaled that reliance on traditional animal experiments will be substantially reduced in favor of NAMs, short for New Approach Methodologies or Novel Alternative Methods: multicellular *in vitro* systems (notably 3D organoid platforms), computational (*in silico*) modeling approaches, and chemical (*in chemico*) assessment techniques. Key programmatic steps include dedicated organoid development centers, FDA Modernization Act 3.0 and regulatory guidance documents for industry that are intended to avoid unnecessary use of animals. These moves together signal a durable pivot toward NAMs in preclinical drug development.

For oncology teams, this transition is particularly relevant. Compared with conventional 2D cell lines, *in vitro* 3D tumor models can preserve tissue architecture, intratumoral heterogeneity and—importantly when applicable—components of the tumor microenvironment (TME), making them powerful tools for immuno-oncology, drug screening and target validation. But different systems are not interchangeable: each model type balances throughput, standardization and fidelity to the native TME in different ways. Here we briefly introduce three widely used 3D systems, their applications in a pragmatic development pipeline, and illustrate how a recent screening study leveraging organotypic tumor spheroids to identify a clinically interesting adjuvant to immune checkpoint therapy—underscoring that, regardless of model, reagent quality (notably antibodies) remains a critical, sometimes underestimated, success factor.

Three types of *in vitro* 3D tumor models

1) Reconstituted tumor organoids (PDTOs/tumoroids) — the workhorse for throughput and engineering

Patient-derived tumor organoids (PDTOs) are typically generated by enzymatically digesting tumor tissue into single cells and allowing them to re-self-organize in an extracellular matrix with defined growth factors. They are relatively straightforward to generate at scale, amenable to automation, and can be genetically manipulated and biobanked. PDTOs retain original tumor characteristics and interpatient variability and are well suited for high-throughput cytotoxicity screens. Their main limitation: conventional PDTOs lack native stromal components, notably immune cells unless co-cultured (reconstitution) or bioengineered.

2) Organotypic tumor spheroids — the "micro battlefield" that preserves original microenvironment

Organotypic tumor spheroids (PDOTSs for patient-derived, MDOTSs for mouse-derived) are partially digested tumor fragments (typically 40–100 μm in diameter) cultured in 3D (often microfluidic devices). They retain autologous immune and stromal populations and can be cultured short-term (days to a couple of weeks). PDOTSs/MDOTSs preserve more of the native spatial variation and immune cell diversity than reconstituted organoids, making them ideal for studies of immunotherapies such as immune checkpoint blockade (ICB), and mechanistic interrogation where immune-tumor interactions matter. Their limits: short culture windows, higher inter-sample heterogeneity and lower amenability to long-term expansion or genetic engineering.

3) Patient-derived tumor fragments (PDTFs) / explants — the most faithful short-term snapshot

PDTFs are minimally processed tumor fragments (~1 mm^3) cultured *in vitro* without enzymatic digestion. They best preserve original architecture and cell composition and are therefore the richest "snapshot" of a patient's tumor biology. PDTFs are typically limited to very short experimental windows and low throughput, so they are most useful for precise, personalized predictions and mechanistic assays rather than broad library screens.

These three systems form a continuum: throughput and standardizability decline from PDTO → PDOTS → PDTF, while preservation of native microenvironment and heterogeneity increases.

While NAMs look more effective and human-relevant, at this stage, *in vitro* 3D models still face challenges in reproducibility and scaling costs. Moreover, organoids cannot be established for all organs. Evidence is often generated within specific contexts of use, such as toxicity prediction for safety assessment.

Implications from a practical case: *in vitro* screening and *in vivo* validation

A recent ***Cancer Cell*** study utilized a multi-tier approach: using *in vitro* 3D models for lead generation and then refining findings in mouse models.

The researchers screened ~3,000 FDA-approved drugs using the MDOTS model, with the goal of identifying a "third agent" that could be added on top of dual immune checkpoint blockade (anti-PD-1 + anti-CTLA-4) to potentiate antitumor efficacy while simultaneously reducing immune-related adverse events (irAEs). MDOTS was deliberately chosen because of its ability to preserve the immune landscape of the TME. By completing the primary screen entirely on MDOTSs, the authors efficiently identified

clofazimine as a candidate drug, avoiding the need to conduct large numbers of animal experiments at the initial discovery stage.

The authors then carried out validation experiments *in vitro* using PDOTSs as well as multiple tumor models *in vivo*. In mice, the combination of clofazimine with dual ICB not only enhanced tumor eradication, but also significantly alleviated irAEs such as colitis, neurotoxicity, and fatal myocarditis. The high degree of concordance between the *in vitro* and *in vivo* results in this study highlights both the efficiency of *in vitro* models for scaled screening and the strength of *in vivo* models in capturing systemic biological complexity.

The NAM era is not simply ending animal testing instantly, but it is a durable re-balancing of discovery paradigms that favors human-relevant, ethically aligned, and probably more effective *in vitro* approaches. For translational teams, success in this landscape depends less on abandoning older models than on smartly combining appropriate models with high-quality reagents and robust validation stages. Notably, the dual ICB antibodies used for drug screening were Bio X Cell's ***InVivo*MAb anti-mouse PD-1 (#BE0146)** and ***InVivo*MAb anti-mouse CTLA-4 (#BE0032)**. High-purity, low-endotoxin functional antibodies provide the essential foundation for ensuring the accuracy and reliability of drug screening results.

Bio X Cell empowers translational research using *in vitro* 3D models

Whether in animal studies or advanced *in vitro* 3D models, high-quality antibodies are fundamental to generating data that researchers can trust. Bio X Cell's premium functional antibodies are equally well suited for both *in vivo* and *in vitro* live-cell models:

- **Exceptional purity and ultra-low endotoxin levels**

Designed to minimize nonspecific interference, Bio X Cell antibodies are ideal for live-cell and immune-competent 3D assays, ensuring that experimental readouts reflect true antigen–antibody interactions.

- **Scalable supply with consistent performance**

Large-format packaging and reliable availability support large scale screening as well as downstream validation studies, while maintaining lot-to-lot consistency.

- **Versatility across experimental systems**

The same antibody can be seamlessly applied across *in vivo* animal studies, *in vitro* 3D models, and downstream analytic assays—reducing variability introduced by reagent switching.

- **Enabling human-relevant research**

Beyond murine targets, Bio X Cell offers a broad portfolio of functional antibodies

against human antigens, including *InVivo*SIM™ products as RUO biosimilars, supporting the transition toward humanized models.

- **Proven and trusted by the scientific community**

Bio X Cell antibodies have been cited in nearly 30,000 scientific publications. The widely used anti-mouse PD-1 (#BE0146) alone appears in ~1,200 studies, providing researchers with a deep and reliable body of published evidence.

From scaled *in vitro* screening to in-depth *in vivo* investigation, Bio X Cell's functional antibodies deliver stable, reliable, end-to-end support for preclinical research. As NAMs reshapes drug discovery, choosing reagents with a proven track record helps ensure confidence in every experiment—and accelerates the development of the next generation of anticancer therapeutics.

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