Journal for ImmunoTherapy of Cancer

Tumor organoids in immunotherapy: from disease modeling to translational research

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To cite: Si Q, Tao S, Wu J, *et al*. Tumor organoids in immunotherapy: from disease modeling to translational research. *Journal for ImmunoTherapy of Cancer* 2025;**13**:e011733. doi:10.1136/jitc-2025-011733

Accepted 01 July 2025

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ABSTRACT

Tumor organoids have emerged as transformative tools in cancer research, enabling the study of tumor biology and immunology in a physiologically relevant, three-dimensional in vitro environment. Derived from patient tumor samples, these self-organizing structures recapitulate the histological and genetic heterogeneity of tumors and their microenvironment, offering significant advantages over traditional two-dimensional cell cultures and animal models. This work provides a comprehensive overview of tumor organoid generation, their characteristics, and their use as models to study tumor-immune interactions. We discuss how tumor organoids faithfully recapitulate tumor heterogeneity, support immune cell infiltration, and simulate immunosuppressive environments, making them ideal platforms for investigating immunotherapy strategies. Emerging technologies, including advanced imaging and single-cell analysis, as well as gene editing tools, further enhance the utility of tumor organoids in dissecting immune-tumor interactions at unprecedented resolution. We also highlight the translational potential of tumor organoids in preclinical immunotherapy research. Organoids offer a promising approach for predicting patient response to immunotherapy and developing personalized treatment strategies. As tumor organoid technology continues to evolve, its application in clinical settings holds great promise for advancing cancer immunotherapy, improving patient outcomes, and overcoming the challenges of drug resistance. Finally, the future direction of tumor organoid development is speculated according to current challenges.

INTRODUCTION

In recent years, immunotherapy has emerged as a promising approach in tumor treatment. This method works by systematically or locally activating the patient's immune system to recognize and eliminate tumor cells, thereby inhibiting tumor progression. Immunotherapy has demonstrated significant efficacy and manageable toxicity in certain cancers, making it a focal point of oncology research.[1] However, tumors are highly heterogeneous, with malignant tumors varying significantly in genotype and phenotype across different patients or even within different regions of the same tumor.[2] This heterogeneity drives divergent patient responses to anticancer regimens, particularly for immunotherapies that depend on intricate crosstalk within the tumor microenvironment (TME).[3] To address these challenges, tumor organoids have emerged as transformative preclinical models.[4] Derived from patient tumor specimens, these self-assembling three-dimensional (3D) structures recapitulate the histopathological and molecular heterogeneity of native tumors and their TME, surpassing the limitations of conventional two-dimensional (2D) cell cultures and animal systems.[5] By emulating the TME's complexity, tumor organoids provide a physiologically relevant platform for dissecting tumor-immune dynamics and evaluating immunotherapeutic efficacy, addressing critical gaps in translational cancer research.

Organoid technology, pioneered in 2009 with the development of intestinal organoids by Hans Clevers and his colleagues, has since evolved to encompass tumor organoids.[6] These patient-derived 3D cultures retain the genetic, transcriptomic, and functional hallmarks of parental tumors, including invasive and metastatic behaviors.[7] Beyond preserving tumor architecture, tumor organoids can recapitulate immune cell infiltration, immunosuppressive signaling, and extracellular matrix remodeling, thereby enabling mechanistic studies of immune evasion and therapeutic resistance.[8 9] Tumor organoids dynamically model TME-specific cell-cell communication, paracrine signaling, and drug responses with high clinical fidelity, establishing them as indispensable tools for drug discovery, biomarker identification, and personalized therapeutic optimization.[10]

This review synthesizes current methodologies for tumor organoid generation, their defining biological and functional attributes,

and their application in elucidating tumor-immune interplay.[8] We evaluate their role in preclinical immunotherapy research, emphasizing their capacity to replicate immune checkpoint activity, cytokine-mediated immunosuppression, and immune cell recruitment. Furthermore, we explore their translational potential in predicting patient-specific immunotherapy responses, guiding combinatorial treatment strategies, and circumventing resistance mechanisms. By integrating multidisciplinary technologies, tumor organoids are advancing mechanistic insights into cancer immunology and accelerating the development of next-generation immunotherapies.[11]

Looking ahead, challenges in standardization, scalability, and immune component integration must be resolved to maximize the clinical utility of tumor organoids.[12] Emerging innovations, such as vascularized co-culture systems,[13] multiomics-driven TME profiling,[14] and bioengineered matrix scaffolds,[15] are anticipated to enhance organoid fidelity and throughput. As these models evolve, their integration into clinical decision-making pipelines may enable precision immunotherapy, tailored to individual TME signatures.[16] Ultimately, tumor organoid technology holds transformative potential for bridging translational gaps, optimizing therapeutic outcomes, and redefining cancer treatment paradigms.

TUMOR ORGANOIDS: FORMATION AND CHARACTERISTICS

Tumor organoids are generated using various methods (figure 1A), with the simplest being the matrix gel immersion technique. Tumor organoids are typically created by dissociating tumor tissue samples into small fragments via mechanical or enzymatic digestion. Mechanical isolation proves more effective than enzymatic methods, yielding higher organoid numbers by preserving cellular connections.[17] The isolated cells are cultured in hydrogels like Matrigel, supplemented with serum-free media containing growth factors (eg, epidermal growth factor EGF, basic fibroblast growth factor FGF) to support organoid formation and stem cell maintenance.[18] Medium composition can be adjusted to influence organoid structure; for example, removing EGF in breast organoid cultures results in more mature luminal cells, while removing heregulin-β1 and p38 mitogen-activated protein kinase inhibitors reduces mature luminal cells.[19] Although Matrigel maintains key tumor characteristics, it primarily supports epithelial cell growth and may not fully replicate the TME, including angiogenesis and immune interactions.[20]

Researchers are advancing organoid models by modifying Matrigel, integrating alternative hydrogels, or designing novel hydrogel systems.[21 22] Recent studies highlight the potential of synthetic hydrogels to enhance organoid growth and differentiation. For example, Eiken *et al* developed a two-step culture method where cells were preaggregated in micropores before embedding in a synthetic hyaluronic acid (HA) hydrogel, achieving high viability and maintained identity in alveolar organoids.[23] Similarly, Carpentier *et al* created gelatin-based hybrid hydrogels that outperformed Matrigel in promoting liver organoid differentiation into hepatocyte-like cells, offering a promising alternative matrix.[24] Lingard *et al* also demonstrated the efficacy of self-assembling peptide hydrogels as a Matrigel substitute for 3D mammary epithelial cell culture, enabling better modeling of mammary gland microenvironments.[25] Additionally, decellularized matrices have been explored to provide physiologically relevant scaffolds for organoid cultures.[26] Together, these efforts underscore the increasing focus on developing hydrogel alternatives that more accurately replicate the TME and support robust organoid growth and differentiation.

Microfluidic technology offers a refined approach for organoid cultivation.[27] Tumor cells are seeded in high density on microfluidic chips with predesigned micropores, using a flowing medium to simulate blood flow, provide nutrients, and remove waste. This method allows the incorporation of diverse cellular elements, such as immune and vascular endothelial cells, making it ideal for vascularized organoids.[28] Microfluidic systems provide a stable growth environment, improving organoid uniformity and controllability, and are advantageous for simulating in vivo conditions and high-throughput drug screening.[29] Brooke *et al* developed an automated microfluidic platform for high-throughput organoid culture and drug sensitivity testing, enabling parallel control of multiple organoids and reducing manual errors.[27] This platform demonstrates significant potential for immunotherapeutic drug development and personalized therapy.

The air-liquid interface (ALI) method, introduced by Calvin J Kuo's group, involves cultivating tissue fragments combined with Matrigel in an upper chamber exposed to air, while a serum-containing medium in the lower chamber provides nutrients.[3] This method simulates epithelial structures and is suitable for tissues requiring gas exchange, such as lung or epidermal cultures.[30] ALI-cultured organoids retain complex tissue structures and cell compositions, including immune and stromal cells, making them valuable for TME research and immunotherapy strategies.

3D bioprinting is another advanced method, using bioink to create organoids with precise tissue structures. Bioink contains cells, biodegradable biopolymers (eg, gelatin, alginate), and growth factors (eg, FGF, vascular EGF (VEGF)).[31] Scaffolding materials like alginate provide structural support, while cells are deposited layer by layer to form organoids. Postprinting, organoids grow in nutrient-rich media, often supported by microfluidic systems or bioreactors for continuous nutrient supply.[32] 3D bioprinting allows precise control over cell spatial layout and supports diverse biological matrices, creating organoids that closely mimic in vivo conditions.

Imaging and single-cell analysis are pivotal methods in tumor organoid research (figure 1B). Imaging includes live and multispectral techniques. Live imaging, such as high-content imaging, allows real-time observation

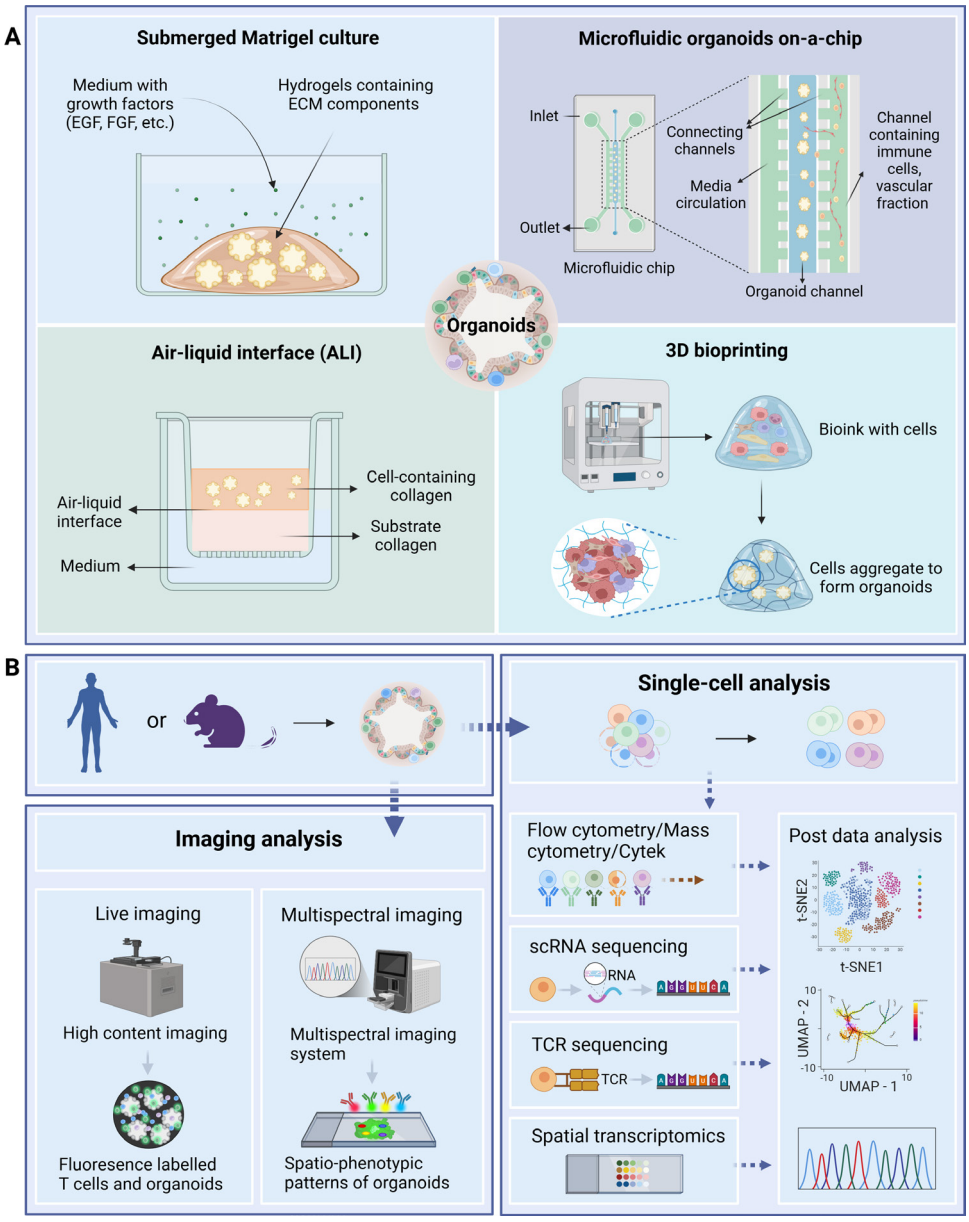

Figure 1 (A) Culture methods of tumor organoids, including submerged Matrigel culture method, microfluid organoids-on-a-chip, air-liquid interface method and three-dimensional bioprinting technique. (B) Imaging analysis and single-cell analysis on tumor organoids. Imaging analysis includes live imaging and multispectral imaging. Single cell analysis techniques including flow cytometry, mass cytometry, Cytek device, scRNA sequencing, TCR sequencing and spatial transcriptomics technology can be used to help understand the biology of tumor organoids. 3D, three-dimensional; ECM, extracellular matrix; EGF, epidermal growth factor; FGF, fibroblast growth factor; scRNA, single-cell RNA; TCR, T cell receptor; t-SNE, t-distribution stochastic neighbor embedding; UMAP, Uniform Manifold Approximation and Projection.

of dynamic processes, like T cell infiltration into tumor organoids, using fluorescent markers.[33] The growth and development of organoids can also be observed through high-content imaging to determine their biological quality.[34] Multispectral imaging has been employed to analyze spatially resolved phenotypic patterns of labeled cells, identifying distinct cell subsets within organoids, providing intuitive biological insights crucial for organoid studies.[35] Single-cell analysis is equally vital. Flow cytometry enables high-dimensional single-cell analysis with unparalleled precision. Spectral flow cytometry (Cytek) represents a major advancement, capturing the full emission spectrum of each fluorophore to dramatically enhance sensitivity and resolution, particularly for dim markers, while overcoming the spectral overlap limitations of conventional flow cytometry.[36] Mass cytometry, such as cytometry by time-of-flight (CyTOF) offers complementary advantages, using metal-tagged antibodies and time-of-flight mass spectrometry to eliminate optical constraints entirely, enabling >40-parameter panels for ultra-deep immune phenotyping.[37] Cytek and CyTOF support massively multiplexed antibody panels, allowing simultaneous detection of dozens of markers with minimal signal interference. This unprecedented analytical power

provides comprehensive, high-dimensional immune profiling in organoid models. These advanced technologies empower researchers to unravel the intricate composition and functional states of immune cells in organoids with exceptional depth, driving transformative insights into tumor immunology and accelerating breakthroughs in immune profiling of tumor organoids.[38] Single-cell RNA sequencing reveals gene expression patterns and tumor heterogeneity.[39] TCR sequencing further explores T cell receptor diversity, enhancing understanding of immune cell specificity.[40] Spatial transcriptomics maps gene expression within tissues, elucidating cell interactions in the tumor TME.[41] Data analysis tools like t-distribution stochastic neighbor embedding and Uniform Manifold Approximation and Projection simplify complex single-cell data into 2D/3D visualizations, highlighting cell population relationships.[42] Together, these techniques provide a high-resolution, comprehensive view of tumor organoid biology and immunotherapy mechanisms.

TUMOR ORGANOIDS AS MODELS FOR STUDYING TUMOR-IMMUNE INTERACTIONS

Recapitulation of tumor heterogeneity and tumor microenvironment in organoids

In tumor research, replicating the complex TME is crucial for developing new therapies. The TME, which includes cancer cells, stromal cells, growth factors like transforming growth factor-beta 1 (TGF-β1), VEGF, and matrix metalloproteinases, dynamically influences tumor growth, metastasis, and prognosis.[43] Tumor heterogeneity, driven by intratumor and intertumor variations in gene mutations and cell composition, complicates treatment responses.[44] Tumor organoid technology offers a promising approach to mimic the TME and advance immunotherapy studies (figure 2A). Stromal cells, particularly cancer-associated fibroblasts (CAFs), play a key role in tumor progression and drug resistance.[45] Co-culture models of CAFs and tumor organoids have been developed to study these interactions. For instance, Luo *et al* used an HA-gelatin hydrogel to co-culture colorectal cancer organoids with CAFs, showing enhanced organoid growth and replication of patient tumor pathways.[46] Similarly, Schuth *et al* demonstrated that co-culturing pancreatic ductal adenocarcinoma (PDAC) organoids with CAFs increased proliferation, reduced cell death, and improved drug resistance, highlighting its potential for personalized medicine.[47] These models improve TME simulation and offer insights into tumor-stromal interactions and drug resistance mechanisms.

A major limitation of tumor organoids is the lack of vasculature, leading to central necrosis due to insufficient oxygen and nutrients.[48] Researchers are developing vascularized organoids to study tumor angiogenesis and VEGFR-targeted drugs.[49] Methods include transplanting organoids into vascular-rich animal tissues, though this is costly and low-throughput.[50] Alternatively, co-culturing endothelial and vascular smooth muscle cells with organoids enhances angiogenesis. Philipp *et al* created vascularized organoids using mesodermal progenitor cells, demonstrating responsiveness to antiangiogenic drugs and hypoxia.[51] Microfluidic technology also enables vascularization by co-culturing organoids with human umbilical vein endothelial cells and fibroblasts, achieving rapid angiogenesis through continuous perfusion.[13] These advancements improve organoid functionality and drug screening potential.

The anticancer effect of immune cells in tumor organoids

Organoid models can be employed to elucidate the interaction between tumor cells and immune cells within the TME. This enables the investigation of immune cell activation, cytotoxicity, recruitment, and proliferation in response to tumor activity (figure 2B). By understanding the anticancer interactions between immune cells and tumor organoids, it will be possible to study in detail the immune cell functions and dysfunctions that determine tumor progression and resistance to therapy.

The activation of the immune system represents a pivotal stage in the development of antitumor immunity. For example, organoids co-cultured with CD8+T cells have been used to study T cell activation via major histocompatibility complex (MHC)-peptide interactions.[52] Dijkstra *et al* demonstrated patient-specific T cell activation by co-culturing tumor organoids with peripheral blood lymphocytes from patients with mismatch repair-deficiency colorectal and non-small cell lung cancer (NSCLC). Similarly, natural killer (NK) cell activation has been modeled using stress ligands on tumor organoids, shedding light on innate immune pathways.[53]

Organoids also facilitate the study of immune cell cytotoxicity. Chakrabarti *et al* co-cultured mouse gastric cancer organoids with dendritic and cytotoxic T cells, creating immune-tumor organoids that mimic in vivo immunocytotoxic effects and predict immune checkpoint inhibitor (ICI) efficacy.[54] This model offers a personalized platform to explore T cell-tumor interactions and immunotherapy mechanisms, including adoptive T cell therapies.[52]

Tumor organoids can be genetically modified to secrete chemokines and cytokines that mediate immune cell recruitment. For example, genetically modified organoids secreting chemokines like CXC motif chemokine ligand (CXCL)9 and CXCL10 have been used to study T cell recruitment.[55] Zhou *et al* optimized co-culture conditions, finding that 10% basement membrane extract maintains organoid morphology while allowing peripheral blood mononuclear cells (PBMCs) to interact with tumor cells.[56] These advancements enhance the study of immune cell infiltration and TME communication, supporting novel immunotherapy development.

Immune cell proliferation is critical for antitumor responses, and some organoids enable the retention and expansion of endogenous immune cells. Ou *et al* used melanoma patient-derived organoids (PDOs) to evaluate tumor-infiltrating lymphocytes (TILs) immunotherapy,

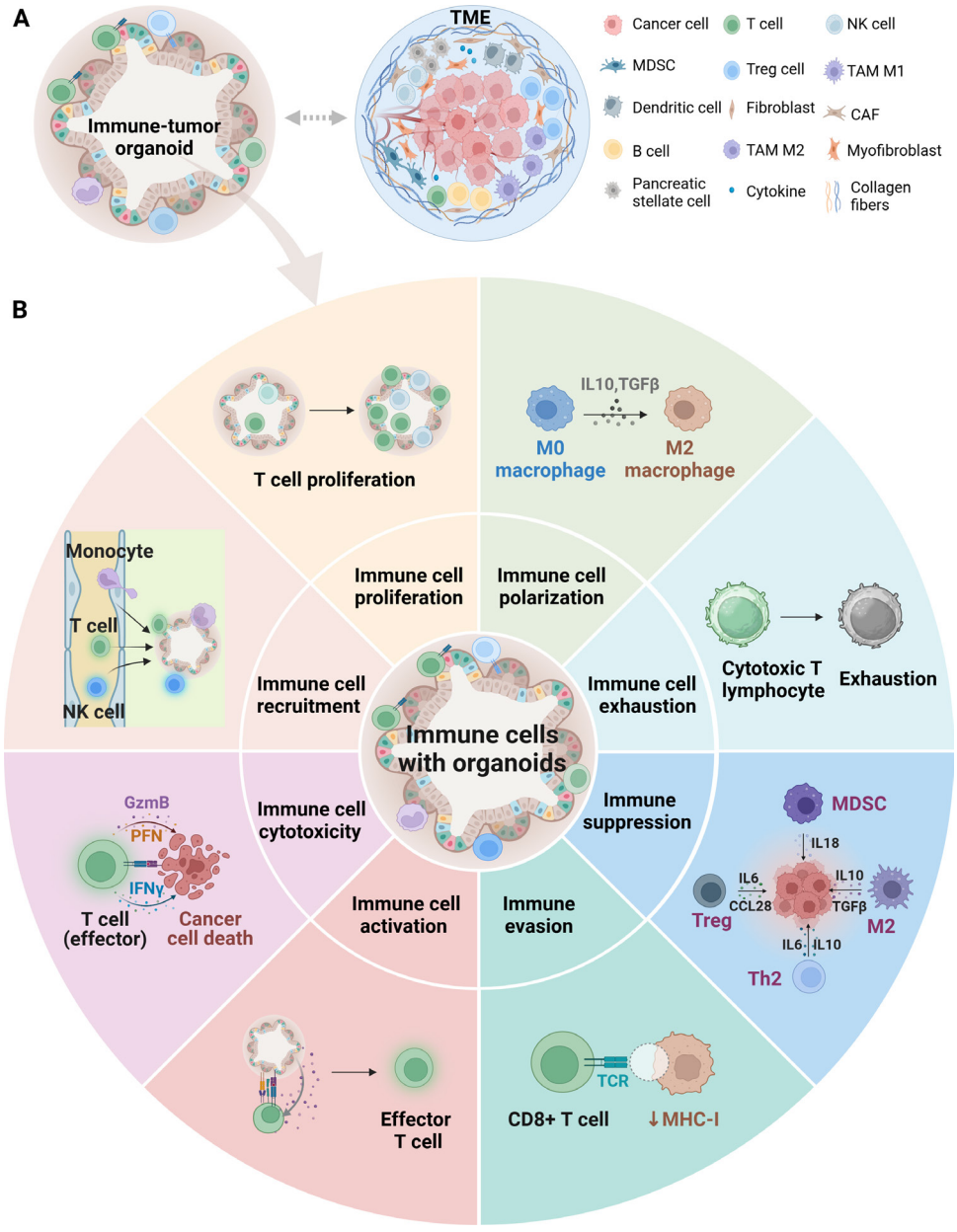

Figure 2 (A) Tumor organoids mimic the tumor microenvironment. (B) Tumor organoids as models for studying tumor-immune interactions. Tumor organoids serve as valuable models for investigating tumor-immune interactions, offering insights into various aspects of immune responses within the tumor microenvironment. Immune cell-tumor organoid models allow the study of immune cell activation, cytotoxicity, recruitment, proliferation, polarization, exhaustion, suppression, and immune evasion. CAF, cancer-associated fibroblast; IFNγ, interferon-gamma; IL-10, interleukin-10; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; NK, natural killer; TAM, tumor-associated macrophage; TCR, T cell receptor; TGFβ, transforming growth factor-beta; Th2, T helper cell 2; TME, tumor microenvironment; Treg, regulatory T cell.

showing that interleukin-2 (IL-2) and anti-programmed cell death protein 1 (αPD-1) stimulation maximized CD8+ T cell expansion.[57] They tested TIL migration in collagen-embedded PDOs and cytotoxicity in Matrigel-cultured PDOs, validating these conditions for TIL therapy. Similarly, Zumwalde *et al* enriched T lymphocytes in breast organoids, observing cytotoxic responses to tumor cells.[58] However, challenges remain, such as maintaining immune cell functionality during extraction and addressing tumor heterogeneity, which affects reproducibility. Future efforts should focus on optimizing culture conditions and establishing standardized protocols for long-term immune component maintenance in organoids.

The immune-suppressive microenvironment in tumor organoids

Immuno-tumor organoids recapitulate key features of the immune TME, enabling in-depth investigation of the dynamic crosstalk between tumor cells and immune components. These models offer a robust platform to dissect mechanisms underlying immune-suppressive

niche formation, including tumor immune evasion, immune cell exhaustion, and phenotypic polarization. However, limitations persist, as they may not fully incorporate all critical immune and stromal elements present in native tumors. Common immune evasion mechanisms in tumors mainly include the expression of immune checkpoint molecules, insufficient antigen presentation, and immune cell depletion.[59] MHC-deficient tumor organoids have been co-cultured with T cells to examine the impact of MHC loss on immune recognition.[52] By inhibiting certain signaling pathways, such as the prostaglandin E2 pathway, or affecting the expression of certain key molecules, such as inhibiting ubiquitin protein ligase E3 component n-recognin 5, and exploring their effects on organoid growth, researchers can reveal the potential role of study objects in tumor development and regulation of tumor immune escape,[60] which helps to discover new therapeutic targets and optimize immunotherapy strategies. Chakrabarti *et al* used a PDO model of gastric cancer to explore the mechanism by which the human epidermal growth factor receptor-2 (HER2) and programmed death-ligand 1 (PD-L1) axis promotes immune escape of tumor cells. The study found that HER2 overexpression is associated with upregulation of PD-L1, which can cause tumors to evade immune surveillance. By knocking down HER2 in organoid models, the expression of PD-L1 can be reduced by disrupting AKT/mTOR signaling, thus reducing the escape of tumor cells from the immune system, creating an environment conducive to immunotherapy, rendering organoids sensitive to PD-1/PD-L1 checkpoint inhibitors, and improving the therapeutic effect of gastric cancer.[61] Frey *et al* combined Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology with organoids to systematically resolve the immune escape mechanism of PDAC and identified two key factors, Vps4b and RING finger protein 31 (Rnf31), that play an important role in evading CD8+ T cell killing. After performing in vivo experiments in mice to verify the role of Rnf31 knockout in human pancreatic cancer, they used engineered Rnf31KO/Rnf31WT human pancreatic PDOs to finally confirm that Rnf31 also protects tumor cells from caspase-8-mediated apoptosis in human pancreatic cancer. With the involvement of organoids, the study found that targeting Vps4b and Rnf31 may be a strategy to make PDAC more vulnerable to immune system attack.[62]

Immune cell suppression in tumor organoids is primarily driven by the presence of immunosuppressive cells and the release of immunosuppressive cytokines. Tumor-derived TGF-β and IL-10 promote immune suppression, suggesting that cytokines play a significant role in shaping the immune landscape within tumor organoids.[63] Furthermore, organoid models have been employed to investigate the role of myeloid-derived suppressor cells (MDSCs) in the suppression of T cell functions. For instance, tumor organoids derived from head and neck squamous cell carcinoma were observed to secrete granulocyte-macrophage colony-stimulating factor, which was shown to induce the expansion and suppressive activity of MDSCs.[64] However, modeling immune suppression in a physiologically relevant context is limited by the absence of stromal and vascular components.

Immune exhaustion is a state of dysfunction characterized by a reduction in effector function and the sustained expression of inhibitory receptors, including PD-1 and TIM-3. For example, organoids derived from PD-L1-high tumors were observed to induce T cell exhaustion, which provides insight into checkpoint inhibitor resistance.[65] Exhaustion studies are limited by a lack of long-term culture systems that mimic antigen exposure. In 3D culture, the removal of nicotinamide can lead to T cell exhaustion, while the addition of 10% human serum supports the growth of PBMCs without adversely affecting the growth and vitality of organoids.[66] Nevertheless, several challenges must be addressed to combat immune cell exhaustion, including lengthy culture times, reliance on a single immune cell type, and the inability to replicate the true TME due to the use of non-tumor cells.

The role of immune polarization, particularly in macrophages and T cells, is increasingly recognized as a pivotal element in shaping the TME. Tumor organoid–macrophage co-cultures have been employed to investigate the polarization of macrophages into either protumor M2 or antitumor M1 phenotypes. Tumor-derived factors such as TGF-β and IL-10 have been identified as promoters of M2 polarization in organoid co-cultures.[63] Similarly, tumor organoids can induce T helper cell (Th)2 or Th17 polarization based on their cytokine profile, aiding the tumor to evade immune attack.[67]

Overall, tumor organoids, as highly simulated 3D cell culture models, have been widely used to study the interaction between tumors and the immune system. Such models can better reproduce the heterogeneity and complex microenvironment of tumor tissue, including stromal cells, vascular components, and immune cell infiltration, allowing researchers to more realistically simulate the immune environment of tumors in vitro. By applying tumor organoids, researchers can explore in depth how tumors fight the host immune system through immune escape mechanisms and how immunotherapy strategies can be optimized to enhance treatment efficacy. Although tumor organoid research faces certain technical challenges, it has great potential to elucidate tumor biology and promote the development of immunotherapy, thus laying a solid foundation for precision medicine and personalized therapy.

TUMOR ORGANOIDS IN PRECLINICAL AND TRANSLATIONAL CANCER IMMUNOTHERAPY STUDIES

Immunotherapy methods in tumor organoids

Tumor organoids can mimic the TME, especially those with added immune components can better simulate

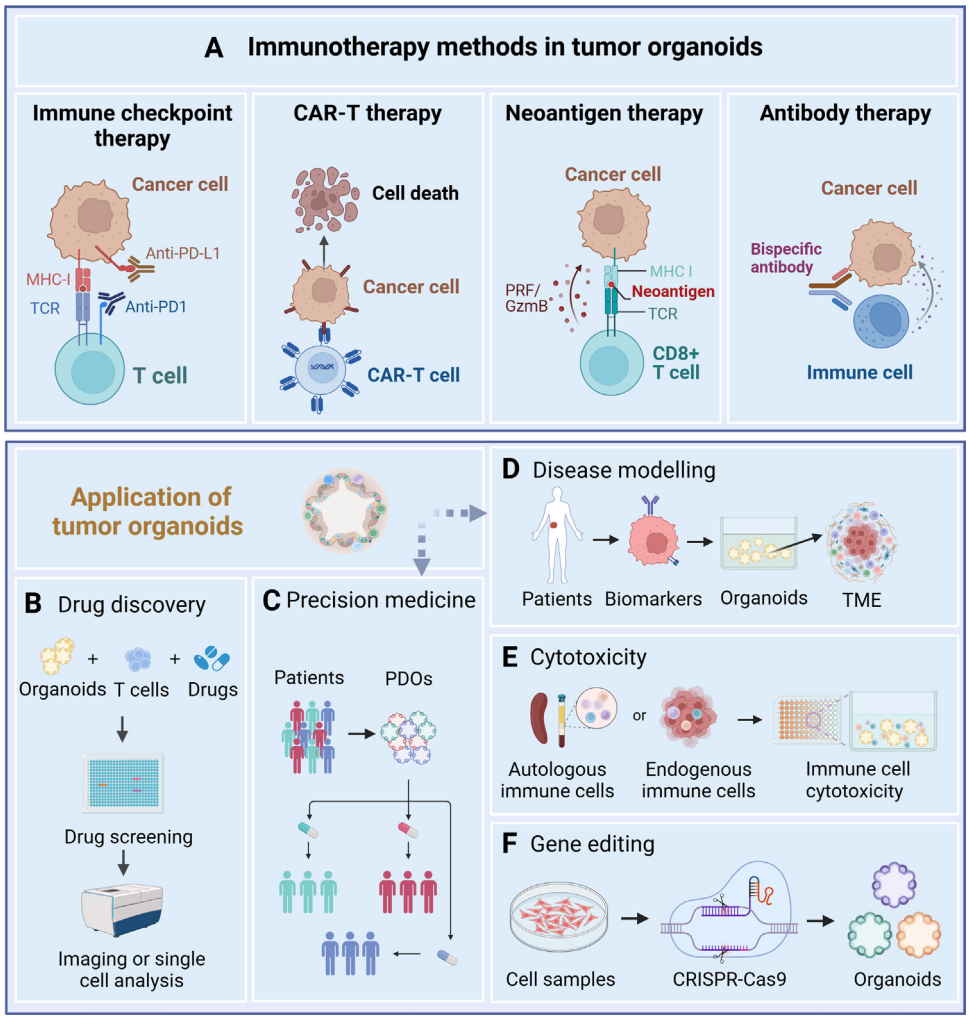

Figure 3 (A) Immunotherapy techniques such as immune checkpoint therapy, CAR-T therapy, neoantigen therapy and antibody therapy are employed in tumor organoids. Application of tumor organoids in preclinical and translational cancer immunotherapy research. (B) Use of tumor organoids for drug discovery. Organoids and immune cells should be co-cultured and treated with different drugs, and the effects of different drugs will be analyzed with high throughput using high-content imaging and other technologies to screen out effective immunotherapy drugs. (C) Tumor organoids can promote the development of personalized medicine. Multiple organoids can be generated from the tumor of a patient, treated with different drugs, predict the therapeutic effect of different treatment schemes in the patient, select the most effective treatment scheme to treat the patient, and guide the selection of clinical treatment strategies. (D) Tumor disease modeling using organoid technology. Patient tumor samples can be obtained by biopsy, such as puncture, and the tumor organoid model is constructed in vitro. (E) Cytotoxicity studies using tumor organoids. Using autologous immune cells extracted from mouse spleen or peripheral blood, or endogenous tumor-infiltrating T cells extracted from tumor tissues to co-culture with tumor organoids to construct immune-enhancing tumor organoids. (F) Combined application of gene editing technology and tumor organoids. Gene editing technology represented by CRISPR-Cas9 can be used to precisely introduce or knock out specific genes into organoids to study the driving effect of the target genes on tumor initiation, progression, and drug resistance. CAR-T, chimeric antigen receptor T; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; MHC, major histocompatibility complex; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PDOs, patient-derived organoids; TCR, T cell receptor; TME, tumor microenvironment.

the interaction between tumor cells and immune cells in tumor tissue and can therefore be used as a reliable model to predict the efficacy of immunotherapy (figure 3A). Tumor organoids have been demonstrated in several studies to predict patient response more accurately to various immunotherapies.

ICIs, such as those targeting PD-1/PD-L1 and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), are a cornerstone of cancer immunotherapy. Neal *et al* used the ALI method to culture PDOs with native immune cells, demonstrating that tumor-specific TILs could induce tumor cell killing when activated with anti-PD-1/PD-L1 antibodies. This validated PDOs as tools for studying ICI efficacy and predicting individual immunotherapy responses.[3] Similarly, Wan *et al* treated high-grade serous ovarian cancer organoids with PD-1/PD-L1 bispecific and monospecific antibodies, identifying NK cells as key players in ICI therapy and

pinpointing BRD1 as a potential therapeutic target.[68] These studies highlight the potential of immune-tumor organoids in exploring immunotherapy mechanisms and efficacy.

Tumor organoids also serve as platforms for evaluating chimeric antigen receptor T (CAR-T) cell therapies, including their specificity, efficacy, and off-target effects. By expressing CAR-T targets like HER2, EGFR, or CD19, organoids enable researchers to assess CAR-T cell cytotoxicity.[69 70] Wehrli *et al* developed MesoFAP CAR-TEAM cells, which target CAFs and activate T cells via TEAM secretion. Using PDAC organoids and patient-derived tumor spheroids, they demonstrated the superior tumor-killing capacity of these cells, underscoring the utility of organoids in analyzing CAR-T therapies in complex TMEs.[71]

Neoantigen-targeted immunotherapy development also benefits from tumor organoid models. Wang *et al* used hepatobiliary tumor organoids to screen immunogenic neoantigen peptides, finding that neoantigen-stimulated CD8+ T cells exhibited enhanced tumor-killing effects, particularly when combined with ICIs.[72] Westcott *et al* developed colorectal cancer organoids with adjustable neoantigen expression, revealing that low neoantigen levels led to T cell dysfunction and immune escape, suggesting that neoantigen vaccination could enhance immunotherapy efficacy.[73] This emphasizes that the expression levels of neoantigens play a key role in immune escape, and that stimulating the initiation of T cells through neoantigen vaccination and other methods can rescue the inhibitory effect on tumor growth. Tumors with a low mutational burden generally produce fewer neoantigens, making them less responsive to immunotherapy. While directly boosting neoantigen expression in tumors remains challenging, recent studies suggest promising indirect strategies. These include using nano-regulators to induce DNA damage[74] or employing epigenetic therapies to reactivate transposable elements.[75] Such methods could enhance neoantigen production and potentially improve immunotherapy outcomes. However, further research is necessary to assess their clinical applicability.

Beyond evaluating immunotherapy responses, immune-tumor organoids help uncover resistance mechanisms. Bispecific antibodies (bsAbs), which target tumor antigens and immune cell receptors like CD3, can be tested in organoid models to assess T cell recruitment and tumor cell killing. For example, HER2-targeting bsAbs have been evaluated in HER2-positive tumor organoids.[76] Chen *et al* found that colorectal cancer organoids responsive to anti-PD-1 therapy had reduced MDSC infiltration, with activated T cells promoting MDSC apoptosis and inhibiting their function via IFN-α/β and TNF-α secretion.[77] These insights suggest that targeting immunosuppressive factors could improve ICI efficacy.[78] Therefore, immune-tumor organoids not only predict immunotherapy responses and aid drug development but also reveal resistance mechanisms, guiding the design of more effective therapeutic strategies.

Use of tumor organoids for drug discovery

Tumor organoids, with their 3D structure, closely mimic the TME, preserving genetic diversity and cellular heterogeneity. This makes them highly reliable for in vitro drug testing, especially in drug screening. The 'immune-tumor organoid' model, created by co-culturing with immune cells, simulates tumor-immune interactions, making it ideal for studying immunotherapies, such as immune checkpoint blockade therapy,[79] CAR-T cell therapy,[80] neoantigen therapy,[73] and bispecific antibody therapy.[81]

While 2D cultures are established tools, organoids uniquely recapitulate the 3D architecture and multicellular interactions of native TME.[82] This spatial and functional complexity enables more physiologically relevant modeling of tumor-immune crosstalk, including immune cell recruitment, infiltration dynamics, and activation states, which cannot be adequately captured in monolayer systems. Equally important, PDOs better preserve the genetic and phenotypic heterogeneity of original tumors.[83] This fidelity is critical for immunotherapy research, as it allows the study of patient-specific variations in response to immunotherapies within a mimicked 3D microenvironment. Such heterogeneity-driven insights into resistance mechanisms or sensitivity biomarkers are often lost in simplified 2D cultures, which lack the native cellular diversity and signaling networks of primary tissue. Finally, organoids provide a distinct advantage by integrating stromal and immune components within their structure.[84] This enables direct investigation of how endogenous stromal cells and immune populations influence tumor behavior and immunotherapy efficacy. For instance, studying immune cell exhaustion or immunosuppressive niche formation in organoids offers clinically actionable insights, particularly for preclinical testing of combination immunotherapies. These insights cannot be replicated in reductionist 2D co-culture models.

Advances now enable high-throughput organoid production, streamlining drug screening. Hans Clevers' group developed a BEHAV3D system to track T cells in PDOs, facilitating investigation of the interaction between T cells and tumor organoids. This system offers a promising tool for characterizing the behavioral-phenotypic heterogeneity of cellular immunotherapies, which can be used for drug screening in terms of T cell-tumor organoid interaction.[33] Zhou *et al* optimized 3D co-culture models using ovalbumin (OVA) positive E0771 organoids and OT-I CD8+ T cells, showing drugs worked in T cell-containing organoids by enhancing antigen presentation.[85] They also created a pancreatic organoid model with T cells, identifying compounds that improve immune responses.[86]

Organoids enhance the efficiency of drug screening and streamline the drug development pipeline. By more faithfully recapitulating the in vivo TME and immune cell interactions, they outperform conventional models in high-throughput drug screening applications (figure 3B). Consequently, tumor organoids serve as a superior platform for evaluating immunotherapy cytotoxicity and

elucidating underlying mechanisms, yielding data that often surpass the limitations of traditional 2D co-culture systems.

Use of tumor organoids to predict responses to immunotherapy and personalized treatment selection

In tumor immunotherapy, the cell interactions and heterogeneity in the TME significantly influence therapeutic outcomes, resulting in variability among individuals.[87] PDOs serve as valuable tools for investigating tumor pathobiology and therapy responses. Although they lack systemic immunity, PDOs enable precise assessment of tumor-specific immunotherapy efficacy. By incorporating immune components, such as autologous T cells or PBMCs, into PDO co-culture systems, researchers can model tumor-immune interactions and stratify patient-specific treatment responses. This approach facilitates the development of tailored immunotherapies, advancing precision medicine.[88] For PDOs to effectively guide treatment, they must retain the biological characteristics of the original tumor. Lee *et al* established a bladder cancer PDO biobank, capturing the mutational spectrum and genetic stability of tumors from non-invasive to invasive stages. Drug responses in these organoids mirrored those in xenografts, demonstrating their potential to predict clinical outcomes and inform personalized treatment strategies.[89]

Organoids also provide a reliable platform for discovering new compounds, crucial for personalized therapy. For example, Sui *et al* identified DKK1 as a biomarker in colorectal cancer, where its high expression inhibits CD8+T cell activation and predicts resistance to PD-1 inhibitors. Testing for DKK1 in serum allows clinicians to tailor treatments, such as combining DKK1 blockade with PD-1 inhibitors for improved efficacy.[90] Additionally, inflammation-related biomarkers like NLR were linked to ICI resistance in colorectal cancer. High NLR expression promotes T cell depletion via the CD80/CD86–CTLA4 axis, predicting poor ICI response. By assessing tumor inflammation and NLR levels, clinicians can select optimal therapies for individual patients, enhancing treatment precision.[91] PDOs offer a robust platform for biomarker discovery, treatment evaluation, and personalized immunotherapy, bridging the gap between preclinical research and clinical application (figure 3C).

Disease modeling using organoid technology

Tumor organoids provide a cost-effective, time-efficient, and ethically favorable alternative to animal models for cancer research. By constructing PDOs from biopsy samples, researchers can replicate the TME and study biomarkers, drug responses, and TME interactions, advancing precision medicine (figure 3D). Yang *et al* established a primary liver cancer biobank using 399 tumor organoids from 144 patients, enabling drug sensitivity screening and pharmacogenomic analysis.[92] Tucci *et al* used bladder tumor organoids to identify NUMB as a tumor suppressor, linking its absence to aggressive bladder cancer.[93] Similarly, Kastenschmidt *et al* developed follicular lymphoma organoids for TME analysis and high-throughput drug screening, highlighting their potential in precision medicine.[94] Organoids retain genomic and transcriptomic features, allowing studies on tumor heterogeneity and drug sensitivity. Li *et al* demonstrated this by creating a biobank from multipoint samples of patients with hepatocellular and cholangiocarcinoma, revealing drug-sensitive heterogeneity across organoids.[95] This approach provides critical insights for drug screening and personalized treatment strategies.

Cytotoxicity studies using tumor organoids

Tumor organoids, particularly those with immune components, effectively replicate tumor biology, cell interactions, and TME complexity, making them ideal for studying immunotherapy cytotoxicity. Co-culturing PDOs with autologous immune cells, such as PBMCs, can induce tumor-reactive cytotoxic T cells, triggering antitumor immune responses. This approach has been applied to colorectal cancer,[88] NSCLC,[52] cholangiocarcinoma,[66] pancreatic cancer[86] and other cancer research.[85] Mouse-derived organoids can also be co-cultured with spleen-derived CD8+ T cells or endogenous immune cells for similar studies (figure 3E).

Immune-tumor organoids enable the evaluation of cytotoxic T cell functionality in immunotherapy. Chalabi *et al* used colorectal cancer PDOs to compare T cell responses in immunotherapy-responsive and non-responsive patients, revealing variability in killing efficacy and highlighting the need for model optimization. Votanopoulos *et al* developed immune-enhanced PDOs (iPTOs) from patients with melanoma, demonstrating their utility in predicting responses to PD-1 inhibitors like nivolumab and pembrolizumab. These studies underscore the potential of organoids in improving immunotherapy evaluation and personalized treatment strategies.[96]

Combined application of gene editing technology and tumor organoids

Gene editing tools like CRISPR-Cas9 enable precise gene knockout, knock-in, or repair, facilitating studies on tumorigenesis, drug resistance, and therapeutic strategies. By editing driver mutations in organoids and selecting based on microenvironmental factors, researchers can create genetically modified organoids that replicate human tumor events when xenografted.[97] In normal tissues, CRISPR-Cas9 helps identify tumor initiation, progression, and drug resistance mechanisms, uncovering new therapeutic targets for personalized therapy (figure 3F).

The combination of gene editing and organoid technology has advanced cancer research significantly. Huang *et al* used CRISPR to create pancreatic cancer organoids with KRAS and TP53 mutations, enabling studies on tumor development and drug screening.[98] Seino *et al* replicated pancreatic carcinogenesis by sequentially editing oncogenes and tumor suppressors in human pancreatic organoids.[99] Duarte *et al* employed CRISPR to construct

BRCA1-deficient breast tumor organoids, exploring Poly(ADP-ribose) polymerase resistance mechanisms to olaparib treatment.[100] Sun *et al* introduced oncogenes like c-MYC and RAS into liver organoids, revealing their roles in hepatocellular carcinoma and cholangiocarcinoma development.[101] In conclusion, the combination of gene editing and organoid technology has brought revolutionary progress in basic tumor research and laid a solid foundation for the clinical translation of tumor therapeutics.

Tumor organoids as a platform for developing combination therapies and overcoming drug resistance

Tumor organoids are valuable for evaluating drug combinations and understanding synergistic effects. Westcott *et al* demonstrated that combination therapy with anti-CD40 and ICIs could effectively reverse immune escape in low-neoantigen colorectal cancer. Using an orthotopic transplantation model, they engrafted organoids into syngeneic immunocompetent mice, which revealed that this combinatorial approach nearly prevented metastasis and showed superior efficacy compared with ICI monotherapy. Importantly, these therapeutic effects were mediated through interactions with the host immune system, highlighting a critical advantage of in vivo organoid models in recapitulating such immune-dependent responses.[73] In addition to in vivo organoid models, Zhou *et al* developed an in vitro platform using pancreatic tumor organoids engineered to express OVA antigens, which were co-cultured with OVA-specific OT-I T cells. The study demonstrated that the combined application of histone deacetylase inhibitor ITF2357 and BET bromodomain inhibitor I-BET151 synergized with anti-PD-1 therapy to markedly augment T cell cytotoxicity and remodel the immunosuppressive TME. These effects translated into robust antitumor efficacy in both in vitro and in vivo settings. Together, these findings highlight the utility of organoid models for optimizing combination immunotherapy regimens and identifying novel synergistic drug interactions.[86] Similarly, combining immunotherapy with other therapies, such as ferroptosis induction, MDSC blockade, or low-dose chemotherapy, has shown promise in enhancing treatment efficacy for liver and prostate cancers.[102]

Organoids also serve as platforms to study drug resistance.[103] By creating treatment-sensitive and resistant organoids, researchers can explore resistance mechanisms and test personalized strategies. For example, Mantilla-Rojas *et al* identified IL-10-induced immune evasion in EGFR-independent colorectal cancer, suggesting that combining EGFR inhibitors with anti-IL-10 antibodies could overcome resistance.[104] Sun *et al* found that targeting TANK-binding kinase 1 (TBK1), a gene linked to immune escape, with TBK1 inhibitors and PD-1 blockers effectively overcame ICI resistance in organoids.[105]

These studies highlight the potential of organoids to uncover resistance mechanisms, optimize drug combinations, and improve clinical outcomes. By simulating the TME and patient-specific genotypes, organoids enhance the accuracy of preclinical drug screening, identify effective immunotherapy targets, and reduce the risk of ineffective treatments entering clinical trials. This accelerates the translation of immunotherapy into personalized clinical strategies, saving time and resources while improving patient care.

CLINICAL APPLICATIONS AND PERSPECTIVES

PDOs offer a powerful platform for personalized drug screening, enabling researchers to test immunotherapy and drug combinations in clinical trials (table 1). This approach helps identify optimal treatments for individual patients, improving efficacy while minimizing adverse effects. For example, Chen *et al* established breast cancer PDOs that mirrored the pathological and genetic features of their parental tumors. Drug responses in these PDOs closely matched clinical outcomes, with sensitivity and resistance patterns aligning with patient histories. By screening drug-resistant and metastatic breast cancer PDOs, effective drug candidates were identified, leading to improved therapeutic outcomes in patients. This demonstrates the potential of PDOs to guide personalized treatment decisions.[106] In another case, patients with gastrointestinal stromal tumor benefited from small intestinal organoid-based drug screening. The organoids predicted patient-specific drug responses and toxicity, enabling the selection of a tailored treatment regimen that outperformed standard Food and Drug Administration-approved therapies. These examples highlight the clinical utility of PDOs in advancing personalized medicine and optimizing treatment strategies.[107] Teijeira *et al* employed patient-derived colon cancer organoids exhibiting varying levels of carcinoembryonic antigen (CEA) expression in a co-culture system with T lymphocytes and autologous fibroblasts. By integrating live confocal microscopy, the researchers quantitatively evaluated the efficacy of CEA-CD3 T-cell bispecific engagers in mediating tumor-specific T-cell cytotoxicity. This experimental platform enables real-time visualization of immune-tumor interactions, providing a physiologically relevant model of the tumor immune microenvironment. Furthermore, the system demonstrates potential utility for preclinical assessment of combinatorial immunotherapeutic strategies under clinical investigation.[108] These clinical cases not only validate the practical value of organoid models in predicting treatment response, but also provide valuable data for the optimization of personalized immunotherapy, demonstrating the potential for translation from laboratory studies to clinical applications, and paving the way for future improvements in therapeutic strategy.

Despite their promise, the integration of organoids into immunotherapy clinical trials remains limited.

Table 1 Registered clinical trials of tumor organoid technology in immunotherapy. The data were analyzed using the ClinicalTrials.gov database (https://clinicaltrials.gov/)

Types of tumor organoid	Techniques	Clinical trial number	Condition or diseases	Relevance to immunotherapy	Phase	Publications
Human lung cancer organoid	PDO	NCT03778814	Advanced lung cancer	TCR-T	Phase 1	Nil
		NCT04826913	Non-small cell lung cancer	Lymphocytes from the patient's blood	Observational	Nil
		NCT05332925		Anti PD-1/L1, anti CTLA-4		Nil
		NCT06405230		Dostarlimab and pembrolizumab	Phase 1/2	Nil
Human gastrointestinal tumor organoid	3D Matrigel culture	NCT05401318	Resectable colon and rectal cancer	Chemotherapy in combination with CAR-T cells	Observational	Nil
		NCT04587128	Metastatic colorectal cancer	Panitumumab or cetuximab	Phase 2	[111]
		NCT05725200	Metastatic colorectal cancer	Pembrolizumab	Phase 2	[112]
	PDO	NCT06435689	Colorectal cancer	Autologous T cells co-culture	Observational	Nil
		NCT06196554	Gastric cancer	Organoid T cell co-culture system	Observational	[113]
	hESCs or hiPSCs	NCT05078866	Lynch syndrome and colorectal cancer	Tumor-specific neoantigen vaccine	Phase 1/2	[114]
	3D Geltrex culture	NCT05630794	Familial adenomatous polyposis or multiple polyps	Akt/ERK inhibitor ONC201; NK cell	Phase 1	Nil
Human breast cancer organoid	PDO	NCT05134779	Breast cancer	Cytokines/chemokines, tumor-specific T cells, and innate immune cell	Observational	Nil
		NCT05429684	HER2+ breast cancer	Anti-PD-1 antibody	Phase 3	[115]
Human glioma organoid	PDO	NCT06156150	Glioma	Therapeutic cancer vaccines	Observational	Nil
	iPSCs	NCT06781372	Glioblastoma	CAR-T; anti-PD-1 and anti-CTLA-4; transposable elements	Observational	[116]
Liver cancer	PDO	NCT05913141	Liver cancer	Tumor-infiltrating lymphocytes	Observational	Nil
		NCT06929845	Hepatocellular carcinoma	Host immune cells	Observational	[117]
Urothelial carcinoma organoid	SPHERTEST	NCT06738797	Advanced or metastatic urothelial carcinoma	UC tumor cells and leukocyte mononuclear cells	Observational	Nil

CAR, chimeric antigen receptor; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; 3D, three-dimensional; HER2, human epidermal growth factor receptor-2; hESCs, human embryonic stem cells; hiPSCs, human induced pluripotent stem cells; NK, natural killer; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PDO, patient-derived organoid; SPHERTEST, using heterotypic spheroid models with PBMC from patients with advanced or metastatic urothelial carcinoma to test the pre-treatment effect; TCR, T cell receptor; UC, urothelial carcinoma.

Challenges in recruiting organoids for clinical trials hinder their broader application, potentially delaying drug development. Addressing these barriers is crucial to fully realize the translational potential of organoid technology in personalized immunotherapy.

CHALLENGES AND FUTURE DIRECTIONS

Currently, the development of tumor organoids still faces some challenges. A key limitation is their inability to fully replicate the TME. The absence of vascular structures leads to oxygen and nutrient deficiencies in larger organoids, resulting in high cell mortality.[48] Additionally, incorporating stromal and immune cells remains poorly understood. Functional immune cells in organoids often differ in type and distribution from those in actual tumors, and maintaining their activity long-term is difficult. This hampers the accurate simulation of tumor-immune interactions, affecting drug efficacy predictions.[3] Furthermore, organoids often fail to represent the full heterogeneity of tumors due

to sample size and location limitations, making drug response evaluations less reliable. To overcome these challenges, further optimization of culture methods is needed to create organoid models that better mimic human biology.

Another major hurdle is the lack of standardized culture protocols. There is no consensus on growth factor addition, culture success criteria, or ethical norms, leading to inconsistent practices across laboratories. While some guidelines and quality control standards exist,[109] more work is needed to establish universal protocols. Standardization will improve reproducibility, reliability, and the success rate of organoid cultures, facilitating their broader application and clinical translation.

Ethical considerations are also critical, especially when using human tissues. Researchers must obtain informed consent from donors, protect privacy, and ensure compliance with legal and ethical guidelines. This is particularly important when generating human embryos or stem cells from organoids, as well as when using CRISPR gene-editing or transplantation techniques.[110] In the future, there is a need to strengthen the ethical review process for organoid research, develop clear norms for use and management, and ensure that all research complies with strict ethical standards to avoid improper use of organoids.

High-throughput screening presents additional challenges, including the lack of standardization and automation in large-scale organoid production. Batch variability and inconsistent quality hinder reproducibility. Integrating 3D printing with organoid technology could address these issues by enabling efficient, large-scale production. Future efforts should focus on optimizing culture conditions, enhancing automation, and reducing human error to fully realize the potential of organoids in biomedical research.

CONCLUSIONS

Tumor organoids are a transformative tool in cancer immunotherapy research, offering a dynamic platform to simulate the TME, study immune cell interactions, and predict treatment outcomes. By replicating tumor heterogeneity and drug resistance mechanisms, organoids accelerate drug development and advance personalized medicine. Future advancements will depend on optimizing culture conditions, incorporating additional immune components, and integrating organoid models into clinical trials. With continued innovation, tumor organoids will become indispensable in cancer immunotherapy, providing more effective and tailored treatment solutions for patients.

Acknowledgements The authors would like to express their gratitude to all members of ZZ's laboratory for their invaluable support.

Contributors QS was responsible for the initial drafting of the manuscript. ST and JW provided valuable feedback during the revision process. JM, ZL, XF, JS and TK offered constructive suggestions regarding the project. ZZ conceived the initial idea, partly drafted, and revised the manuscript. ZZ is the guarantor.

Funding The study was supported by the Research Initiative fund to ZZ from the Zhejiang University—University of Edinburgh Institute, and the Joint Fund of Zhejiang Provincial Natural Science Foundation of China (Grant No. LHDMZ25H160001) to ZZ and the Key Program of Basic Research Fund in Shenzhen Natural Science Foundation (JCYJ20241202124303005).

Competing interests No, there are no competing interests.

Patient consent for publication Not applicable.

Ethics approval Not applicable.

Provenance and peer review Commissioned; externally peer reviewed.

Data availability statement All figures have been generated using the BioRender platform. Permission has been granted for publication.

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