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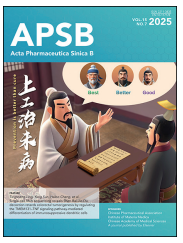

REVIEW

Immune organoid for cancer immunotherapy

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Abstract Cancer immunotherapy, which harnesses the patient's own immune system to target malignant cells, has shown remarkable promise in reducing tumor burden and extending survival. However, the complex tumor microenvironment (TME) limits therapeutic benefits to a subset of patients, making it challenging to develop accurate *in vitro* models for drug response prediction, drug discovery, and personalized medicine. Organoids, three-dimensional (3D) "mini-organs" derived from individual patients that faithfully recapitulate the structural, molecular, and gene expression profiles of primary tumors along with their complex TME *in vitro*, have emerged as powerful tools for patient-specific drug screening and therapeutic strategy development. Their versatility has led to widespread adoption across both clinical and basic cancer research. However, a key limitation of traditional organoid models is their lack of immune system components. Recent years have seen significant efforts to address this challenge through the integration of immune cells with organoids, aiming to create more physiologically relevant models. This review describes 3D culture methods for immunocompetent organoids, explores organoid−immune cell interactions, and discusses their applications in cancer immunotherapy and drug screening, along with recent advances in related clinical studies.

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1. Introduction

Malignant diseases continue to rank among the primary factors contributing to worldwide death, with both incidence and fatality rates on the rise[1]. Immunotherapy has transformed the treatment of cancer within the last ten years[2]. The introduction of checkpoint-targeting immunotherapies has transformed oncology, showing remarkable effectiveness across a wide range of cancers[3]. However, despite the unprecedented success of these therapies, only a small subset of cancer patients has experienced significant benefits[4]. Treatment resistance, both intrinsic and acquired, is often linked to the presence of tumor heterogeneity[5]. The tumor microenvironment (TME) is a complex, evolving system consisting of heterogeneous cancer cells, stromal cells, secreted factors, and extracellular matrix (ECM) components[6]. It reduces the effectiveness of immunotherapy by suppressing immune function and promoting tumor growth through intricate cellular, molecular, and metabolic networks[7]. While conventional cell culture and animal models have been foundational in cancer research, they still have several limitations[8-11]. For instance, traditional two-dimensional (2D) cancer cell lines are difficult to maintain over the long term and fail to fully replicate the interactions between tumor cells[8]. Additionally, they are prone to genetic drift[8]. Animal models face their own set of challenges, including high costs, time requirements, technical difficulties, and issues with immunocompatibility[9-11]. Consequently, there is an urgent need for dynamic, generalized modeling systems that can provide deeper insights into the TME, advancing preclinical research and improving immunotherapy strategies.

Organoids are defined as three-dimensional (3D) *in vitro* cultures formed from stem cells, organ-specific progenitor cells, or tumor tissue-isolated cells through self-organization[11-13]. As "miniature organs in a dish" organoids serve a key function in pharmacological research, especially in areas like novel drug discovery and efficacy prediction[13]. In 2022, the Food and Drug Administration (U.S. FDA) approved a clinical study (NCT04658472) for the first new drug to enter clinical trials based entirely on preclinical data from "organ-on-a-chip" models[14]. Organoids have now been successfully developed for a wide range of human tumor types, including lung[15], breast[16], colon[17], gastric[18], bladder[19], ovarian[20] cancers. Unlike conventional 2D cancer cell cultures and animal models, tumor organoids are quicker to generate, better preserve tumor characteristics, provide broader amplification of the tumor genome, and maintain long-term stability[11,16]. They also facilitate the creation of biobanks and enable high-throughput screening (HTS)[11,16] (Table 1). Tumor organoids are increasingly used as high-throughput platforms to study tumor genomic and phenotypic heterogeneity, as well as the ecological niche of the TME[21]. They are also valuable for testing and developing emerging immunotherapies[22]. Despite these advantages, an important limitation of conventional organoid cultures is the shortage of immune components[23-25]. Recent research has focused on integrating immune cells with organoids, underscoring the necessity for a comprehensive review of the latest advances in this rapidly evolving field[24-26].

Immune organoids are advanced 3D culture systems that integrate immune components, reconstruct tissue architecture, and model immune interactions, serving as a physiologically relevant platform for investigating immune responses and therapeutic interventions[13,26-29]. This review summarizes the research progress of immune organoids, with a focus on their construction and cultivation methods. Recent studies have highlighted the utility of co-culture models to investigate tumor−immune interactions, offering valuable insights into the TME[24-26]. Immune organoids represent a promising preclinical model for cancer immunotherapy, offering capabilities to evaluate immunotherapy potential, support drug screening, identify novel antigenic targets, advance immunotherapeutic strategies, and inform personalized treatment approaches[26]. The review concludes with a detailed analysis of immune organoids evaluated in clinical cancer trials.

2. Construction and cultivation of immune organoids

Organoid technology is advancing from traditional tumor models to more sophisticated immune organoids, aiming to better capture the intricate relationships that exist between tumors and the immune system[26]. Tumor organoids derived from patient tissues or engineered through CRISPR modification form the basis for developing immunocompetent models[30,31]. The incorporation of immune elements into these organoid systems represents a significant advancement in studying tumor immunology and evaluating responses to immunotherapy[13,26].

To better preserve the structural characteristics of tumor tissue *in vitro* and more accurately mimic the *in vivo* TME, various culture systems have been developed, including submerged[32], air-liquid interface (ALI)[33], and 3D microfluidic[34] models. In submerged culture, tumor cells are embedded in a 3D ECM and incubated with customized growth factor supplements[32] (Fig. 1). The ECM can consist of materials such as Matrigel, collagen, hydrogels, or basement membrane extract type 2 (BME2)[32]. ALI

Table 1 Comparison among 2D cell culture, organoid and animal model.

Item	2D cell culture	Organoid	Animal model
Physiology structure	Limited	Similar	Similar
Gene phenotype	Similar, genetic drift occurs in long-term cultures	Similar	Similar
HTS	Practicable	Practicable	Limited
Biobanking	Practicable	Practicable	Practicable, but only at cellular level
Cost	Low	Middle	High
Establishment time	Short	Middle	Long
Treatment responses	Limited	Accurate	Accurate
Modeling human immune system	Limited	Similar	Similar, but merely offers a mouse physiological environment
Modeling cellular communications	Similar	Similar	Limited

HTS, high-throughput screening.

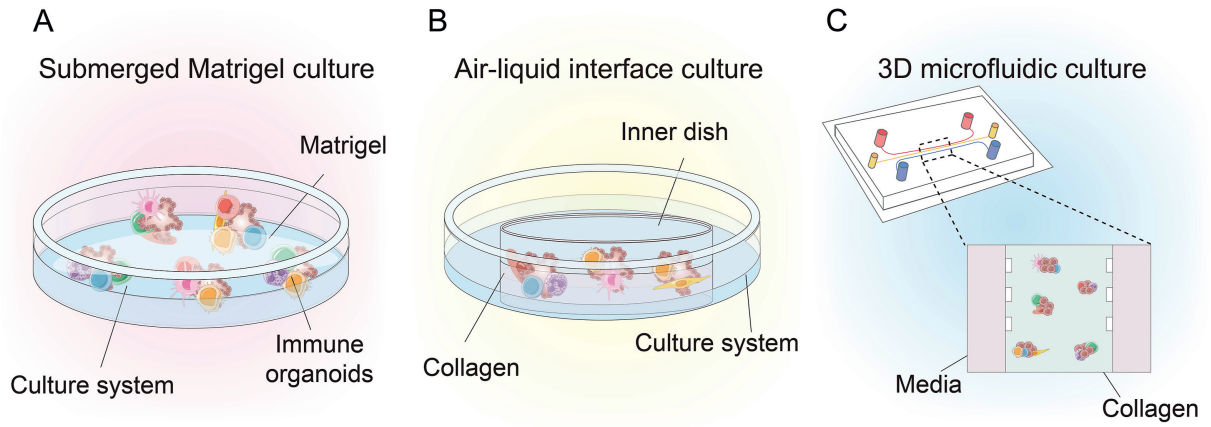

Figure 1 Three methods of culture of immune organoids. (A) The submerged Matrigel method cultures free tumor cells in a 3D matrix, either spherical or flat, in tissue culture medium. (B) The ALI method places tumor tissue in a Transwell, allowing the outer medium to permeate and exposing the inner collagen gel to air for oxygenation. (C) The 3D microfluidic method cultures tumor spheroids in a central 3D gel, flanked by parallel channels for media flow and waste removal.

culture involves embedding the tissue in a collagen matrix, exposing it to air, and placing a medium containing the desired growth factors in an outer dish[33]. The medium then passes through a permeable membrane into a transwell. In 3D microfluidic systems, tissue is grown in a central zone surrounded by parallel channels that transport nutrients and remove metabolites[34]. Additionally, microfluidic-based technology facilitates the construction of micro-organospheres (MOSs), enabling the rapid generation of large numbers of MOSs from minimal tissue input[35].

Conventional submerged Matrigel-based patient-derived organoids (PDOs) often fail to maintain stromal components, focusing primarily on epithelial carcinoma cells[23]. As a result, the co-culture of immune cell types is crucial for accurately recapitulating the TME in these models[23]. In contrast, 3D microfluidic and ALI cultures can maintain both tumor epithelium and native immune components as part of an integrated ecosystem without the need for reconstitution[27,36]. Single-cell RNA sequencing of multiple samples showed that a substantial portion of tumors in ALI organoid cultures remained in their original state, preserving a range of immune elements such as T cells, B cells, NK cells, and macrophages[27]. Immunofluorescence and confocal imaging further demonstrated that the cellular environment in 3D microfluidic cultures closely matched the tumor and stromal cell ratios, as well as the activation status typically observed *in vivo* within the mammary TME[36]. This model successfully incorporated major cellular components of the *in vivo* mammary TME, including macrophages, T cells, fibroblasts, and epithelial cells[36]. At present, two distinct strategies have been developed for modeling the TME in organoids. The reconstituted TME approach (RTa) adds exogenous stromal cells to conventional submerged stromal gels to study specific cell−cell interactions in systemic TME[25]. The native TME approach (NTa) maintains and expands endogenous stromal cells in ALI and 3D microfluidic (including MOSs) media to investigate complex crosstalk between multiple distinct cell populations *in vivo*[25] (Fig. 2).

3. Immune organoids for exploring the TME

Immune cells within tumors include those that mediate adaptive immunity, such as T cells, natural killer (NK) cells, and B cells, as well as effectors of innate immunity, such as neutrophils, macrophages, and dendritic cells (DCs)[37]. These immune cells can influence tumor growth and therapeutic responses by directly interacting with tumor cells or indirectly releasing chemokines and cytokines[37]. Cancer cells, as the central component of TME, not only exhibit significant heterogeneity in their molecular and phenotypic characteristics, but also actively shape the immune landscape through direct cell−cell contacts and secretion of various factors[37,38]. These factors not only convert anti-tumor immune cells into a pro-tumoral phenotype but also recruit immunosuppressive cell populations, which may significantly contribute to resistance to immune checkpoint blockade (ICB) therapy[37,38]. Immune organoids provide important insights into studying cancer cell heterogeneity and their interactions with immune components[36]. Studies have shown that immune organoids cultured in 3D microfluidic systems successfully maintain their primary cellular constituents, including various phenotypic cancer cells[36]. These cancer cell subpopulations exhibit distinct molecular characteristics, which may influence their interactions with immune cells[36]. Therefore, accurately mimicking the intricate relationships between tumor cells and their environment is crucial for advancing novel treatment strategies[36]. This section summarizes key studies on various organoid culture platforms used to study tumor−immune cell interactions within the TME (Fig. 3 and Table 2[20,39-56]).

3.1. *T cells in immune organoids*

Recent studies have developed tumor organoid−T cell co-culture platforms that not only recapitulate T cell-mediated cytotoxicity through direct contact and cytokine secretion but also enable HTS for immunotherapy evaluation and drug discovery[39-42]. T cells achieve specific recognition and killing of tumor cells through cytotoxic effector functions and cytokine production[57].

To better replicate the immunological function of T cells in organoids, researchers have developed co-culture systems combining tumor organoids with circulating immune cells[39]. Tumor-reactive T cells can not only be expanded using tumor organoids, but their activity against matched tumor cells can also be assessed[40]. The incorporation of effector T cells derived from peripheral blood into patient-derived cholangiocarcinoma (CCA)

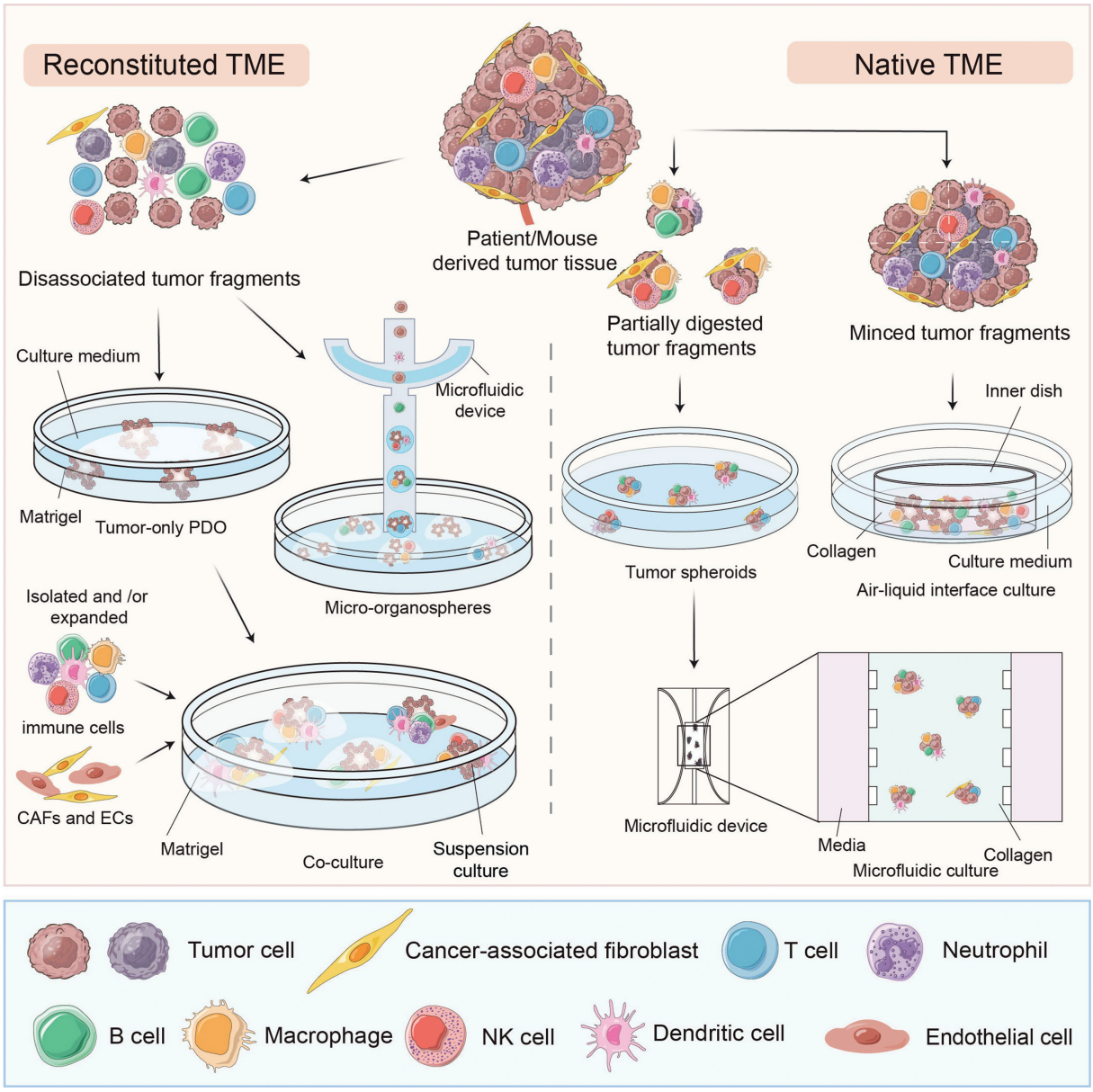

Figure 2 Different methods for constructing the immune microenvironment in tumor organoids. TME can be constructed in organoids by two routes. The reconstituted TME approach is by co-culturing isolated and/or expanded exogenous immune cells (*e.g.*, autologous peripheral blood cells) with tumor organoids, typically submerged and cultured within Matrigel or in suspension. Moreover, MOSs can be fabricated through microfluidic techniques by encapsulating sparse tumor and stromal cell populations within ECM droplets, followed by cultivation in nutrient-rich media. The native TME approach involves the generation of organoid models capable of maintaining and expanding native immune cells by cultivating enzymatically cleaved or physically excised tumor fragments. This approach includes 3D microfluidic culture and ALI culture.

organoids demonstrated that both $CD4^+$ and $CD8^+$ T cells could induce apoptosis in tumor organoids through direct contact or secretion of cytokines like tumor necrosis factor-α (TNF-α) and interferon gamma (IFN-γ)[40]. Additionally, significant heterogeneity was observed in the expression of co-inhibitory and co-stimulatory molecules, as well as RNA expression, in CCA organoids following both direct and indirect interactions with immune cells[40]. These discoveries may help to assess the efficacy of novel immune checkpoint inhibitors (ICIs) or inform the development of personalized ICIs treatment regimens[40].

The organoid platform enables HTS of T cells for tumor-killing efficiency[41,42]. Experiments using microfluidic microwell arrays have facilitated high-throughput studies of the heterogeneity in immune-tumor cell interactions[41]. These studies revealed heterogeneity in T-cell cytotoxicity efficiency and temporal dynamics of target cell elimination at the single-cell level[41]. Furthermore, tumor cell killing activity and tumor antigen presentation can be assessed through interactions between autologous tumor cells and tumor-specific cytotoxic T cells derived from peripheral blood[42]. In this study, screening revealed that the epigenetic inhibitors BML-210, CUDC-101, and GSK-LSD1 exhibited anti-tumor activity in tumor organoids, suggesting that the tumor-organoid-T-cell platform enables standardized measurements of tumor cell killing activity[42].

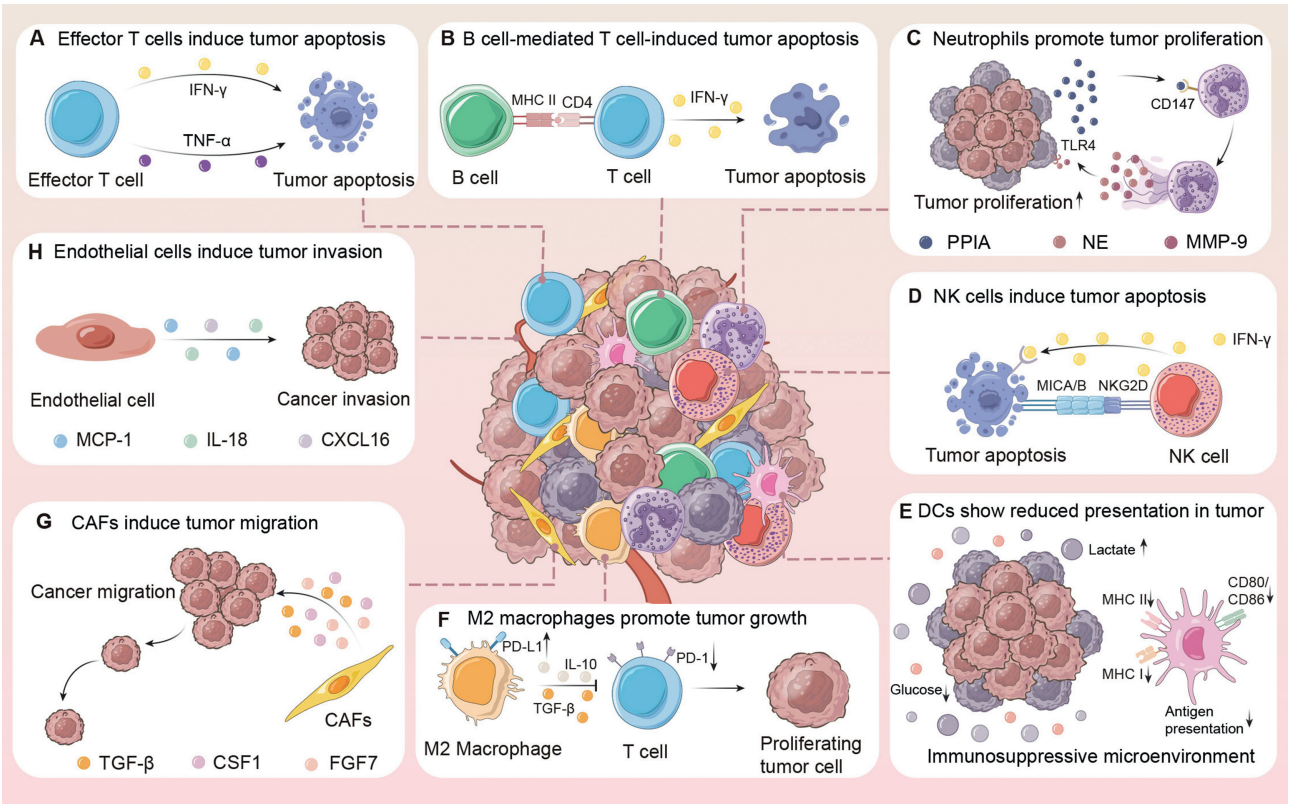

Figure 3 Behavior of various immune cells in TME studied with immune organoids. (A) Effector T cells secrete IFN-γ and TNF-α to tumors to induce tumor cell apoptosis. (B) B cells bind to T cells through MHC II molecules and CD4 molecules, activating T cells, which exert cytotoxicity by secreting IFN-γ. (C) NQO1-deficient tumor cells secreted PPIA that activated neutrophils by binding to CD147, which in turn induced NET formation and release of NE and MMP-9. (D) Activated NK cells scientific research induces tumor apoptosis by activating the receptor NKG2D-MICA/B with simultaneous release of IFN-γ. (E) DCs exhibit decreased expression of MHC molecules and co-stimulatory molecules (CD80, CD86) in an immunosuppressive microenvironment of high lactate and low glucose, resulting in decreased antigen presentation. (F) M2 macrophages release IL-10 and TGF-β to produce immunosuppressive effects on T cells and promote tumor growth. (G) CAFs secrete cytokines and chemokines in the microenvironment, which affect tumor cell migration and invasion. (H) Endothelial cells secrete MCP-1, IL-8, and CXCL16, inducing tumor invasion and metastasis.

Table 2 Overview of the possibilities of studying various immune cells in different tumor organoids.

Cell type	Cell source	Co-culture type	Tumor category	Ref.
T cells	PBMCs, $CD3^+$ T, OHI-T, $CD8^+$ T	NTa, RTa	NSCLC, AML, CCA, BC	39–42
B cells	PBMCs	NTa, RTa	DLBCL, CRC	43,44
Neutrophils	PBMCs	NTa, RTa	OC, BA	20,45
NK cells	PBMCs	NTa, RTa	pedCA, CRC, BCa	46–48
DCs	mBMC	NTa	PDAC	49
TAMs	THP-1, PBMCs	NTa, RTa	PDAC,BC, MM, OC, Mel	50–52
CAFs	mPSC, hCRC-TS	RTa, NTa	PDAC, CRC	53,54
ECs	HUVEC	RTa	HCC, PDAC	55,56

NTa, native TME approach; RTa, reconstituted TME approach; AML, acute myeloid leukemia; BCa, bladder cancer; NSCLC, non-small cell lung cancer; BC, breast cancer; DLBCL, large B cell lymphoma; CRC, colorectal cancer; pedCA, pediatric cancer; MM, multiple myeloma; Mel, melanoma; PBMCs, peripheral blood mononuclear cells; GC, gastric cancer; PDAC, pancreatic ductal adenocarcinoma; OC, ovarian cancer; BMC, mouse bone marrow cells; mPSC, mouse pancreatic stellate cells; hCRC-TS, human colorectal cancer tumor specimens; HUVEC, human umbilical vein endothelial cells.

3.2. B cells in immune organoids

Recent advances in organoid technology have enabled the development of various pan-cancer and lymphoid tissue models that can recapitulate complex B cell functions and evaluate immunotherapy responses[43,44]. As crucial components of tertiary lymphoid structure (TLS), B cells enhance anti-tumor immune responses through multiple mechanisms, including antigen presentation, cytokine secretion, and antibody production[58].

Organoids provide a valuable platform for studying B cell responses in health and disease[43]. In one innovative study, researchers developed humanized immune organoids using synthetic

hydrogels instead of conventional Matrigel, successfully maintaining long-term *in vitro* culture functionality[43]. B cell compartmentalization and maturation were achieved using microfluidic chips[43]. Through experiments establishing CXCL12 chemokine gradients and influenza antigen stimulation, the study demonstrated that B cells from healthy donors could form distinct germinal center structures and produce antibodies, while these functions were significantly impaired in B cells from large B cell lymphoma (DLBCL) patients[43].

B cell-formed TLS in tumors play a crucial role in enhancing ICB therapy responses[58-60]. A study investigating the mechanisms of B cell involvement in ICB therapy during colorectal cancer (CRC) metastasis utilized PDO humanized mouse models[44]. The results revealed that B cells mediate $CD4^+$ T cell anti-tumor immune responses by forming TLS in the TME and expressing MHC II molecules[44]. The activated T cells then exert cytotoxicity through IFN-γ secretion[44]. This finding offers new therapeutic strategies for improving ICB therapy outcomes[44]. However, research using organoid-B cell co-culture models to study anti-tumor mechanisms remains limited in this field and warrants further exploration by future scientists.

3.3. Neutrophils in immune organoids

Recent studies using organoid−neutrophil co-culture systems have uncovered diverse mechanisms by which neutrophils modulate tumor development, including cytokine secretion, neutrophil extracellular trap (NET) formation, and immune response regulation[20,45,61,62]. As key immune cells, neutrophils play a dual role in cancer: they can inhibit tumor growth through antigen presentation and reactive oxygen species (ROS) generation, while also promoting tumor progression *via* angiogenic factors and matrix metalloproteinases (MMPs) that enhance metastasis[63].

In recent years, growing attention has been focused on the links between cancer cells and tumor-associated neutrophils, which have opened up promising avenues for therapeutic intervention[64]. A study explored an organoid model derived from patients with high microsatellite instability (MSI-H) CRC, alongside 142 surgically resected cases[61]. Their results revealed that neutrophils in the inflammatory TME suppress immune responses through the CD80/CD86−CTLA4 axis, thereby contributing to resistance to ICIs in MSI-H CRC[61]. NETs are responses of activated neutrophils, and research indicates that NET-associated proteases can promote cancer metastasis by disrupting endothelial cell contacts or the ECM[62]. In a co-culture system of neutrophils and ovarian tumor cells within a microfluidic device, neutrophils were observed to produce NETs that accelerated the migration of ovarian tumor cells[20]. To further investigate the effects of NETs on cancer cell metastasis and proliferation, researchers established a co-culture model using MMTV-PyMT breast cancer (BC) organoids and neutrophils[45]. The study revealed that peptidyl-prolyl *cis−trans* isomerase A (PPIA) secreted by NQO1-deficient tumor cells triggers neutrophil activation through CD147 binding[45]. This interaction subsequently induces NET formation and the release of neutrophil elastase (NE) and MMP-9[45]. Additionally, PPIA both promotes interleukin (IL)-6 production through the TLR4−NF-κB pathway and enhances neutrophil maturation in bone marrow with directed migration to the lungs, creating a metastasis-promoting microenvironment[45].

3.4. NK cells in immune organoids

Organoid−NK cell co-culture systems have revealed NK cells' therapeutic potential through their ability to kill tumor cells *via* receptor recognition and cytokine release[46-48,65]. As critical immune cells, NK cells target tumors through receptors like NKG2D and can either stimulate or suppress target cells, resulting in cytotoxicity or immune activation[66].

These co-culture systems enable dynamic studies of the anti-tumor immune response in patient-derived models[46,47]. Research has shown that activated allogeneic NK cells can penetrate tumor spheroids and exhibit strong cytotoxic activity[46]. Notably, the NKG2D receptor plays a crucial role in recognizing CRC spheroids, whether derived from primary tumor biopsies or established cell lines such as DLD1 and HT29[46]. However, tumor cells within the spheroids may evade immune detection through NKG2A−HLA-E interactions[46]. Current strategies targeting both the inhibitory NKG2A receptor on NK cells and the NKG2D ligands MICA/B on tumor cells have shown a synergistic effect in suppressing tumor growth, even in the presence of the NKG2A ligand HLA-E on tumor cells[47].

The tumor-killing mechanisms of NK cells can be clearly observed using organoid−NK cell co-culture systems[48,65]. In a monolayer cytotoxicity assay, it has been shown that NK cells effectively target and kill bladder cancer organoids (BCOs) through NKG2D receptor activation, resulting in the release of granzyme B and IFN-γ[48]. Additionally, NK cells secrete chemokines, including C−C-motif ligand (CCL)1, CCL2, and CCL20, which promote T cell chemotaxis[48]. Furthermore, a microfluidic device was used to culture pancreatic ductal adenocarcinoma (PDAC) spheroids to assess the immunotherapeutic effects of a novel immunomodulator, the Tri-specific Killer Engager (TriKE), in combination with NK cells[65]. This study demonstrated that two antibody fragments of TriKE-targeting B7−H3 and CD16 form a synapse between NK cells and cancer cells, thereby enhancing the cytolytic activity of NK cells against tumor organoids and facilitating tumor suppression[65]. These findings underscore the potential of microfluidic technology in personalized medicine and provide a valuable experimental foundation for the development of novel immunotherapies[48,65].

3.5. DCs in immune organoids

Organoid−DC co-culture models have provided valuable insights into both DC-mediated antitumor immunity and tumor-induced regulation of DCs in a physiologically relevant context[49,67]. As the most potent antigen-presenting cells, DCs are crucial for initiating and regulating both innate and adaptive immunity within the TME[68]. These cells are capable of presenting tumor-associated antigens (TAAs) on major histocompatibility complex molecules and providing soluble and costimulatory signals that help shape T cell-mediated antitumor responses[68].

The development of organoid-tumor immune co-culture platforms has deepened our understanding of DC−cancer cell crosstalk[49,67]. Through a co-culture model of patient-derived PDAC organoids and immune cells, researchers discovered that PDAC cells create an immunosuppressive microenvironment characterized by high lactate and low glucose through enhanced glycolysis[49]. This metabolic reprogramming directly impairs DC function by disrupting mitochondrial function and ATP

production, leading to decreased expression of MHC molecules and costimulatory molecules (CD80, CD86)[49]. When lactate dehydrogenase A (LDHA) was inhibited in PDAC organoids, the metabolic profile of the co-culture environment improved and DC antigen presentation function was restored, suggesting a novel therapeutic approach targeting tumor metabolism[49]. In another study, a novel co-culture system was established to study the biological behavior of DCs in CRC[67]. This system enabled researchers to recreate and analyze the crosstalk between DCs and patient-derived metastatic colorectal cancer (mCRC) organoids[67]. The behavior, phenotype, and function of monocyte-derived DCs in a collagen matrix are influenced and modulated by mCRC organoids, as demonstrated by flow cytometric analysis and other methods[67]. The study demonstrated that the DCs exhibited high viability and extensive interaction with cancer cells, providing valuable insights into the tumor−immune interaction within the TME[67].

3.6. Tumor-associated macrophages (TAMs) in immune organoids

Organoid−TAM co-culture platforms have demonstrated how TAMs regulate tumor progression through cytokine secretion and phenotype polarization, providing a means to investigate TAM heterogeneity and immunosuppressive mechanisms[50-52]. TAMs regulate tumor progression in the TME through secreted factors[69,70]. They can polarize into two main subtypes: anti-tumor M1 macrophages that promote cell death, and pro-tumor M2 macrophages that support tissue repair[69]. While both subtypes exist in tumors, the TME typically promotes the M2 phenotype through factors like IL-4 and hypoxia, facilitating tumor growth[70].

Recent advances in TME research have underscored the complex interactions between TAMs and cancer cells, leading to new insights into cancer progression and metastasis[50-52]. A recent study has constructed a pancreatic cancer organoid model by combining THP-1-derived macrophages, patient-derived PDAC cells, and human-induced pluripotent stem cells (hiPSC) derived stromal cells in ALI[50]. Single-cell RNA sequencing revealed that this model preserves the diversity of TAMs within the TME, identifying five characteristic subpopulations (*e.g.*, SPP1-TAM) that don't fit the traditional M1/M2 classification[50]. These TAM subpopulations support cancer cell survival by promoting angiogenesis and inhibiting cell death[50]. Another study developed a novel mammary carcinoma organoid and TAM co-culture model to investigate the mechanisms by which TAMs enhance invasion and metastasis in the TME[51]. The researchers found that TAMs promoted the self-renewal and pluripotency of mammary carcinoma stem cells by upregulating stem cell markers such as ALDH1A1, SOX2, and CD166[51]. Additionally, TAMs secreted IL-18, fibroblast growth factor-2 (FGF-2), and CCL17, which synergistically induced the expression of MMP9 in cancer cells, enhancing cell migration and persistence, as shown in transwell migration assays[51]. These findings provide new insights for developing anti-metastasis strategies[51]. Furthermore, 3D TME simulation models have revealed that TAMs suppress T cell antitumor functions through dual mechanisms of PD-L1 upregulation coupled with T cell PD-1 downregulation, while simultaneously releasing immunosuppressive cytokines transforming growth factor-β (TGF-β) and IL-10[52]. This immunosuppression reduces T cell-mediated tumor cell destruction, thereby promoting tumor growth and metastasis[52].

3.7. Cancer-associated fibroblasts (CAFs) in immune organoids

Organoid−CAF co-culture systems have demonstrated how CAFs support tumor growth through diverse mechanisms, including niche factor secretion and phenotypic regulation[53,54]. CAFs are found in virtually all solid tumors and are among the most prominent stromal cells within the TME[53]. Organoid co-culture experiments have been widely utilized to study the interactions between CAFs and different cancers[53,71].

Advanced 3D co-culture systems have revealed the diverse roles of CAFs in tumor progression through their heterogeneous phenotypes and secreted factors[53,54]. For instance, a novel 3D co-culture system combining mouse pancreatic stellate cells (mPSCs) with PDAC organoids was developed using Matrigel and Transwell platforms[53]. This system identified two distinct CAF phenotypes[53]. One subpopulation, located near tumors, highly expresses α-smooth muscle actin (α-SMA) and stimulates tumor growth, while the other loses myofibroblast properties and secretes inflammatory cytokines[53]. This culture model highlights the symbiotic interactions between CAFs and cancer cells *in vivo*, offering opportunities for drug development targeting specific CAF subtypes[53]. In another study, co-culturing patient-derived CRC tissues with matched fibroblasts demonstrated that fibroblasts could support the growth of PDOs without the addition of classical ecological niche factors[54]. This support was attributed to fibroblasts' secretion of small niche factors such as Noggin, R-Spondin1, EGF, and WNT3a[54]. Additionally, CAFs secrete chemokines and cytokines, including TGF-β1, colony-stimulating factor 1 (CSF1), and FGF7, which facilitate intercellular communication through ligand−receptor interactions[54]. These factors contribute to tumor growth, maintain tumor cell stemness, and enhance tumor cell invasion and migration[54]. Deciphering the complex interplay of CAFs within the TME provides valuable insights for developing targeted therapeutic strategies[54].

3.8. Endothelial cells (ECs) in immune organoids

Organoid−EC co-culture platforms have demonstrated how endothelial cells modulate tumor progression through vascular formation and secretory signaling, enabling investigation of TME and therapeutic targeting[55,56]. ECs form blood vessels that supply tumors with oxygen and nutrients, while also promoting tumor progression through paracrine signaling with cancer cells and stromal components[72].

Given the high vascularity of certain tumors and the overexpression of angiogenesis-promoting factors[72], tumor organoid−EC co-culture models provide powerful tools for investigating angiogenesis and exploring the TME[55,56]. Recent research has shown that the intricate relationship between cancer cells and the surrounding blood vessel environment is essential for tumor progression and metastasis[55,56]. One study utilized hydrogels to co-culture patient-derived xenograft organoids (PDXOs) of hepatocellular carcinoma (HCC) with ECs, effectively mimicking the vascular secretory crosstalk between ECs and HCC *in vitro*[55]. The results revealed increased expression of genes related to *TNF* signaling, suggesting that ECs contribute to the creation of an inflammatory milieu in HCC by recruiting immune cells[55]. Through antibody detection, the study identified elevated expression of vascular secretory factors, including C−X−C motif ligand (CXCL) 16, IL-8, and monocyte chemoattractant protein-1 (MCP-1), in the co-culture system[55]. These factors promoted the

polarization of macrophages toward pro-inflammatory and pro-angiogenic phenotypes, resembling tumor-associated macrophage subpopulations commonly seen in HCC[55].

Cancer-initiating cells (CICs), characterized by self-renewal and differentiation abilities, correlate with drug resistance and poor prognosis[56]. A recent study established a co-culture model of ECs and patient-derived PDAC organoids using a conventional medium devoid of artificial stem cell growth factors[56]. This model notably preserved $CD44^{+}$CICs compared to the PDAC organoid model alone[56]. Further investigations indicated that organoids maintain CICs through paracrine effects or Notch/Wnt signaling interactions with ECs[56]. In conclusion, understanding the biology of ECs in TME may provide new therapeutic targets for controlling tumor development.

4. Immune organoids for evaluating immunotherapy efficacy

4.1. *Immune organoids in drug-based immunotherapy*

Tumor immunotherapy drugs traditionally include monoclonal antibodies and certain small molecule immunological agents[26]. Monoclonal antibodies targeting programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) are commonly used in clinical practice[26]. These antibodies enhance anti-tumor immunity by blocking critical immune checkpoints, thus modulating immune cell function[26]. In recent years, strategies combing ICIs with anti-angiogenic agents are taking center stage, and Khan and Kerbel[73] systematically described the bidirectional synergy between ICIs and anti-angiogenic drugs, proposing that they can form a positive feedback loop of "immune stimulation-vascular remodeling". Furthermore, this theory has been proven in recent phase III clinical trials, demonstrating that the success of combination therapy focuses on balancing the dual effects of anti-angiogenic drugs on tumor immunity[74]. Organoid has become a powerful tool for studying pharmacological immunotherapy, offering an efficient platform for evaluating the effects of immunotherapeutic agents such as anti-angiogenic drugs[75-79] (Fig. 4). By closely mimicking the TME, organoids provide deeper insights into cancer−immunity interactions and facilitate the development of novel, more effective immunotherapies and pharmacological targets[75-79].

In recent years, cancer organoids have been increasingly utilized to study the mechanisms of novel immune therapies and to explore optimal therapeutic combinations[75-79]. One study demonstrated that a novel antibody−drug conjugate specifically targets CRC organoids[75]. The drug, Cet-ZA, combines the anti-

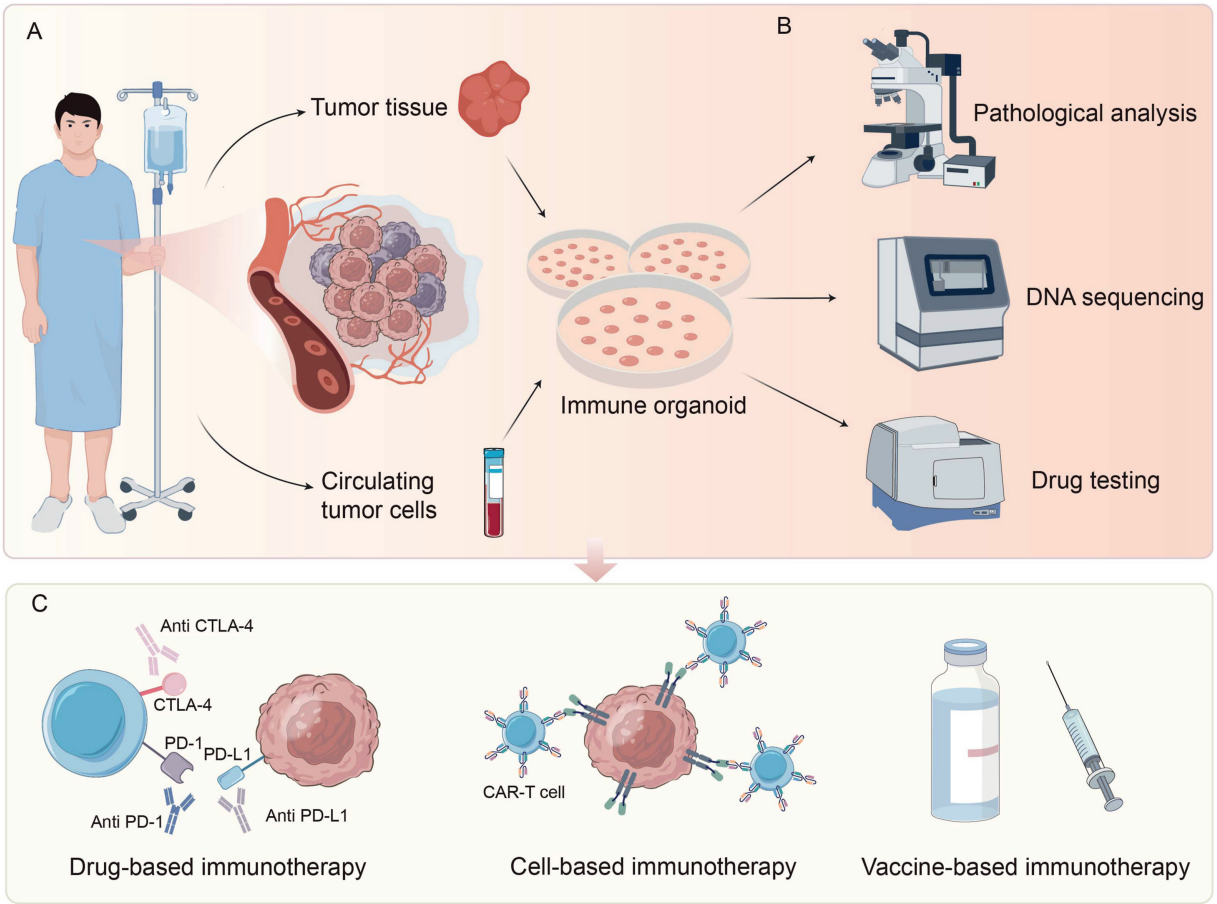

Figure 4 Immune organoids to assess the efficacy of different immunotherapies. Organoid modeling encapsulates physiological features and functions and provides a more realistic platform for preclinical *in vitro* techniques. (A) Generation of tumor organoids from patient tumor tissue or body fluids by various methods. (B) Analysis of differently treated tumor organoids and their immune microenvironment using different techniques. (C) It can be used to evaluate the effectiveness of pharmacological and cellular immunotherapies and cancer vaccines and to guide clinical treatment regimens.

epidermal growth factor receptor (EGFR) antibody cetuximab with zoledronic acid[75]. When added to a co-culture system consisting of patient-derived CRC organoids and allogeneic Vδ2 T cells, Cet-ZA activated Vδ2 T cells *via* T cell receptor (TCR) and FcγRIIIA (CD16), leading to degranulation of cytolytic enzymes and subsequent anti-tumor effects[75]. Additionally, patient-derived BCOs, with preserved T cells, were used to assess the immunosynergistic effects of combining PD-1 inhibitors with platinum-based (Pt) compounds[76]. Histological analysis revealed that PtIV complexes enhanced IFN-γ secretion from T cells within BCOs, activated immune-related pathways, and promoted TCR clonal expansion[76]. These findings underscore the value of cancer organoid models in monitoring and reproducing responses to ICIs and in identifying potential therapeutic combinations[76]. Moreover, a novel *ex vivo* 3D culture system, the organotypic tumor spheroid (MDOTS/PDOTS), was developed to maintain key features of the natural immune TME and guide the selection of optimal clinical treatment regimens for ICB therapy[77]. Cellular profiling of MDOTS from anti-PD-1 partially sensitive CT26 mouse tumor models revealed elevated CCL2 expression[77]. Profiling of PDOTS derived from melanoma and Merkel cell carcinoma patient samples, post-ICB treatment, showed upregulation of cytokines and chemokines (*e.g.*, CCL19 and CXCL13), closely resembling clinical findings[77].

Stimulator of interferon genes (STING), a small-molecule drug target, has gained significant attention as a promising cancer treatment strategy[80]. Activated STING pathway promotes the production of type I IFN and other inflammatory cytokines, which in turn activates DCs and T cell-mediated antitumor immune responses[81]. A preclinical study investigating STING function demonstrated enhanced migration and cytotoxicity of NK cells in a 2D malignant pleural mesothelioma (MPM) cell line model when STING-expressing MPM cells were included in the NK cell co-culture medium[78]. Additionally, studies using mouse and human MMR gene-deficient CRC organoid models confirmed that acquired STING variants enhance intrinsic STING pathway activity in cancer cells[79]. These enhanced variants were able to transform "cold" TMEs into "hot" TMEs, sensitizing drug-resistant tumors to ICI therapy[79]. Under this strategy, chemokines (*e.g.*, CXCL11, CXCL10, CXCL9, and CCL5) and other cytotoxic molecules that recruit NK cells and cytotoxic T lymphocytes (CTLs) were strongly upregulated within the TME[79]. Furthermore, infiltrating CTLs exhibited increased expression of *Gzmb* and *Ifng*[79]. These findings suggest that organoids can serve as a powerful tool for studying novel TME regulatory strategies and are poised to provide accurate preclinical *in vitro* models that better represent cancer patients' responses to therapy[79].

Organoids can offer a theoretical basis for the discovery of new clinical therapeutic targets for future tumor treatments[82,83]. Aurora kinase A (AURKA) has been identified as a potential target for treating hepatic metastatic CRC using a tumor organoid model[82]. In this study, PDOs were employed to replicate the acquisition of chemotherapy resistance, and the efficacy of AURKA inhibitors in inducing apoptosis under drug-resistant conditions was evaluated[82]. The results demonstrated that AURKA inhibitors could restore chemotherapy sensitivity in drug-resistant CRC cells, particularly those harboring *KRAS* mutations and exhibiting elevated AURKA/c-MYC expression[82]. These findings suggest that combining EGFR pathway blockade with AURKA inhibitors could offer an effective second-line therapeutic strategy[82]. This research provides crucial insights that could inform the development of novel treatment approaches for CRC[82]. Additionally, a separate experiment investigated the off-target tumor toxicity of T cell-binding bispecific antibodies using both tumor and healthy organoid models[83]. Single-cell resolution immunofluorescence staining revealed efficient T-cell infiltration in healthy organoids, resulting in a toxic apoptotic response[83]. Interestingly, despite the lower expression of epithelial cell adhesion molecule and carcinoembryonic antigen in healthy organoids compared to tumor organoids, the enhanced T-cell infiltration and subsequent toxicity response were still observed[83]. These gut-derived 3D organoids not only offer valuable insights into the exploration of new therapeutic targets but also contribute to a deeper understanding of disease treatment mechanisms[83].

4.2. Immune organoids in cell-based immunotherapy

Adoptive cell transfer therapy (ACT) represents an innovative approach to cancer treatment, where immune cells are isolated from either autologous or allogeneic sources and undergo *in vitro* modification, expansion, and activation before being reinfused into cancer patients[84]. This therapeutic strategy employs T cells engineered with chimeric antigen receptors (CARs) or high-affinity TCRs to recognize multiple tumor antigens, or autologous tumor-infiltrating lymphocytes (TILs) selected for tumor specificity[85]. Recent years have witnessed the emergence of CAR-T/NK cell therapy as a promising anti-cancer approach, particularly in treating hematologic malignancies[86]. However, its effectiveness against solid tumors remains uncertain[86]. A significant challenge lies in the dynamic nature of tumor phenotypes, which can vary considerably among patients and evolve throughout disease progression and treatment[86]. Current *in vitro* solid tumor models are limited by their inability to replicate cellular heterogeneity and maintain mutations that drive changes in surface antigens[86]. 3D organoid models offer a solution to these limitations by successfully reproducing the parental cellular heterogeneity and preserving critical intercellular interactions[87]. These models have become valuable tools for investigating the efficacy of ACT immunotherapy against cancer[11,87] (Fig. 4).

Organoids have emerged as powerful platforms for evaluating ACT, with numerous studies validating their utility in assessing CAR-T cell functionality and therapeutic efficacy[88-91]. In a novel clinical trial design, investigators generated patient-derived glioblastoma organoids (GBOs) that could be tested for CAR-T cell therapy while the patient was being treated to assess treatment response in real time[88]. The study found that the treatment response of GBOs, including the degree of tumor cell death and cytokine release, was strongly correlated with the patient's clinical prognosis[88]. This study shows the great potential of organoids for real-time assessment of CAR-T cell bioactivity and immunotherapy efficacy[88]. Using BCOs models, researchers evaluated CAR-T cell immunological effects through cytotoxicity and ELISA assays[89]. When MUC1-targeting CAR-T cells were co-cultured with MUC1$^+$ BCOs, measurements of LDH and cytokine levels demonstrated specific antigen recognition and immune activation, establishing a reliable platform for preclinical CAR-T cell assessment[89]. Similar results were observed in studies using GBOs, where CAR-T cells targeting the mutant antigen *EGFRvIII* showed remarkable specificity in eliminating *EGFRvIII*$^+$ organoids[90]. CRC PDOs provided both a highly mimetic TME and a platform for real-time observation of CAR−NK cell-mediated tumor cell destruction[91]. Through the combination of confocal live-cell imaging and luciferase measurements, researchers could precisely evaluate CAR−NK cell

killing efficiency and specificity, validating the crucial role of organoids in optimizing CAR−NK cell therapeutic strategies[91].

Emerging evidence suggests that TIL therapy shows promise in treating solid tumors, potentially offering advantages over CAR-T/NK and TCR-T approaches, which may be limited by their single-antigen recognition capability[92]. The strength of TIL therapy lies in its ability to target multiple tumor antigens simultaneously, including potentially undiscovered ones, through naturally occurring T cells[92]. Recent developments in organoid technology have altered the evaluation of TIL therapy efficacy[93,94]. A study developed an innovative bioprinting platform that integrates GC PDOs and TILs in a unique dual-region design[93]. Through confocal microscopy and multiple cytokine analysis techniques, the platform allows for a comprehensive assessment of TILs migration patterns and functional activities[93]. The results of the study show the chemotactic migration and degranulation processes of TILs, as well as the dynamics of key effector molecules including granzymes and perforins[93]. While current TCR-T therapies primarily target known antigens, their limited success against aggressive malignancies like pancreatic cancer has prompted further investigation into TIL-based approaches[94]. The clonal diversity of TCRs in TILs, ranging from 0.1% to 50%, combined with intratumoral TIL heterogeneity, creates favorable conditions for developing tumor-specific immune responses[95,96]. In a ground-breaking study using patient-derived pancreatic tumor organoids, researchers successfully stimulated the clonal expansion of tumor-reactive T cells from peripheral blood[97]. Patient peripheral T cells were co-cultured with autologous tumor organoids to generate organoid-primed T cells (opTs)[97]. The killing effects of opT and TCR specificity were detected using flow cytometry for clonal expansion, ELISA assays, and deep sequencing of gDNA extracted from T cells[97]. Additionally, NKG2A blockade significantly elevated IFN-γ expression, identifying the NKG2A−HLA-E axis as a key checkpoint for $CD8^+$ T cells in pancreatic cancer[97].

4.3. Immune organoid in cancer vaccines-based immunotherapy

Cancer vaccines have emerged as a powerful immunotherapeutic approach that activates patient-specific adaptive immunity against tumor antigens to control cancer progression and promote regression of established malignancies[92,98,99]. Researchers have developed several categories of cancer vaccines, including antigen-specific tumor vaccines, whole tumor cell vaccines, viral vector vaccines, peptide vaccines, and cellular vaccines[100]. Considering the genetic and environmental differences between species, many vaccine candidates that show promise in animal models do not fare well in human trials[101]. This has highlighted the critical need for platforms that can effectively recapitulate key elements of human adaptive immunity. It has now been found that the development of immune organoids using human tonsils can accurately summarize the key features of *in vitro* germinal centers and support in-depth research into the vital mechanisms of adaptive immunity, as well as rapid screening of vaccine candidates[102]. These engineered immune constructs provide a robust platform for modeling germinal center functions by recreating precise immunological tissue microenvironments, enabling high-throughput evaluation with reduced timeframes[102]. In particular, studies have shown that sugar-conjugated vaccine candidates successfully induced germinal center B cell development and B cell receptor clustering within 3D biomaterial-based mouse B cell follicular analogs[35]. This approach enhanced cellular activation, downstream signaling pathway transduction, diverse immunoglobulin expression, and the identification of high-affinity, specific monoclonal antibodies[35]. These results highlight the potential of immunological organoids as rapid predictive tools for vaccine efficacy assessment and specific antibody discovery[35].

Organoid models have proven to be powerful platforms for identifying mutation-associated neoantigens for cancer vaccination[103-105]. Studies have demonstrated this utility through the establishment of small human-derived pre-cervical carcinoid and tumor biobanks that successfully preserved and replicated both genomic features and disease-specific tissue characteristics[103]. Through co-culture experiments with human papillomavirus (HPV) peptide-stimulated PBMCs, researchers observed differential responses between tumor organoids to immune PBMCs and identified varying efficacies of antigenic peptides in tumor organoid elimination[103]. These findings support the significant potential of organoid platforms in screening therapeutic HPV vaccines[103] (Fig. 4). In a separate investigation, researchers developed an innovative α-lactalbumin (α-LA) engineered exosome system incorporating Hiltonol (TLR3 agonist) and ELANE (human neutrophil elastase) to create an *in situ* DC vaccine (HELA-Exos) for BC treatment[104]. The α-LA component, serving as a tumor-specific homing protein, was enriched on the exosomal surface, leading to improved targeting capabilities and immunogenicity[106]. When evaluated in a HELA-Exos and PDO-PBMCs co-culture system, HELA-Exos effectively stimulated antigenic cross-presentation in DCs and induced robust $CD8^+$ T cell activation, resulting in significant growth inhibition of autologous PDOs[104]. This approach, combining TLR3 agonists and immunogenic cell death (ICD) inducers within cell-free exosomes, presents a promising strategy for developing DC vaccines against BC[104]. Further advancing the field, researchers cultivated four CRC organoid clones from a single patient, each harboring identical exonic mutations[105]. The presence of distinct mutations among these organoid clones effectively modeled intratumor heterogeneity, demonstrating their genetic diversity[105]. Integration of organoid proteomics data with corresponding peptide ligand profiles revealed that cancer cells predominantly produced tumor-specific ligands derived from DNA damage control and tumor suppressor proteins[105]. This observation may be linked to compromised cytoprotective functions within the tumor cells[105]. The study highlighted the variability in HLA-peptide expression within individual patients and suggested that immunization strategies targeting multiple peptides derived from highly conserved tumor suppressors could potentially reduce immune evasion[105]. These findings are promising and demonstrate the great potential of organoid applications in cancer vaccine research and development[105].

5. Immune organoids in HTS

Cancer cell line-based HTS has provided valuable insights into *in vitro* drug responses across diverse human cancer cells[98]. However, genetic drift resulting from prolonged culturing significantly limits their clinical relevance[8,107]. While patient-derived xenografts (PDXs) offer an alternative, their widespread implementation in HTS is constrained by low throughput, high costs, and their animal-based nature[10]. Organoids have emerged as a promising technology in cancer research[108-110]. These organ-on-chip systems more accurately replicate tumor drug responses and capture individual patient variations compared to conventional

cell culture methods[108,109]. They are often combined with HTS platforms for large-scale compound testing and gene function screening, providing precise strategies for disease treatment[108,109]. For instance, microdroplet emulsion technology can generate thousands of MOSs from minimal tumor biopsy material, allowing rapid assessment of tumor responses to anti-PD-1 and bispecific antibodies within approximately 10−14 days[110].

Some of the latest innovative drug screening studies use organoids to screen drugs in a high-throughput manner, providing promising avenues for cancer treatment[111-113]. A platform for high-throughput drug screening utilizing mouse PDAC organoids was reported in a recent study[111]. The platform screened 6000 compounds and found that perhexiline maleate interfered with cholesterol metabolism *via* sterol-regulatory-element-binding protein 2 (SREBP2) downregulation and specifically inhibited the growth of PDAC organoids carrying the *KRAS G12D* mutation[111]. Another significant advancement came from a drug repurposing initiative using CRC PDOs[112]. The study tested 335 drugs, identified 34 drugs with anti-CRC effects, and explored the mechanism of drug action in conjunction with transcriptome analysis[112]. It suggests that organoid combined with next-generation sequencing technology provides a valuable pathway for drug screening and identification[112]. In addressing lenvatinib-resistant HCC, researchers developed a sophisticated multi-dimensional HTS platform incorporating patient-derived cell (PDC)-PDO-PDX models to evaluate 80 mechanistically diverse compounds[113]. This comprehensive approach identified romidepsin, a histone deacetylase inhibitor, as a potent inhibitor of drug-resistant tumor growth at low concentrations[113]. Subsequent transcriptomic analysis revealed a crucial relationship between *HDAC2* and *EGFR* expression, demonstrating that romidepsin's therapeutic effect stems from EGFR pathway inhibition and induction of ICD[113]. This finding not only provides a new strategy to overcome lenvatinib resistance, but also validates the value of this platform in drug screening and mechanism studies[113].

The analysis of specific mechanisms of effective drugs can provide molecular diagnostic pathways for diseases[114,115]. A novel screening approach was developed using microfluidic chip-cultured human hepatocyte organoids, integrating CRISPR-based drug screening with high-throughput gene expression sequencing[114]. This innovative platform identified *FADS2* as a crucial gene in regulating hepatic fat accumulation, offering new therapeutic possibilities for non-alcoholic fatty liver disease[114]. In addition to exploring the accuracy of organoid generalization of *in vivo* tumor response to a given drug, an HTS platform has been constructed to study drugs using organoid technology[115]. This platform enabled the identification of relationships between cancer-selective growth inhibitors and their molecular targets in CRC, specifically focusing on aberrantly activated genes[115]. These associations allowed accurate prediction of drug response based on molecular characteristics of the tumor. Through multivariate analysis of variance, researchers examined associations between various genotypes and molecularly targeted therapeutics[115]. Their finding of a significant correlation between TP53 mutations and MDM2 inhibitors confirmed the value of organoid biobanks in HTS[115].

6. Immune organoids in clinical trials

High-throughput sequencing, which can accurately identify somatic mutations, has been widely used in the field of cancer therapy[116]. However, genomic profiling alone has shown limitations in predicting targeted therapy responses and guiding clinical drug selection, constraining its applications in precision medicine[117]. In recent years, many scientists have constructed organoid biobanks of cancer patients that recapitulate the histopathological manifestations and genetic features of the original tumor[90,118]. These PDOs demonstrate drug response patterns that closely mirror patients' clinical outcomes.

Currently, several clinical trials have been conducted utilizing this technique[119-121] (Table 3). In one notable study, $BRAF^{V600E}$ CRC patients treated with triple therapy (spartalizumab, dabrafenib, and trametinib-PD-1, BRAF, and MEK inhibitors, respectively) showed an overall confirmed response rate of 24.3%[119]. Among microsatellite-stable patients without prior BRAF-targeted therapy or ICB therapy, the confirmed response rate was 25%[119]. Research on lung cancer (LC) organoids has yielded promising results in predicting patient responses to chemotherapy and targeted treatments[120]. The predictive model achieved impressive

Table 3 Overview of immune organoids used in immunotherapy clinical trial applications.

Trial number	Research topics	Study type	Cancer type	Co-culture of organoids	Therapy assessment
NCT06268652	PDO-guided personalized treatment *vs* physician's choice	Int.	BC	−	Drug-based
NCT05725200	Research on individualized treatment	Int.	CRC	−	Drug-based
NCT03026140	Neoadjuvant ICI and IO combinations	Int.	CC	RTa (PBMCs)	Drug-based
NCT03668431	Dabrafenib + trametinib + pdr001	Int.	CRC	−	Drug-based
NCT06196554	Organoids in the screening of neoadjuvant drugs	Obs.	GC	RTa (T cells)	Drug-based
NCT06315868	PDO characterization in BC subtypes	Obs.	BC	RTa (PBMCs)	Drug-based
NCT03778814	TCR-T cell immunotherapy in LC and solid tumors	Int.	LC	RTa (TILs)	Cell-based
NCT05332925	Using *ex vivo* tumoroids to predict immunotherapy response	Obs.	NSCLC	−	Drug-based
NCT04951115	Chemo-immuno-radiotherapy in stage IV trial	Int.	SCLC	−	Drug-based
NCT05401318	Tailoring treatment in CRC	Obs.	CRC	−	Cell-based
NCT06156150	The role of B7−H4 in tumor vaccine	Obs.	Glioma	−	Vaccine-based
NCT05508399	Perioperative tislelizumab-chemotherapy biomarkers	Obs.	GAC/GEJAC	RTa (TILs)	Drug-based
NCT05955196	CD47−SIRPα inhibitors in CC microenvironment	Obs.	CC	−	Drug-based

RTa: reconstituted TME approach; Int., interventional; Obs., observational; *vs*, *versus*; ICIs, immune checkpoint inhibitors; BC, breast cancer; LC, lung cancer; PDO, patient derived organoid; CRC, colorectal cancer; GC, gastric cancer; CC, colon cancer; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; GAC/GEJAC, G/GEJ adenocarcinoma.

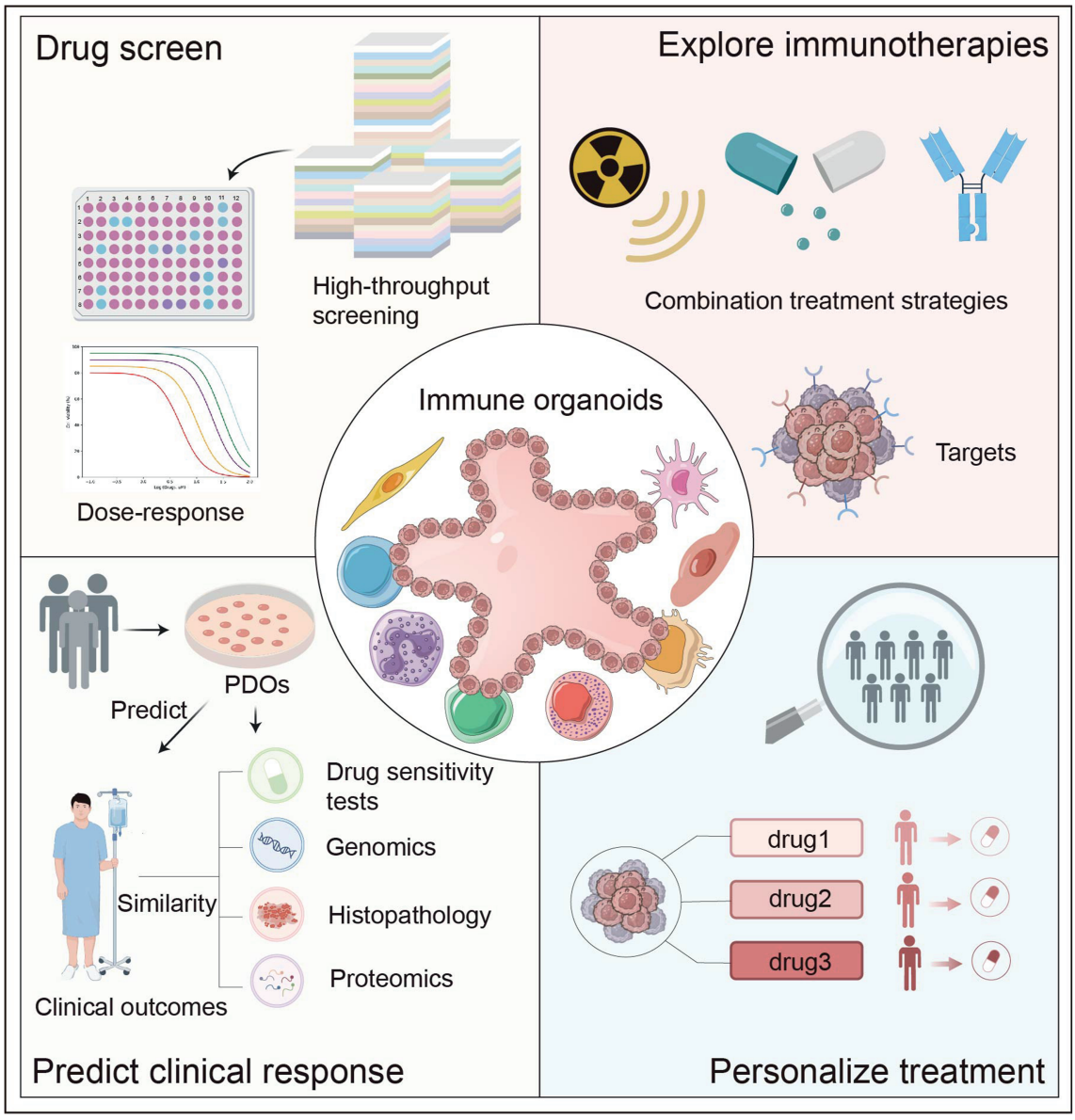

Figure 5 Integrated platform for the development and clinical translation of immunotherapy based on immune organoids. Drug screen: Immune organoids enable high-throughput drug screening through multi-well plate, allowing systematic evaluation of drug candidates using dose-response curves to assess therapeutic efficacy. Exploration of immunotherapies: development of combination treatment strategies or immunotherapeutic targets using immune organoid models. Predict clinical response: Patient-derived organoids (PDOs) serve as predictive models by demonstrating similar responses to clinical outcomes through integrated analysis of drug sensitivity testing, genomic profiling, histopathological examination, and proteomic characterization. Personalize treatment: Evaluation system that tests multiple treatment options using patient-specific organoids to determine optimal therapeutic strategies for individual patients.

metrics: 83.3% accuracy, 84.0% sensitivity, and 82.8% specificity, demonstrating strong concordance with clinical outcomes and highlighting the potential of organoid-guided clinical applications[120]. The NICHE trial further validated immunotherapy applications in CRC[121]. This study employed drug screening in PDO–immune cell co-cultures, using IFN-γ production by $CD8^+$ T cells as a marker to predict clinical responses to combination therapy with nivolumab and ipilimumab[121]. The findings revealed potential synergistic effects between BRAF/MAPK pathway inhibition and immune response, suggesting new therapeutic strategies for CRC immunotherapy[121]. Currently, numerous clinical studies are in recruitment phases[122]. Organoid platforms show considerable promise in providing personalized treatment recommendations, potentially revolutionizing clinical decision-making and improving patient outcomes[119-121].

However, several challenges remain in translating immune organoid technology to clinical practice[119-121]. The success rate of organoid culture varies significantly between different tissue sources, with insufficient cell numbers being a common limitation[120]. Additionally, the lack of standardized protocols for drug sensitivity testing and inconsistent drug regimens across patient groups make it difficult to define precise cutoff values for

determining treatment efficacy[119,120]. Furthermore, clinical validation studies are often limited by small cohort sizes and short follow-up periods, making it difficult to establish robust long-term correlations between organoid-based predictions and patient outcomes[121]. The time and resources required to establish and maintain immune organoid cultures also present practical challenges for routine clinical implementation.

7. Conclusion and perspectives

7.1. Current technical barriers of immune organoid

While tumor organoids represent powerful tools for cancer immunology research and show great potential for next-generation drug screening and immunotherapy applications, several significant challenges must be addressed[13,123-129]. A primary challenge is the low efficiency in generating organoids from tumor tissues[123]. The success rate of culturing organoids from patient tissues has been reported to be about 36.8% in different cancer types[123]. Multiple factors contribute to this limited success in organoid formation. To enhance the success rate of tumor organoid culture, it is essential to optimize the culture conditions by incorporating specific regulatory factors and utilizing conditional media that account for the heterogeneity of tumors[124]. Another limitation is the difficulty in reproducing the full complexity of tumor tissue within organoids[124]. Since organoids are typically derived from small tumor tissue samples, they may not completely capture the diverse spectrum of tumor gene mutations or temporal evolution observed *in vivo*[124]. To address this limitation, tumor samples from multiple regions of primary and secondary sites were collected to reflect intratumoral heterogeneity[124]. Additionally, the development of paired pre- and post-treatment PDOs has provided valuable insights into drug resistance mechanisms[13]. Furthermore, tumor-derived organoids currently lack exposure to critical *in vivo* external stresses, such as hypoxia and biomechanical stimuli[125]. These environmental factors play crucial roles. Hypoxic conditions can drive cancer progression and influence therapeutic responses, while biophysical forces can alter cellular behavior and promote tumor metastasis[126,127]. However, replicating these external features in organoid systems remains technically challenging[125]. Addressing these limitations requires a multidisciplinary approach, potentially drawing on insights and innovations from the field of bioengineering[128,129].

7.2. Future therapeutic perspectives of immune organoid

The fast advancement of organoid technology is ushering in an exciting era of biomedical research, providing breakthrough opportunities and tools for drug screening, exploring novel therapeutic strategies, predicting patient clinical response, and personalizing treatment[130-135] (Fig. 5). Recent studies have demonstrated the technology's remarkable potential[130,131]. For instance, patient-derived PDAC organoids successfully predicted patient responses to lysosomal adenovirus therapy and its synergistic effects with chemotherapy[130]. The organoids showed response patterns identical to those observed in xenografts and *in situ* tumor models, validating their value as preclinical models for lysosomal virus research[130]. The development of advanced organoid models to recapitulate TLS remains challenging due to their complex cellular composition and organization, but recent progress with high endothelial venule organoids demonstrates the feasibility of engineering key components of lymphoid tissues[131]. As research tools, organoids provide unique platforms for omics research and fundamental scientific investigations. While genomics and whole-genome screening of tumor organoids have been well established[132], emerging applications of single-cell sequencing and proteomics to tumor organoids are particularly promising for immunotherapy research[133]. These advanced technologies enable detailed analysis of cellular diversity and protein interaction networks within the TME, an approach that has already proven valuable in predicting treatment responses and identifying potential therapeutic targets[134,135]. Through the development of patient-specific tumor organoids, researchers can now better predict individual responses to various treatment regimens, significantly advancing the field of precision medicine.

Additionally, the applications of immune organoid platforms extend beyond tumor research, showing promise in investigating autoimmune disorders[136], infectious diseases[137], and regenerative medicine[138,139]. Importantly, studying these non-malignant conditions with integrated immune-competent organoid systems may offer unique perspectives on the early stages of tumorigenesis[13,136-139]. The combination of interdisciplinary applications of these organoid platforms and other technologies may reveal new mechanisms shared between cancer and other diseases, thereby expanding our understanding of disease pathogenesis[140,141].

In conclusion, 3D organoid cultures derived from both healthy and tumor biopsy tissues are transforming *in vitro* cancer research and clinical trials[11-13]. To recapitulate the intricacy of TME, the ideal approach is to incorporate an array of immune cells (T cells, neutrophils, macrophages, NK cells, DCs), CAFs, and ECs in a co-culture model[11-13]. This co-culture model can enhance our insights into TME cellular interactions and, crucially, its translational application to the field of immunotherapy[13,26]. In this field, tumor organoids can be used to construct personalized models based on drugs, cells, and cancer vaccines[75-79]. Not only accelerating the discovery of new therapeutic targets, but the organoids may also accurately reflect or predict the clinical responses, providing ideas for the development of effective cancer treatment strategies[119-121]. Looking ahead, current and future organoid technologies will continue to advance both fundamental immuno-oncology research and clinical applications, ultimately helping to realize the full potential of personalized cancer immunotherapy.

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Author contributions

Xiaohe Wang: Writing - review & editing, Writing - original draft, Visualization. Wuyin Wang: Writing - review & editing, Project administration. Zhijun Sun: Writing - review & editing, Project administration.

Conflicts of interest

The authors declare that they have no competing interests.

References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2024;**74**:229−63.
2. Topalian SL, Forde PM, Emens LA, Yarchoan M, Smith KN, Pardoll DM. Neoadjuvant immune checkpoint blockade: a window of opportunity to advance cancer immunotherapy. *Cancer Cell* 2023;**41**: 1551−66.
3. Kubli SP, Berger T, Araujo DV, Siu LL, Mak TW. Beyond immune checkpoint blockade: emerging immunological strategies. *Nat Rev Drug Discov* 2021;**20**:899−919.
4. Ruella M, Korell F, Porazzi P, Maus MV. Mechanisms of resistance to chimeric antigen receptor-T cells in haematological malignancies. *Nat Rev Drug Discov* 2023;**22**:976−95.
5. Vitale I, Shema E, Loi S, Galluzzi L. Intratumoral heterogeneity in cancer progression and response to immunotherapy. *Nat Med* 2021; **27**:212−24.
6. de Visser KE, Joyce JA. The evolving tumor microenvironment: from cancer initiation to metastatic outgrowth. *Cancer Cell* 2023;**41**: 374−403.
7. van Weverwijk A, de Visser KE. Mechanisms driving the immunoregulatory function of cancer cells. *Nat Rev Cancer* 2023;**23**: 193−215.
8. Ben-David U, Siranosian B, Ha G, Tang H, Oren Y, Hinohara K, et al. Genetic and transcriptional evolution alters cancer cell line drug response. *Nature* 2018;**560**:325−30.
9. Odunsi A, McGray AJR, Miliotto A, Zhang Y, Wang J, Abiola A, et al. Fidelity of human ovarian cancer patient-derived xenografts in a partially humanized mouse model for preclinical testing of immunotherapies. *J Immunother Cancer* 2020;**8**:e001237.
10. Liu Y, Wu W, Cai C, Zhang H, Shen H, Han Y. Patient-derived xenograft models in cancer therapy: technologies and applications. *Signal Transduct Target Ther* 2023;**8**:160.
11. LeSavage BL, Suhar RA, Broguiere N, Lutolf MP, Heilshorn SC. Next-generation cancer organoids. *Nat Mater* 2022;**21**:143−59.
12. Yan HHN, Chan AS, Lai FP, Leung SY. Organoid cultures for cancer modeling. *Cell Stem Cell* 2023;**30**:917−37.
13. Polak R, Zhang ET, Kuo CJ. Cancer organoids 2.0: modelling the complexity of the tumour immune microenvironment. *Nat Rev Cancer* 2024;**24**:523−39.
14. Querol L, Lewis RA, Hartung HP, Van Doorn PA, Wallstroem E, Luo X, et al. An innovative phase 2 proof-of-concept trial design to evaluate sar445088, a monoclonal antibody targeting complement c1s in chronic inflammatory demyelinating polyneuropathy. *J Peripher Nerv Syst* 2023;**28**:276−85.
15. Kim M, Mun H, Sung CO, Cho EJ, Jeon HJ, Chun SM, et al. Patient-derived lung cancer organoids as *in vitro* cancer models for therapeutic screening. *Nat Commun* 2019;**10**:3991.
16. Chen P, Zhang X, Ding R, Yang L, Lyu X, Zeng J, et al. Patient-derived organoids can guide personalized-therapies for patients with advanced breast cancer. *Adv Sci* 2021;**8**:e2101176.
17. Teijeira A, Migueliz I, Garasa S, Karanikas V, Luri C, Cirella A, et al. Three-dimensional colon cancer organoids model the response to CEA-CD3 T-cell engagers. *Theranostics* 2022;**12**:1373−87.
18. Zhang W, Wang S, Zhang H, Meng Y, Jiao S, An L, et al. Modeling human gastric cancers in immunocompetent mice. *Cancer Biol Med* 2024;**21**:553−70.
19. Minoli M, Cantore T, Hanhart D, Kiener M, Fedrizzi T, La Manna F, et al. Bladder cancer organoids as a functional system to model different disease stages and therapy response. *Nat Commun* 2023;**14**: 2214.
20. Surendran V, Rutledge D, Colmon R, Chandrasekaran A. A novel tumor-immune microenvironment (TIME)-on-Chip mimics three dimensional neutrophil-tumor dynamics and neutrophil extracellular traps (NETs)-mediated collective tumor invasion. *Biofabrication* 2021;**13**:035029.
21. Yang H, Cheng J, Zhuang H, Xu H, Wang Y, Zhang T, et al. Pharmacogenomic profiling of intra-tumor heterogeneity using a large organoid biobank of liver cancer. *Cancer Cell* 2024;**42**:535.
22. Li H, Liu H, Chen K. Living biobank-based cancer organoids: prospects and challenges in cancer research. *Cancer Biol Med* 2022; **19**:965−82.
23. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, et al. Single lgr5 stem cells build crypt-villus structures *in vitro* without a mesenchymal niche. *Nature* 2009;**459**:262−5.
24. Magré L, Verstegen MMA, Buschow S, van der Laan LJW, Peppelenbosch M, Desai J. Emerging organoid−immune co-culture models for cancer research: from oncoimmunology to personalized immunotherapies. *J Immunother Cancer* 2023;**11**:e006290.
25. Yuki K, Cheng N, Nakano M, Kuo CJ. Organoid models of tumor immunology. *Trends Immunol* 2020;**41**:652−64.
26. Dao V, Yuki K, Lo YH, Nakano M, Kuo CJ. Immune organoids: from tumor modeling to precision oncology. *Trends Cancer* 2022;**8**: 870−80.
27. Neal JT, Li X, Zhu J, Giangarra V, Grzeskowiak CL, Ju J, et al. Organoid modeling of the tumor immune microenvironment. *Cell* 2018;**175**:1972.
28. Bar-Ephraim YE, Kretzschmar K, Clevers H. Organoids in immunological research. *Nat Rev Immunol* 2020;**20**:279−93.
29. Zhou Z, Pang Y, Ji J, He J, Liu T, Ouyang L, et al. Harnessing 3D *in vitro* systems to model immune responses to solid tumours: a step towards improving and creating personalized immunotherapies. *Nat Rev Immunol* 2024;**24**:18−32.
30. Lo YH, Kolahi KS, Du Y, Chang CY, Krokhotin A, Nair A, et al. A CRISPR/Cas9-engineered *ARID1A*-deficient human gastric cancer organoid model reveals essential and nonessential modes of oncogenic transformation. *Cancer Discov* 2021;**11**:1562−81.
31. Mizutani T, Boretto M, Lim S, Drost J, González DM, Oka R, et al. Recapitulating the adenoma-carcinoma sequence by selection of four spontaneous oncogenic mutations in mismatch-repair-deficient human colon organoids. *Nat Cancer* 2024;**5**:1852−67.
32. Zhao H, Jiang E, Shang Z. 3D co-culture of cancer-associated fibroblast with oral cancer organoids. *J Dent Res* 2021;**100**:201−8.
33. Santos AJM, van Unen V, Lin Z, Chirieleison SM, Ha N, Batish A, et al. A human autoimmune organoid model reveals IL-7 function in coeliac disease. *Nature* 2024;**632**:401−10.
34. Quintard C, Tubbs E, Jonsson G, Jiao J, Wang J, Werschler N, et al. A microfluidic platform integrating functional vascularized organoids-on-chip. *Nat Commun* 2024;**15**:1452.
35. Moeller TD, Shah SB, Lai K, Lopez-Barbosa N, Desai P, Wang W, et al. Profiling germinal center-like B cell responses to conjugate vaccines using synthetic immune organoids. *ACS Cent Sci* 2023;**9**:787−804.
36. Berger Fridman I, Kostas J, Gregus M, Ray S, Sullivan MR, Ivanov AR, et al. High-throughput microfluidic 3D biomimetic model enabling quantitative description of the human breast tumor microenvironment. *Acta Biomater* 2021;**132**:473−88.
37. Hanahan D, Michielin O, Pittet MJ. Convergent inducers and effectors of T cell paralysis in the tumour microenvironment. *Nat Rev Cancer* 2024;**25**:41−58.
38. Wee Y, Wang J, Wilson EC, Rich CP, Rogers A, Tong Z, et al. Tumour-intrinsic endomembrane trafficking by ARF6 shapes an immunosuppressive microenvironment that drives melanomagenesis and response to checkpoint blockade therapy. *Nat Commun* 2024;**15**: 6613.
39. Cattaneo CM, Dijkstra KK, Fanchi LF, Kelderman S, Kaing S, van Rooij N, et al. Tumor organoid-T-cell coculture systems. *Nat Protoc* 2020;**15**:15−39.
40. Zhou G, Lieshout R, van Tienderen GS, de Ruiter V, van Royen ME, Boor PPC, et al. Modelling immune cytotoxicity for

cholangiocarcinoma with tumour-derived organoids and effector T cells. *Br J Cancer* 2022;**127**:649−60.
41. Tu H, Wu Z, Xia Y, Chen H, Hu H, Ding Z, et al. Profiling of immune-cancer interactions at the single-cell level using a microfluidic well array. *Analyst* 2020;**145**:4138−47.
42. Zhou Z, Van der Jeught K, Fang Y, Yu T, Li Y, Ao Z, et al. An organoid-based screen for epigenetic inhibitors that stimulate antigen presentation and potentiate T-cell-mediated cytotoxicity. *Nat Biomed Eng* 2021;**5**:1320−35.
43. Zhong Z, Quiñones-Pérez M, Dai Z, Juarez VM, Bhatia E, Carlson CR, et al. Human immune organoids to decode B cell response in healthy donors and patients with lymphoma. *Nat Mater* 2024;**24**:297−311.
44. Küçükköse E, Heesters BA, Villaudy J, Verheem A, Cercel M, van Hal S, et al. Modeling resistance of colorectal peritoneal metastases to immune checkpoint blockade in humanized mice. *J Immunother Cancer* 2022;**10**:e005345.
45. Wang X, Qu Y, Xu Q, Jiang Z, Wang H, Lin B, et al. NQO1 triggers neutrophil recruitment and net formation to drive lung metastasis of invasive breast cancer. *Cancer Res* 2024;**84**:3538−55.
46. Särchen V, Shanmugalingam S, Kehr S, Reindl LM, Greze V, Wiedemann S, et al. Pediatric multicellular tumor spheroid models illustrate a therapeutic potential by combining BH3 mimetics with natural killer (NK) cell-based immunotherapy. *Cell Death Discov* 2022;**8**:11.
47. Courau T, Bonnereau J, Chicoteau J, Bottois H, Remark R, Assante Miranda L, et al. Cocultures of human colorectal tumor spheroids with immune cells reveal the therapeutic potential of MICA/B and NKG2A targeting for cancer treatment. *J Immunother Cancer* 2019;**7**:74.
48. Wang F, Zhang G, Xu T, Ma J, Wang J, Liu S, et al. High and selective cytotoxicity of *ex vivo* expanded allogeneic human natural killer cells from peripheral blood against bladder cancer: implications for natural killer cell instillation after transurethral resection of bladder tumor. *J Exp Clin Cancer Res* 2024;**43**:24.
49. Zhang B, Ohuchida K, Tsutsumi C, Shimada Y, Mochida Y, Oyama K, et al. Dynamic glycolytic reprogramming effects on dendritic cells in pancreatic ductal adenocarcinoma. *J Exp Clin Cancer Res* 2024;**43**:271.
50. Tabe S, Takeuchi K, Aoshima K, Okumura A, Yamamoto Y, Yanagisawa K, et al. A pancreatic cancer organoid incorporating macrophages reveals the correlation between the diversity of tumor-associated macrophages and cancer cell survival. *Biomaterials* 2024;**314**:122838.
51. Xu NY, Li J, Wang ML, Chen XY, Tang R, Liu XQ. Fabrication of a coculture organoid model in the biomimetic matrix of alginate to investigate breast cancer progression in a tams-leading immune microenvironment. *ACS Appl Mater Interfaces* 2024;**16**:11275−88.
52. Li YR, Yu Y, Kramer A, Hon R, Wilson M, Brown J, et al. An *ex vivo* 3D tumor microenvironment-mimicry culture to study TAM modulation of cancer immunotherapy. *Cells* 2022;**11**:1583.
53. Öhlund D, Handly-Santana A, Biffi G, Elyada E, Almeida AS, Ponz-Sarvise M, et al. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. *J Exp Med* 2017;**214**:579−96.
54. Atanasova VS, de Jesus Cardona C, Hejret V, Tiefenbacher A, Mair T, Tran L, et al. Mimicking tumor cell heterogeneity of colorectal cancer in a patient-derived organoid-fibroblast model. *Cell Mol Gastroenterol Hepatol* 2023;**15**:1391−419.
55. Lim JTC, Kwang LG, Ho NCW, Toh CCM, Too NSH, Hooi L, et al. Hepatocellular carcinoma organoid co-cultures mimic angiocrine crosstalk to generate inflammatory tumor microenvironment. *Biomaterials* 2022;**284**:121527.
56. Choi JI, Jang SI, Hong J, Kim CH, Kwon SS, Park JS, et al. Cancer-initiating cells in human pancreatic cancer organoids are maintained by interactions with endothelial cells. *Cancer Lett* 2021;**498**:42−53.
57. van der Leun AM, Thommen DS, Schumacher TN. $CD8^+$ T cell states in human cancer: insights from single-cell analysis. *Nat Rev Cancer* 2020;**20**:218−32.
58. Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, et al. B cells and tertiary lymphoid structures promote immunotherapy response. *Nature* 2020;**577**:549−55.
59. Cabrita R, Lauss M, Sanna A, Donia M, Skaarup Larsen M, Mitra S, et al. Tertiary lymphoid structures improve immunotherapy and survival in melanoma. *Nature* 2020;**577**:561−5.
60. Petitprez F, de Reyniès A, Keung EZ, Chen TW, Sun CM, Calderaro J, et al. B cells are associated with survival and immunotherapy response in sarcoma. *Nature* 2020;**577**:556−60.
61. Sui Q, Zhang X, Chen C, Tang J, Yu J, Li W, et al. Inflammation promotes resistance to immune checkpoint inhibitors in high microsatellite instability colorectal cancer. *Nat Commun* 2022;**13**:7316.
62. Yang L, Liu Q, Zhang X, Liu X, Zhou B, Chen J, et al. DNA of neutrophil extracellular traps promotes cancer metastasis *via* CCDC25. *Nature* 2020;**583**:133−8.
63. Yu X, Li C, Wang Z, Xu Y, Shao S, Shao F, et al. Neutrophils in cancer: dual roles through intercellular interactions. *Oncogene* 2024;**43**:1163−77.
64. Hedrick CC, Malanchi I. Neutrophils in cancer: heterogeneous and multifaceted. *Nat Rev Immunol* 2022;**22**:173−87.
65. Choi D, Gonzalez-Suarez AM, Dumbrava MG, Medlyn M, de Hoyos-Vega JM, Cichocki F, et al. Microfluidic organoid cultures derived from pancreatic cancer biopsies for personalized testing of chemotherapy and immunotherapy. *Adv Sci* 2024;**11**:e2303088.
66. Bald T, Krummel MF, Smyth MJ, Barry KC. The NK cell-cancer cycle: advances and new challenges in NK cell-based immunotherapies. *Nat Immunol* 2020;**21**:835−47.
67. Ding L, Chakrabarti J, Sheriff S, Li Q, Thi Hong HN, Sontz RA, et al. Toll-like receptor 9 pathway mediates $Schlafen^+$-MDSC polarization during helicobacter-induced gastric metaplasias. *Gastroenterology* 2022;**163**:411−25.
68. Wculek SK, Cueto FJ, Mujal AM, Melero I, Krummel MF, Sancho D. Dendritic cells in cancer immunology and immunotherapy. *Nat Rev Immunol* 2020;**20**:7−24.
69. Yang Q, Guo N, Zhou Y, Chen J, Wei Q, Han M. The role of tumor-associated macrophages (TAMs) in tumor progression and relevant advance in targeted therapy. *Acta Pharm Sin B* 2020;**10**:2156−70.
70. Han S, Wang W, Wang S, Yang T, Zhang G, Wang D, et al. Tumor microenvironment remodeling and tumor therapy based on M2-like tumor associated macrophage-targeting nano-complexes. *Theranostics* 2021;**11**:2892−916.
71. Luo X, Fong ELS, Zhu C, Lin QXX, Xiong M, Li A, et al. Hydrogel-based colorectal cancer organoid co-culture models. *Acta Biomater* 2021;**132**:461−72.
72. Yang D, Guo P, He T, Powell CA. Role of endothelial cells in tumor microenvironment. *Clin Transl Med* 2021;**11**:e450.
73. Khan KA, Kerbel RS. Improving immunotherapy outcomes with anti-angiogenic treatments and *vice versa*. *Nat Rev Clin Oncol* 2018;**15**:310−24.
74. Kuo HY, Khan KA, Kerbel RS. Antiangiogenic−immune-checkpoint inhibitor combinations: lessons from phase III clinical trials. *Nat Rev Clin Oncol* 2024;**21**:468−82.
75. Benelli R, Costa D, Salvini L, Tardito S, Tosetti F, Villa F, et al. Targeting of colorectal cancer organoids with zoledronic acid conjugated to the anti-EGFR antibody cetuximab. *J Immunother Cancer* 2022;**10**:e005660.
76. Zhao Z, Zhang S, Jiang N, Zhu W, Song D, Liu S, et al. Patient-derived immunocompetent tumor organoids: a platform for chemotherapy evaluation in the context of T-cell recognition. *Angew Chem Int Ed Engl* 2024;**63**:e202317613.
77. Jenkins RW, Aref AR, Lizotte PH, Ivanova E, Stinson S, Zhou CW, et al. *Ex vivo* profiling of PD-1 blockade using organotypic tumor spheroids. *Cancer Discov* 2018;**8**:196−215.
78. Knelson EH, Ivanova EV, Tarannum M, Campisi M, Lizotte PH, Booker MA, et al. Activation of tumor-cell sting primes NK-cell therapy. *Cancer Immunol Res* 2022;**10**:947−61.

79. Vornholz L, Isay SE, Kurgyis Z, Strobl DC, Loll P, Mosa MH, et al. Synthetic enforcement of STING signaling in cancer cells appropriates the immune microenvironment for checkpoint inhibitor therapy. *Sci Adv* 2023;**9**:eadd8564.
80. van der Zanden SY, Luimstra JJ, Neefjes J, Borst J, Ovaa H. Opportunities for small molecules in cancer immunotherapy. *Trends Immunol* 2020;**41**:493−511.
81. Diamond MS, Kinder M, Matsushita H, Mashayekhi M, Dunn GP, Archambault JM, et al. Type I interferon is selectively required by dendritic cells for immune rejection of tumors. *J Exp Med* 2011;**208**: 1989−2003.
82. Boos SL, Loevenich LP, Vosberg S, Engleitner T, Öllinger R, Kumbrink J, et al. Disease modeling on tumor organoids implicates Aurka as a therapeutic target in liver metastatic colorectal cancer. *Cell Mol Gastroenterol Hepatol* 2022;**13**:517−40.
83. Harter MF, Recaldin T, Gerard R, Avignon B, Bollen Y, Esposito C, et al. Analysis of off-tumour toxicities of T-cell-engaging bispecific antibodies *via* donor-matched intestinal organoids and tumouroids. *Nat Biomed Eng* 2023;**8**:345−60.
84. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat Rev Immunol* 2020;**20**:651−68.
85. Majzner RG, Mackall CL. Clinical lessons learned from the first leg of the CAR T cell journey. *Nat Med* 2019;**25**:1341−55.
86. Maalej KM, Merhi M, Inchakalody VP, Mestiri S, Alam M, Maccalli C, et al. Car-cell therapy in the era of solid tumor treatment: current challenges and emerging therapeutic advances. *Mol Cancer* 2023;**22**:20.
87. Jacob F, Ming GL, Song H. Generation and biobanking of patient-derived glioblastoma organoids and their application in CAR T cell testing. *Nat Protoc* 2020;**15**:4000−33.
88. Logun M, Wang X, Sun Y, Bagley SJ, Li N, Desai A, et al. Patient-derived glioblastoma organoids as real-time avatars for assessing responses to clinical CAR-T cell therapy. *Cell Stem Cell* 2024;**32**: 181−90.
89. Yu L, Li Z, Mei H, Li W, Chen D, Liu L, et al. Patient-derived organoids of bladder cancer recapitulate antigen expression profiles and serve as a personal evaluation model for CAR-T cells *in vitro*. *Clin Transl Immunology* 2021;**10**:e1248.
90. Jacob F, Salinas RD, Zhang DY, Nguyen PTT, Schnoll JG, Wong SZH, et al. A patient-derived glioblastoma organoid model and biobank recapitulates inter- and intra-tumoral heterogeneity. *Cell* 2020;**180**:188−204.
91. Schnalzger TE, de Groot MH, Zhang C, Mosa MH, Michels BE, Röder J, et al. 3D model for CAR-mediated cytotoxicity using patient-derived colorectal cancer organoids. *EMBO J* 2019;**38**:e100928.
92. Klobuch S, Seijkens TTP, Schumacher TN, Haanen J. Tumour-infiltrating lymphocyte therapy for patients with advanced-stage melanoma. *Nat Rev Clin Oncol* 2024;**21**:173−84.
93. Flores-Torres S, Dimitriou NM, Pardo LA, Kort-Mascort J, Pal S, Peza-Chavez O, et al. Bioprinted multicomponent hydrogel co-culture tumor−immune model for assessing and simulating tumor-infiltrated lymphocyte migration and functional activation. *ACS Appl Mater Interfaces* 2023;**15**:33250−62.
94. Comoli P, Chabannon C, Koehl U, Lanza F, Urbano-Ispizua A, Hudecek M, et al. Development of adaptive immune effector therapies in solid tumors. *Ann Oncol* 2019;**30**:1740−50.
95. Balachandran VP, Beatty GL, Dougan SK. Broadening the impact of immunotherapy to pancreatic cancer: challenges and opportunities. *Gastroenterology* 2019;**156**:2056−72.
96. Beausang JF, Wheeler AJ, Chan NH, Hanft VR, Dirbas FM, Jeffrey SS, et al. T cell receptor sequencing of early-stage breast cancer tumors identifies altered clonal structure of the T cell repertoire. *Proc Natl Acad Sci U S A* 2017;**114**:E10409−17.
97. Meng Q, Xie S, Gray GK, Dezfulian MH, Li W, Huang L, et al. Empirical identification and validation of tumor-targeting T cell receptors from circulation using autologous pancreatic tumor organoids. *J Immunother Cancer* 2021;**9**:e003213.
98. Liu C, Shi Q, Huang X, Koo S, Kong N, Tao W. mRNA-based cancer therapeutics. *Nat Rev Cancer* 2023;**23**:526−43.
99. Yaremenko AV, Khan MM, Zhen X, Tang Y, Tao W. Clinical advances of mRNA vaccines for cancer immunotherapy. *Med* 2025;**6**:100562.
100. Saxena M, van der Burg SH, Melief CJM, Bhardwaj N. Therapeutic cancer vaccines. *Nat Rev Cancer* 2021;**21**:360−78.
101. Jameson SC, Masopust D. What is the predictive value of animal models for vaccine efficacy in humans? Reevaluating the potential of mouse models for the human immune system. *Cold Spring Harbor Perspect Biol* 2018;**10**:a029132.
102. Wagar LE, Salahudeen A, Constantz CM, Wendel BS, Lyons MM, Mallajosyula V, et al. Modeling human adaptive immune responses with tonsil organoids. *Nat Med* 2021;**27**:125−35.
103. Hu B, Wang R, Wu D, Long R, Fan J, Hu Z, et al. A promising new model: establishment of patient-derived organoid models covering HPV-related cervical pre-cancerous lesions and their cancers. *Adv Sci* 2024;**11**:e2302340.
104. Huang L, Rong Y, Tang X, Yi K, Qi P, Hou J, et al. Engineered exosomes as an *in situ* DC-primed vaccine to boost antitumor immunity in breast cancer. *Mol Cancer* 2022;**21**:45.
105. Demmers LC, Kretzschmar K, Van Hoeck A, Bar-Epraïm YE, van den Toorn HWP, Koomen M, et al. Single-cell derived tumor organoids display diversity in HLA class I peptide presentation. *Nat Commun* 2020;**11**:5338.
106. Permyakov EA. A-lactalbumin, amazing calcium-binding protein. *Biomolecules* 2020;**10**:1210.
107. Ling A, Huang RS. Computationally predicting clinical drug combination efficacy with cancer cell line screens and independent drug action. *Nat Commun* 2020;**11**:5848.
108. Chen S, Li Z, Zhang S, Zhou Y, Xiao X, Cui P, et al. Emerging biotechnology applications in natural product and synthetic pharmaceutical analyses. *Acta Pharm Sin B* 2022;**12**:4075−97.
109. Wang Y, Gao Y, Pan Y, Zhou D, Liu Y, Yin Y, et al. Emerging trends in organ-on-a-chip systems for drug screening. *Acta Pharm Sin B* 2023;**13**:2483−509.
110. Ding S, Hsu C, Wang Z, Natesh NR, Millen R, Negrete M, et al. Patient-derived micro-organospheres enable clinical precision oncology. *Cell Stem Cell* 2022;**29**:905.
111. Duan X, Zhang T, Feng L, de Silva N, Greenspun B, Wang X, et al. A pancreatic cancer organoid platform identifies an inhibitor specific to mutant KRAS. *Cell Stem Cell* 2024;**31**:71−88.e78.
112. Mao Y, Wang W, Yang J, Zhou X, Lu Y, Gao J, et al. Drug repurposing screening and mechanism analysis based on human colorectal cancer organoids. *Protein Cell* 2024;**15**:285−304.
113. Sun L, Wan AH, Yan S, Liu R, Li J, Zhou Z, et al. A multidimensional platform of patient-derived tumors identifies drug susceptibilities for clinical lenvatinib resistance. *Acta Pharm Sin B* 2024;**14**:223−40.
114. Hendriks D, Brouwers JF, Hamer K, Geurts MH, Luciana L, Massalini S, et al. Engineered human hepatocyte organoids enable CRISPR-based target discovery and drug screening for steatosis. *Nat Biotechnol* 2023;**41**:1567−81.
115. Toshimitsu K, Takano A, Fujii M, Togasaki K, Matano M, Takahashi S, et al. Organoid screening reveals epigenetic vulnerabilities in human colorectal cancer. *Nat Chem Biol* 2022;**18**:605−14.
116. Xu J, Liao K, Yang X, Wu C, Wu W. Using single-cell sequencing technology to detect circulating tumor cells in solid tumors. *Mol Cancer* 2021;**20**:104.
117. Jia Q, Chu H, Jin Z, Long H, Zhu B. High-throughput single-cell sequencing in cancer research. *Signal Transduct Target Ther* 2022;**7**:145.
118. Yan HHN, Siu HC, Law S, Ho SL, Yue SSK, Tsui WY, et al. A comprehensive human gastric cancer organoid biobank captures tumor subtype heterogeneity and enables therapeutic screening. *Cell Stem Cell* 2018;**23**:882−97.
119. Tian J, Chen JH, Chao SX, Pelka K, Giannakis M, Hess J, et al. Combined PD-1, BRAF and MEK inhibition in $BRAF^{V600E}$ colorectal cancer: a phase 2 trial. *Nat Med* 2023;**29**:458−66.
120. Wang HM, Zhang CY, Peng KC, Chen ZX, Su JW, Li YF, et al. Using patient-derived organoids to predict locally advanced or

metastatic lung cancer tumor response: a real-world study. *Cell Rep Med* 2023;**4**:100911.
121. Chalabi M, Fanchi LF, Dijkstra KK, Van den Berg JG, Aalbers AG, Sikorska K, et al. Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. *Nat Med* 2020;**26**:566−76.
122. Weng G, Tao J, Liu Y, Qiu J, Su D, Wang R, et al. Organoid: bridging the gap between basic research and clinical practice. *Cancer Lett* 2023;**572**:216353.
123. Pauli C, Hopkins BD, Prandi D, Shaw R, Fedrizzi T, Sboner A, et al. Personalized *in vitro* and *in vivo* cancer models to guide precision medicine. *Cancer Discov* 2017;**7**:462−77.
124. Xu H, Jiao D, Liu A, Wu K. Tumor organoids: applications in cancer modeling and potentials in precision medicine. *J Hematol Oncol* 2022;**15**:58.
125. Zhang J, Tavakoli H, Ma L, Li X, Han L, Li X. Immunotherapy discovery on tumor organoid-on-a-chip platforms that recapitulate the tumor microenvironment. *Adv Drug Deliv Rev* 2022;**187**:114365.
126. Petrova V, Annicchiarico-Petruzzelli M, Melino G, Amelio I. The hypoxic tumour microenvironment. *Oncogenesis* 2018;**7**:10.
127. Ren X, Ghassemi P, Babahosseini H, Strobl JS, Agah M. Single-cell mechanical characteristics analyzed by multiconstriction microfluidic channels. *ACS Sens* 2017;**2**:290−9.
128. Hofer M, Lutolf MP. Engineering organoids. *Nat Rev Mater* 2021;**6**:402−20.
129. Brassard JA, Nikolaev M, Hübscher T, Hofer M, Lutolf MP. Recapitulating macro-scale tissue self-organization through organoid bioprinting. *Nat Mater* 2021;**20**:22−9.
130. Raimondi G, Mato-Berciano A, Pascual-Sabater S, Rovira-Rigau M, Cuatrecasas M, Fondevila C, et al. Patient-derived pancreatic tumour organoids identify therapeutic responses to oncolytic adenoviruses. *EBioMedicine* 2020;**56**:102786.
131. Wang X, Li X, Zhao J, Li Y, Shin SR, Ligresti G, et al. Rapid generation of hPSC-derived high endothelial venule organoids with *in vivo* ectopic lymphoid tissue capabilities. *Adv Mater* 2024;**36**:e2308760.
132. Michels BE, Mosa MH, Streibl BI, Zhan T, Menche C, Abou-El-Ardat K, et al. Pooled *in vitro* and *in vivo* CRISPR-Cas9 screening identifies tumor suppressors in human colon organoids. *Cell Stem Cell* 2020;**26**:782−92.
133. Landon-Brace N, Li NT, McGuigan AP. Exploring new dimensions of tumor heterogeneity: the application of single cell analysis to organoid-based 3D *in vitro* models. *Adv Healthc Mater* 2023;**12**:e2300903.
134. Ji S, Feng L, Fu Z, Wu G, Wu Y, Lin Y, et al. Pharmaco-proteogenomic characterization of liver cancer organoids for precision oncology. *Sci Transl Med* 2023;**15**:eadg3358.
135. Pellegrini L, Bonfio C, Chadwick J, Begum F, Skehel M, Lancaster MA. Human CNS barrier-forming organoids with cerebrospinal fluid production. *Science* 2020;**369**:eaaz5626.
136. Santos AJM, van Unen V, Lin Z, Chirieleison SM, Ha N, Batish A, et al. A human autoimmune organoid model reveals IL-7 function in coeliac disease. *Nature* 2024;**632**:401−10.
137. Elbadawi M, Efferth T. Organoids of human airways to study infectivity and cytopathy of SARS-CoV-2. *Lancet Respir Med* 2020;**8**:e55−6.
138. Sun Z, Chen Z, Yin M, Wu X, Guo B, Cheng X, et al. Harnessing developmental dynamics of spinal cord extracellular matrix improves regenerative potential of spinal cord organoids. *Cell Stem Cell* 2024;**31**:772.
139. Liu H, Su J. Organoid and organoid extracellular vesicles for osteoporotic fractures therapy: current status and future perspectives. *IMed* 2023;**1**:e20230011.
140. Zhang P, Xiao Y, Sun X, Lin X, Koo S, Yaremenko AV, et al. Cancer nanomedicine toward clinical translation: obstacles, opportunities, and future prospects. *Med* 2023;**4**:147−67.
141. Hsu JC, Tang Z, Eremina OE, Sofias AM, Lammers T, Lovell JF, et al. Nanomaterial-based contrast agents. *Nat Rev Methods Primers* 2023;**3**:30.