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REVIEW

Organoid models in oncology: advancing precision cancer therapy and vaccine development

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ABSTRACT Organoids are three-dimensional stem cell-derived models that offer a more physiologically relevant representation of tumor biology compared to traditional two-dimensional cell cultures or animal models. Organoids preserve the complex tissue architecture and cellular diversity of human cancers, enabling more accurate predictions of tumor growth, metastasis, and drug responses. Integration with microfluidic platforms, such as organ-on-a-chip systems, further enhances the ability to model tumor-environment interactions in real-time. Organoids facilitate in-depth exploration of tumor heterogeneity, molecular mechanisms, and the development of personalized treatment strategies when coupled with multi-omics technologies. Organoids provide a platform for investigating tumor-immune cell interactions, which aid in the design and testing of immune-based therapies and vaccines. Taken together, these features position organoids as a transformative tool in advancing cancer research and precision medicine.

KEYWORDS Organoids; oncology; precision therapy; vaccine

Introduction

Organoids are tissue analogs with a defined spatial structure that are generated through the three-dimensional (3D) culture of adult or pluripotent stem cells *in vitro*. Organoids can be derived from embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), or tumor cells, and are cultivated in specialized 3D culture systems. While organoids are not fully representative of human organs, organoids effectively mimic the structure and function of real organs and tissues *in vivo*. Additionally, organoids can be stably sub-cultured over extended periods[1]. Organoids display more physiologically relevant cellular compositions and behaviors compared to two-dimensional (2D) cultures, which offers considerable potential for applications in biomedicine, regenerative medicine and tissue engineering, precision medicine, and developmental biology.

Organoids have proven to be highly valuable in cancer modeling and precision medicine. Researchers have used organoid platforms to conduct comprehensive studies on molecular pathways, tumor cell heterogeneity, and cell-to-cell interactions in gastric, colorectal, lung, and pancreatic cancer[2-5]. In addition, organoid models simulate the *in vivo* environment more accurately in pharmaceutical research, which facilitate the exploration of new therapies and mechanisms of drug resistance. Notably, organoid immune co-culture models have been developed to simulate the tumor immune microenvironment (TIME), which enables the assessment of individual responses to immunotherapy through co-culture of peripheral blood lymphocytes and tumor organoids, showing promising prospects for future development[6]. Advances in organoid technology have revitalized cancer research, significantly enhancing both mechanistic studies and drug screening.

Organoids are particularly valuable in predicting the efficacy of neoadjuvant and adjuvant therapies for tumors. Adjuvant

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therapy given after primary treatment targets residual cancer cells to reduce recurrence, while neoadjuvant therapy administered before primary treatment shrinks tumors using chemotherapy, radiotherapy, or targeted therapy[7]. Neoadjuvant treatment can improve surgical outcomes and make inoperable patients candidates for surgery[8]. Both approaches help lower recurrence rates and enhance survival[9]. Research and evaluation of patient-derived organoids (PDOs) can facilitate the selection of neoadjuvant and adjuvant chemotherapy agents, as well as explore the mechanisms underlying chemoradiotherapy resistance in tumors[10]. For example, PDOs can be used to evaluate responses to chemotherapy, which enables the personalization of adjuvant chemotherapy regimens[11]. By integrating organoid culture technology with genomic data, researchers can more accurately predict treatment responses and further optimize neoadjuvant and adjuvant strategies.

Research on immunotherapy has revolutionized cancer treatment protocols in recent years, yielding promising outcomes for many cancer patients. Tumor vaccines, as a form of immunotherapy, hold significant development potential due to high specificity and durability. Tumor vaccines can be delivered through viral, nanoparticle, and cellular carrier vectors. Designing effective vaccines has become a major challenge in immunotherapy.

Organoid technology has shown significant potential in tumor vaccine research, especially in antigen screening, vaccine design, and personalized immunotherapy. Organoids can create miniature tumor models with three-dimensional structures derived from patient tumor tissues, which preserves the genetic and phenotypic characteristics of the original tumor, while reconstructing the tumor microenvironment (TME). Organoids can be used to screen for tumor-specific antigens with high immunogenicity in tumor vaccine development and assess vaccine-induced immune responses through co-culture with patient immune cells. Personalized antigen prediction is essential for vaccine development but the clinical application is limited by the scarcity and poor quality of resected samples[12]. Patient-derived organoid models offer a promising solution to these challenges given the ability to expand efficiently while preserving the molecular characteristics of the original tumor. Organoids simulate individual patient responses to vaccines as an *in vitro* platform, which facilitate the optimization of vaccine design and enhance the ability to predict efficacy. This positions organoids as essential tools in the development of personalized tumor vaccines, offering new research directions and clinical applications for precision cancer immunotherapy[13].

An organoid is a realistic model that more accurately reflects *in vivo* conditions and provides more reliable complementary data than traditional 2D cell lines and animal models. In addition, organoids are more cost-effective and technically less demanding than patient-derived xenograft (PDX) models. In this review organoid advantages and advances in the development of precision cancer treatments and vaccines are summarized and future directions and challenges are discussed. **Figure 1** provides an overview of the functional characteristics, applications, and limitations of PDOs.

Comparative analysis: organoids and traditional models

Cell models and animal models are commonly used for cancer studies in the current medical research landscape. However, these models fail to adequately replicate the *in vivo* tumor environment, resulting in a significant gap between basic research and clinical application. As a result, identifying more ideal models for cancer research has become a key focus in oncology. Cell models, while advantageous for ease of manipulation and expansion, are limited by genomic alterations that arise during prolonged passaging and fail to replicate the original tumor structures. Animal models, although capable of providing a complete tumor environment, face challenges that include long cultivation cycles, low tumorigenic rates, high costs, and early clonal selection, which alters tumor heterogeneity. As a result, these models do not represent the optimal research model[14]. Organoid models, as the latest advance in tumor research, offer significant advantages over traditional models. Organoid models bridge the gap between cell lines and animal models by better preserving the structure and heterogeneity of the original tumor. As a result, organoid models demonstrate greater physiologic relevance in establishing human disease models and predicting drug responses. Compared to animal models, organoids can shorten experimental timelines, simplify experimental procedures, and are well-suited for large-scale expansion and passaging. This makes organoid models ideal for biobanking and high-throughput screening.

Cell line-based models

Cell lines, which consist of immortalized cell lines grown as monolayers in culture dishes, are widely used in cancer research due to their simplicity, low cost, short cultivation periods, high

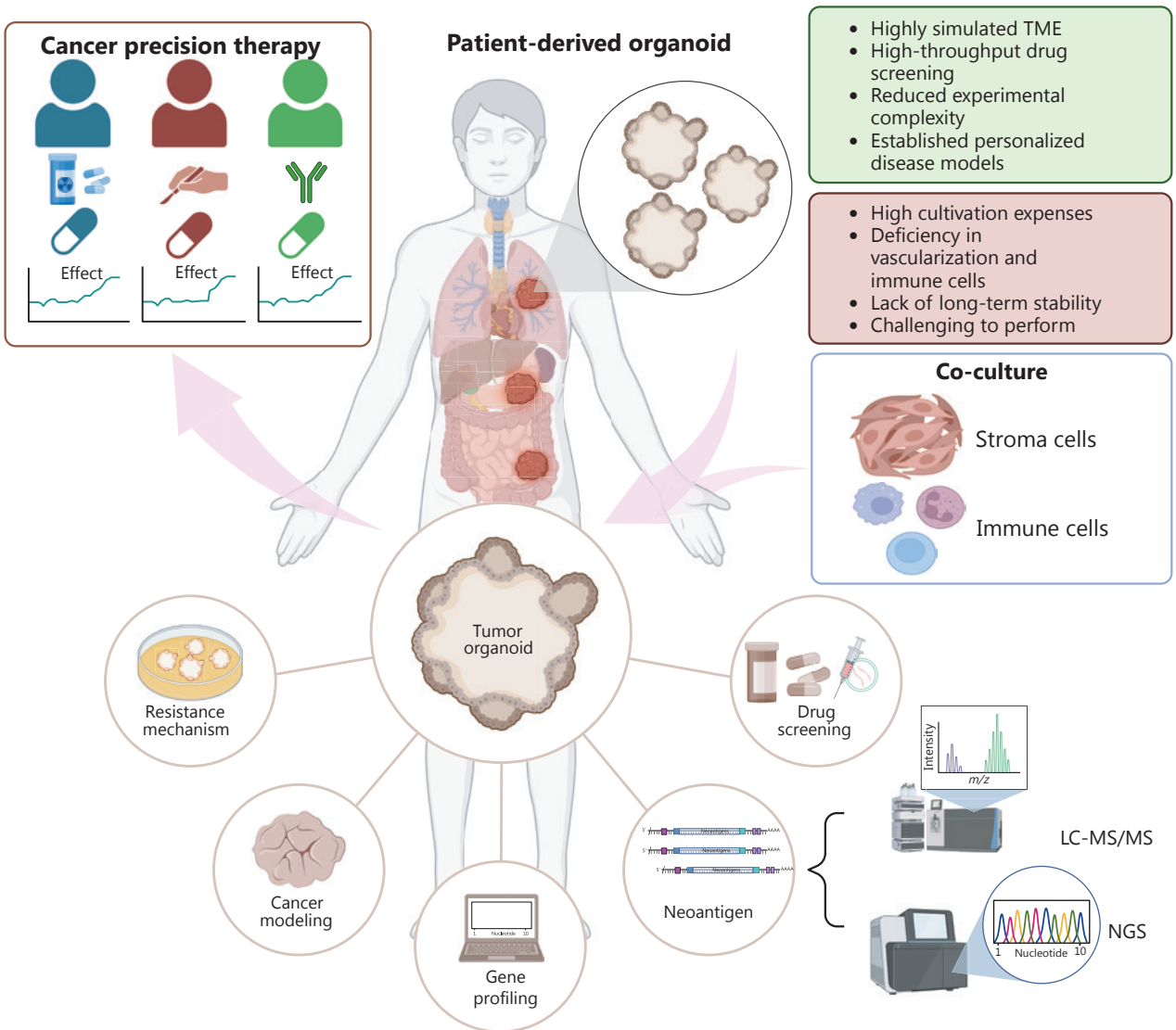


Figure 1 Functional characteristics, applications, and limitations of patient-derived tumor organoids in cancer modeling and therapeutic discovery. This figure illustrates the central role of tumor organoids in patient-specific cancer research. Patient-derived organoids (PDOs) established from gastrointestinal, pulmonary, breast, and other tumor types simulate key aspects of the tumor microenvironment (TME), enabling applications in drug screening, neoantigen identification, resistance mechanism analysis, and cancer progression modeling. Organoids preserve tumor heterogeneity and can be profiled using next-generation sequencing (NGS), transcriptomics, and mass spectrometry-based proteomics to identify therapeutic targets and biomarkers. Co-culturing tumor organoids with stromal and immune cells allows reconstitution of cellular interactions in the TME. Advantages of the system include personalized disease modeling, reduction in experimental variability, and compatibility with high-throughput platforms. However, high cultivation costs, lack of vascularization, limited immune component integration, and technical challenges in maintaining long-term culture stability continue to be system limitations. Incorporating immune and stromal cells can help mitigate these limitations. This model provides a powerful tool for linking patient-derived tumor biology with translational therapeutic development. Created with BioRender.com.

survival rates, and suitability for high-throughput screening. However, the most significant drawback of cell lines is the lack of complexity found in real organ tissues, which limits the ability to accurately mimic the intricate environment of the human body. Tumors are highly heterogeneous with the TME consisting of blood vessels, immune cells, and extracellular matrix components that interact in complex and intimate ways with tumor cells[15]. Cell models, which lack key

microenvironmental elements, are unable to replicate some functions and signaling pathways. Moreover, cell lines may undergo genetic mutations during long-term culture and passaging, resulting in biological characteristics that differ from the biological characteristics of the original tumor cells. As a result, while cell models are useful for studying basic cellular functions and drug screening, cell models have limitations in simulating real disease environments and complex cellular interactions[16].

The multicellular tumor spheroid (MCTS) model is a 3D culture system in which cells are introduced into a porous, biocompatible scaffold. This set-up allows the cells to grow in suspension and develop in a 3D structure, which more closely mimics the *in vivo* tumor environment. 3D culture systems provide a more accurate representation of the natural growth environment of the tumor compared to 2D culture systems. In these systems, tumor cells can form spheroids from a single cell type or a combination of different cell types. This structure allows for more realistic simulations of cell-cell and cell-matrix interactions, which are crucial for understanding tumor behavior, drug resistance, and response to treatments[17]. The MCTS model is a versatile tool in cellular biology research, especially in tumor biology. The MCTS model has a crucial role in studying fundamental processes, such as cell adhesion, migration, and epithelial morphology. This model serves as an excellent *in vitro* screening system, which enables evaluation of key aspects, such as angiogenesis, tumor invasion, and metastasis. Furthermore, the MCTS model is instrumental in assessing the efficacy of various therapies and understanding the underlying mechanisms of treatment responses, making the MCTS model a valuable resource in cancer research and drug development[18]. MCTS model accessibility and ease of operation make the MCTS model an excellent tool for high-throughput screening, contributing to more targeted and personalized tumor treatments. However, the MCTS model does come with some limitations. Controlling the size, density, and uniformity of the spheroids can be difficult in experimental cultures. These factors significantly impact the consistency of the results and the reproducibility of drug responses. Variations in spheroid characteristics can lead to discrepancies in experimental outcomes, which can complicate data interpretation and affect the overall reliability of the model in therapeutic studies[19]. These challenges with the MCTS model highlight some of the limitations in tumor research. The difficulty in imaging multicellular spheroids due to light scattering and poor antibody penetration can hinder accurate analysis and visualization of tumor responses, especially in complex drug screening scenarios. The variability in spheroid formation and response to drugs also complicates the interpretation of experimental results because inconsistencies between trials are introduced. Furthermore, the lack of physical stimulation from a true extracellular matrix, which is crucial for mimicking the *in vivo* TME, can lead to high rates of necrosis and apoptosis within the spheroids. This finding makes long-term passaging of these spheroids difficult, reducing the potential for repeated, large-scale experimentation. Despite these drawbacks, continued advances in scaffold materials, imaging technologies, and culture methods may help overcome these limitations in the future.

Mouse subcutaneous, metastatic, and orthotopic tumor models

A mouse subcutaneous tumor model is created by injecting a defined quantity of tumor cells beneath the skin of mice, resulting in tumor growth over time. This model is categorized into syngeneic and xenograft subcutaneous tumor models. The syngeneic subcutaneous tumor model involves inoculating murine-derived cells into immunocompetent mice, offering advantages like low cost and simplicity in establishing the model. In contrast, a xenograft subcutaneous tumor model involves inoculating human-derived cells into immunodeficient mice. While this method is more expensive, the human-derived cells more closely mimic the *in vivo* human environment, thereby enhancing the reliability and relevance of the experimental outcomes[20]. A mouse metastatic tumor model is established by injecting tumor cells into the tail veins of mice, enabling the cells to circulate through the bloodstream and form tumors in various organs, resulting in multiple metastatic foci throughout the body. This method is especially suitable for creating lung metastasis or leukemia models and is widely used in intravital imaging techniques[21]. However, tail vein injection is technically challenging and may result in mouse mortality. An orthotopic tumor model involves transplanting tumor cells into physiologically relevant sites, such as implanting hepatocellular carcinoma (HCC) cells into the liver or lung cancer cells into the lungs[22]. This model more effectively simulates tumor development within the human body, providing a more accurate prediction of drug efficacy and reducing the incidence of false positives. However, more complex surgical procedures are required, which are less suitable for observation and measurement and incur higher costs.

In summary, these three types of animal models enable the direct acquisition of critical data, including animal body weight, tumor growth curves, tumor weight, and tumor inhibition rates. Additionally, these models allow for the analysis of blood and tissue samples, making them widely applicable in biomedical research and drug development[23]. However, significant differences between mice and humans in terms of genetics, physiology, and metabolism may affect the clinical relevance of the experimental outcomes. Additionally, the use of mice in cancer research raises concerns related to animal welfare, ethics, and experimental costs, which can limit the design and scope of experiments.

PDX models

A PDX model is a type of animal model in which fresh human tumor tissues are processed and transplanted into immunodeficient mice, allowing the tumors to grow in the murine environment. A PDX model is directly derived from human tumor tissues and retains the original TME and characteristics, which preserves more intact biological features and offers higher clinical relevance. As a result, PDX models more authentically reflect the characteristics of patients' tumors. PDX models have significantly advanced personalized therapy by enabling drug sensitivity screening for individual patients, providing clinicians with optimal treatment strategies and thereby improving treatment success rates. Additionally, PDX models mimic the drug resistance pathways found in human primary tumors, offering a more accurate simulation of patients' tumor responses and drug sensitivities[24]. Currently, PDX models are highly valuable for personalized therapy and studying drug responses in specific tumor types. PDX models provide an ideal *in vivo* model for investigating tumor mechanisms, treatment strategies, and drug screening. However, PDX models have several limitations. Tumor samples are primarily sourced from surgical resections and cannot be repeatedly obtained. The modeling process is technically challenging, requiring significant expertise from researchers. Additionally, the engraftment success rate of PDX models is relatively low and the modeling cycle is longer, limiting widespread application. While PDX models effectively mimic the histopathology, genomic structure, and drug sensitivity of primary tumors, the clonal distribution of PDX tumors may differ from the original patient tumor during transplantation[25]. These disadvantages and limitations must be taken into account when designing experiments and interpreting results to ensure the accuracy and reliability of the research. However, with ongoing technological advances, new methods may emerge in the future that address these challenges, thereby expanding the potential applications of PDX models in tumor research.

Organoid models

Organoids represent a significant breakthrough in cancer research, offering a powerful platform to accurately model the complexities of tumor growth and the TME. Organoids are derived from patient tumor tissues or iPSCs and faithfully retain the pathologic structure and key genetic mutations of the original tumors. For example, a 2023 study using single-cell sequencing demonstrated a high degree of consistency in copy number variations (CNVs) and driver mutations (e.g., *APC* and *KRAS*) between colorectal cancer (CRC) organoids and the corresponding primary tumors[26]. Patient-derived melanoma organoid models (MPDOs) closely resemble the morphology and immune cell composition of the parental melanoma tissues with similar levels of PD-1, PD-L1, and CTLA-4 expression in lymphoid and myeloid lineages[27]. Glioblastoma organoids (GBOs) retain critical features that enable the study of patient-specific treatment strategies, while also recapitulating stromal-epithelial interactions within the TME, such as collagen deposition and extracellular matrix remodeling[28]. Additionally, organoids maintain tumor heterogeneity through polyclonal co-culture systems, as occurs in breast cancer models in which different sub-clonal populations exhibit varied responses to targeted therapies and provide a unique platform for studying clonal evolution[29]. Importantly, organoids hold immense potential in personalized cancer research because organoids integrate genetic information and microenvironmental factors, making organoids ideal for advancing precision oncology. Prospective clinical studies have validated the predictive capabilities of organoids. For example, a 2023 trial involving advanced gastric cancer (GC) patients showed an 87% concordance between organoid drug sensitivity testing and actual clinical responses to chemotherapy[30]. Similarly, pancreatic cancer organoids have been used to identify patient-specific molecular subtypes and guide combination therapies[31].

The recent development of organoid-immune co-culture systems has revolutionized the study of tumor-immune interactions. While traditional organoids preserve tumor histology and genetic features, traditional organoids lack critical immune components. This limitation has been addressed by

co-culturing patient-derived immune cells, such as T cells, tumor-associated macrophages (TAMs), and natural killer (NK) cells, with organoids[32]. For example, Dijkstra et al.[33] reported that co-culturing non-small cell lung cancer organoids with autologous peripheral blood T cells accurately predicts clinical responses to PD-1 inhibitors with *in vitro* sensitivity strongly correlating with improved patient survival. PD-L1 blockade significantly enhances T cell-mediated tumor killing in CRC organoid-$CD8^+$ T cell co-cultures, an effect that correlates with the tumor mutational burden[34]. In addition to T cell research, a 2023 study utilizing a macrophage-organoid co-culture model revealed the role of the macrophage-CCL5-Sp1-AREG signaling loop in promoting cancer stem cell-like properties and gemcitabine resistance, which is mediated through activation of the CCL5/CCR5/Sp1/CD44 axis. Leveraging this model, researchers identified drugs, such as gefitinib and cisplatin that effectively target this signaling loop and mitigate gemcitabine resistance, offering a novel strategy to overcome chemotherapy resistance[35]. Recent technological advances, such as microfluidic chip-based 3D co-culture systems, have further enhanced the utility of the model by enabling spatial analysis of T cell infiltration dynamics and providing high-resolution tools to study immune cell migration and targeted cytotoxicity[36]. Despite challenges related to sample heterogeneity and standardization, organoid-immune co-culture systems in combination with genomics, single-cell transcriptomics, and real-time imaging are becoming indispensable for bridging basic research and clinical translation with significant potential in high-throughput drug screening, immunotherapy biomarker discovery, and resistance mechanism studies.

However, the reproducibility of organoid cultures remains a major challenge. The complexity of culturing protocols, high material costs, and dependence on sample quality hinder standardization. Prolonged culture periods can also lead to issues, such as cellular senescence, incomplete differentiation, or genetic mutations, which may compromise the stability and reliability of the models.

In conclusion, organoid models provide innovative tools for studying tumor heterogeneity, exploring drug mechanisms, and evaluating therapeutic efficacy. Researchers can gain deeper insights into individual cancer variations by culturing organoids from patient-derived tumor cells, laying the foundation for precision medicine and personalized treatment strategies. Importantly, organoids complement rather than replace traditional models. 2D cell cultures and organoids can be used for initial drug screening and mechanistic studies, while subsequent validation in animal models ensures clinical relevance and safety, accelerating the translation of research findings into clinical applications. A comparative summary of organoid models and traditional models can be seen in **Table 1**.

Technological integration: organoids with genomic and molecular profiling

Organoid chips

Microfluidic chip technology integrates essential operational units, such as sample preparation, reaction, separation, and detection, onto a microscale platform, enabling the automated execution of biological, chemical, and medical analysis processes. As microfluidic chip technology advances, the integration of organoid models with chip technology has opened new opportunities for cancer research. This integration is commonly referred to as organoid-on-a-chip technology. Building on traditional organoid culture, organoid-on-a-chip technology utilizes the microfluidic platform to precisely control fluid dynamics, oxygen supply, nutrient exchange, and cell-to-cell interactions, thereby replicating the dynamic processes involved in tumor development. This finding provides an innovative platform for studying the complex TME and enables applications, such as high-throughput cultivation and drug screening.

Organoid-on-a-chip systems have emerged as a powerful tool for rapid drug sensitivity testing. Researchers have developed a novel integrated superhydrophobic microporous array (InSMAR)-chip, which significantly reduces sample consumption and culture time due to the nanoliter-scale volume of the micropores[37]. This innovation enables the prediction of drug sensitivity in tumor organoids within 1 week, significantly improving the efficiency and timeliness of assessing the clinical efficacy of anticancer drugs for patients. In oncology, organoid-on-a-chip technology can also simulate tumor invasion and metastasis, study the interactions between tumors and the TME, and evaluate the efficacy and toxicity of anticancer drugs. For example, researchers can examine how tumor cells traverse the vascular wall by integrating tumor organoids with vascular network models on a single chip, effectively mimicking the metastatic process. A study published in the journal, *Small*, introduced a tumor organoid chip designed to assess

Table 1 Comparison of different experimental model properties

	Cell models	Subcutaneous tumor models	Metastatic tumor models	Orthotopic tumor models	PDX models	MCTS models	Organoids models
Model type	2D cell culture	Animal model	Animal model	Animal model	Animal model	3D cell culture	3D cell culture
Microenvironment simulation	Lacks tumor microenvironment	Limited	Circulating tumor cells, metastatic sites	Organ-specific microenvironment	Preserved tumor microenvironment	Simulating cell-cell and cell-matrix interactions	Closely resembling the physiologic state of actual tumors
Heterogeneity	Low	Moderate	High (heterogeneity in metastatic spread)	Moderate (more representative of clinical tumors)	High (maintains tumor diversity)	High (multiple cell types)	High (patient-derived, diverse)
Cultivation periods	Short	Moderate	Moderate	Long	Long	Short	Moderate
Complexity of culture	Low	Moderate	High	High	High	Moderate	High
Genetic integrity	Can undergo mutations	Preserved in xenografts	Can reflect metastasis patterns	Maintains tumor features	Preserved from patient tissue	Retains genetic complexity	Retains genetic complexity
Cost	Low	Moderate	High	High	High	Moderate	High
Success rates	High	Moderate	Low	Low	Low	High	Moderate
Application in drug screening	Yes, high-throughput	Yes, for basic efficacy studies	Yes, for metastasis-specific therapies	Yes, for organ-specific tests	Yes, personalized drug testing	Yes, for 3D tumor biology and therapy testing	Yes, personalized and predictive drug screening
Scalability	High	Low	Low	Low	Low	Moderate	High
Immune system integration	No (lack of immune cells)	Depends on the type of mouse model	Depends on the type of mouse model	Depends on the type of mouse model	Limited (immune-deficient models)	Low (lacks full immune response)	Moderate (co-culture with immune cells)
Clinical relevance	Low (oversimplified)	Moderate (basic model)	Moderate-to-high (for metastasis)	High (mimics organ-specific tumors)	Very high (reflects patient-specific features)	Moderate (lacks full clinical representation)	Very high (mimics patient-specific tumors)
Other features	Susceptible to contamination or misidentification	Direct acquisition of critical data Ethical concerns	Technical challenges; direct acquisition of critical data Ethical concerns	Complex surgical procedures; direct acquisition of critical data; ethical concerns	Useful for personalized therapy and studying drug responses in specific tumor types; tests the technical skills of the researchers	Supporting the assessment of angiogenesis, invasion, and metastasis; challenging to control the size, density, and uniformity; high rates of necrosis/apoptosis	In conjunction with liquid biopsy techniques for early cancer diagnosis and monitoring of mutated genes

tumor metastasis. This chip can simulate the physiologic processes of tumor growth and metastasis in the human body, effectively evaluating the invasive and proliferative capabilities of patient-derived tumor cells. The chip provides a valuable tool for studying tumor metastasis and advancing the development of targeted cancer treatments and drug research[38]. Furthermore, organoid-on-a-chip technology can replicate the TME and capture subtle dynamic changes, making organoid-on-a-chip technology essential for studying the interactions between tumors and immune cells[39]. By co-culturing tumor organoids and immune cells on the chip, the recruitment of immune cells can be simulated, allowing for the identification of epigenetic changes and providing experimental evidence to support cancer immunotherapy[36].

Organoid-on-a-chip models are designed to closely replicate the microenvironment under physiologic and pathologic conditions in the human body, making organoid-on-a-chip models valuable for evaluating drug efficacy and safety. By integrating multiple organoid modules on a single chip, these models enable high-throughput drug testing while reducing the reliance on experimental animals[40]. This technology can also be applied in personalized medicine. Customized drug response predictions for individual patients is enabled by combining PDOs with chip technology. This approach can significantly shorten the drug development cycle, reduce the risk of drug screening failure, and revolutionize new drug development.

Organoids and proteomics

Proteomics is the scientific study of proteomes, which encompass the entire set of proteins produced by a cell, tissue, or organism. Proteomics involves the large-scale analysis of protein characteristics, including levels of expression, post-translational modifications, and protein-protein interactions. This approach offers a comprehensive understanding of processes, such as disease development and cellular metabolism at the protein level. Organoids are widely used in oncology to model cellular heterogeneity within the TME, as well as drug screening and precision medicine research. Proteomic technologies provide a powerful method for the comprehensive analysis of protein expression and modifications in cells or tissues, which is essential for understanding the molecular mechanisms underlying tumors and predicting drug responses[11].

Researchers can explore the molecular characteristics of tumors by integrating organoid and proteomic technologies and predict the efficacy of personalized treatments. For example, the creation of a liver cancer organoid biobank (LICOB) has enabled a comprehensive analysis of the histologic and molecular features of liver cancer, including genomics, epigenomics, transcriptomics, and proteomics. These organoids not only replicate the original tumors in morphology but also show a high degree of concordance with the original tissues in terms of somatic mutations, copy number variations, DNA methylation, transcriptomic, and proteomic features. Through proteogenomic analysis, researchers have identified organoid subtypes linked to patient prognosis and uncovered distinct drug response patterns for each subtype, which are associated with specific multi-omics signatures[41]. The integration of organoid and proteomic technologies marks a significant advance in precision medicine. Proteomics based on patient-derived models (PDMs) has emerged as a cutting-edge approach. The Zhejiang University School of Medicine has developed an integrated database that provides proteomic data for PDMs. This resource systematically offers proteomic profiles, such as expression, function, and interaction, for all proteins analyzed in the studied proteomics, while comprehensively presenting the raw data from organoid proteomic studies across various diseases[42]. The development of such platforms facilitates the comparison of protein expression patterns within and across datasets, providing a crucial data foundation for advancing precision medicine.

However, the cultivation of organoids typically requires a substance known as Matrigel, which serves as an essential extracellular matrix for the preparation and embedding of organoids. Matrigel contains a variety of growth factor proteins that can significantly interfere with ion activity, potentially leading to inaccurate experimental outcomes. While proteomics is commonly applied to tumor tissue samples, the advantages of organoids in proteomic studies lie primarily in the convenience of sample acquisition. Organoid technology addresses the challenge of obtaining rare tumor samples, thereby facilitating more efficient and effective research progress[43]. In addition, organoids are well-suited for high-throughput screening, enabling precise prediction of drug responses and the evaluation of potential drug combination treatment strategies[44]. When combined with proteomics, this approach offers valuable guidance for drug combination treatments in clinical patients, thereby advancing the development of precision medicine[45]. Proteomics, by identifying key biomarkers, signaling molecules, and pathways, has a crucial role in understanding the behavior of organoids. This

technology has significantly advanced our understanding of the mechanisms involving proteins, genes, and signaling pathways in organoids.

Organoids and single-cell sequencing

Single-cell sequencing technology involves the sequencing and analysis of the genome, transcriptome, and epigenome at the individual cell level. In contrast to traditional sequencing, which provides an average signal from a population of cells, single-cell sequencing can detect cellular heterogeneity within mixed samples. Single-cell sequencing is an essential tool for exploring biological questions related to tissue development and tumor heterogeneity. The combination of organoid models with single-cell sequencing technology has emerged in recent years as a powerful tool for oncology research. This integration allows researchers to explore the molecular level, uncovering cellular heterogeneity and the TME. Applications of this combination include identifying cellular subpopulations within tumor organoids, studying mechanisms of tumor drug resistance, understanding key decision points in fate determination, and investigating the heterogeneity of the tumor immune microenvironment[46]. Researchers can precisely identify and characterize distinct cellular subpopulations within tumor organoids, including cancer, stromal, and immune cells, through single-cell RNA sequencing. This approach enables a deeper understanding of the roles of cell subpopulations in tumor development and therapeutic responses. Such insights are crucial for developing personalized medical treatments and precision therapeutic strategies.

Single-cell sequencing technology is essential for exploring the TME when integrated with organoids. Comparative analysis of single-cell data from PDOs and primary tumors in GC has revealed both similarities and differences across various lineages, providing new insights into the molecular boundaries of experimental models. Additionally, a comprehensive single-cell atlas has been instrumental in uncovering cellular heterogeneity, the TME, and subtype-specific expression programs in GC. These findings offer valuable molecular resources for molecular typing and personalized therapy[47]. Researchers have discovered that prostate epithelial organoids exhibit tumor-associated epithelial cell states that differ from the parental tissues in terms of cell types and states. Single-cell analysis has revealed the heterogeneity of these tumor-associated epithelial cell states, providing deeper insights into the complexities of prostate cancer[48]. The application of organoid models in CRC has been particularly extensive, using patient-derived tumor organoids to evaluate the antitumor effects of PPAR inhibitors, as well as the self-renewal and differentiation capabilities of stem-like tumor cells. By combining single-cell RNA sequencing with targeted cDNA Sanger sequencing for single-cell point mutation identification, significant phenotypic differences have been uncovered between cancer cells with and without critical point mutations, such as *KRAS* and *TP53*, within the same patient[49]. Similarly, liver, pancreas, renal, and ovarian cancers and glioblastoma studies have utilized the combination of single-cell sequencing technology and organoids to investigate the TME[50-54].

The application of organoid models in drug screening is closely tied to single-cell sequencing technology. Researchers have successfully established organoid models of dopamine receptor agonist-resistant prolactinomas and screened 180 small molecule compounds using 8 types of organoids to identify potential therapeutic drugs. Transcriptome and single-cell sequencing analyses revealed that dopamine receptor agonist (DRA) resistance in prolactinomas is linked to upregulation of the focal adhesion (FA) signaling pathway. Furthermore, genistein has been shown to exert antitumor effects by inhibiting expression of the FA pathway[55]. Additionally, a study in 2021 explored the mechanisms underlying heterogeneity and drug resistance in liver and bile duct tumors using single-cell sequencing combined with organoids. The study described the heterogeneity within liver and bile duct tumor organoids and found that interactions among intra-tumoral heterogeneous subpopulations may contribute to the emergence of malignant phenotypes and drug resistance[56]. Co-culturing primary pancreatic ductal adenocarcinoma (PDAC) organoids with patient-matched tumor-associated fibroblasts (CAFs) and utilizing single-cell RNA sequencing has revealed the impact of CAFs on the sensitivity to treatments with gemcitabine, 5-fluorouracil, and paclitaxel. This approach provides valuable insights into how the TME influences the drug response in PDAC[57]. Researchers can assess the effects of drugs on various cell types within the TME by exposing organoids to different drug treatment conditions and subsequently performing single-cell sequencing. This approach enables the prediction of drug efficacy and the identification of potential resistance mechanisms, offering a more comprehensive understanding of treatment outcomes.

Single-cell sequencing is also invaluable for tumor lineage research. Professor Hans Clevers[58] utilized human intestinal organoids and a clustered regularly interspaced short

palindromic repeats (CRISPR) gene knockout screening system to identify transcription factors involved in enteroendocrine cell (EEC) lineage differentiation. It was shown that the transcription factor, ZNF800, acts as a primary inhibitor of EEC differentiation through single-cell transcriptome sequencing and has a pivotal role in regulating EEC lineage commitment[58]. This work highlights the potential application of organoids in CRISPR functional screening and opens new research avenues for studying gastrointestinal and endocrine diseases.

In summary, offers a powerful tool for biomedical research. The integration of organoids and single-cell sequencing technology unveils the complexity at both the cellular and molecular levels, facilitates the development of new therapies, and lays a solid foundation for advancing precision medicine in the future. This field will expand further as technology continues to advance, offering deeper biological insights and more effective treatment strategies. This progress promises to significantly enhance our understanding of diseases and improve outcomes for patients. Some combined applications of organoids with chip technology, proteomics, and single-cell sequencing are shown in **Figure 1**.

Predicting targeted efficacy using PDOs

Targeted cancer therapy utilizes chemotherapeutic agents that selectively target genetic biomarkers associated with specific cancers, either directly or indirectly. This approach includes a range of strategies, such as monoclonal antibodies, small molecule inhibitors, and antibody-drug conjugates, all designed to precisely disrupt the molecular pathways driving tumor growth. However, like immunotherapy, targeted cancer therapies face challenges in preclinical validation, as the translational relevance of animal models is often limited.

Tyrosine kinase inhibitors (TKIs)

TKIs are targeted therapies designed to interfere with cellular pathways that regulate the growth of malignant cells. Epidermal growth factor receptor (EGFR) mutations are prevalent in malignant solid tumors with lung cancer being the most notable. Organoids have served as effective models for clinical pre-validation of efficacy and for investigating resistance mechanisms in TKI development.

The study of the resistance mechanisms of TKIs is of great significance to targeted cancer therapy. Organoid models have a crucial role in this research area and have demonstrated considerable value in examining changes in the immune microenvironment associated with TKI resistance. Ding et al.[59] revealed that lipid metabolism reprogramming mediated by unconventional prefoldin RPB5 interactor (URI) confers resistance to TKI-induced ferroptosis in HCC. Their study demonstrated that the combination of the SCD1 inhibitor, aramchol, and the deuterated sorafenib derivative, donafenib, shows promising antitumor effects in class organoids and xenograft tumors derived from p53-wild-type HCC patients. Wang et al.[60] utilized PDOs from ALK-rearranged lung adenocarcinoma patients to demonstrate the therapeutic potential of a specific GSTP1 inhibitor, ezatiostat, in combination with the ALK inhibitor, crizotinib. Yan et al.[61] restored the sensitivity of TKI-resistant tumor cells and organoids by inhibiting DCLK1 activity, highlighting the key role in TKI resistance. Jin et al.[62] utilized functional targeting of the BDNF-TrkB pathway with neutralizing antibodies against BDNF and TrkB in PDO models to enhance the sensitivity of GC cells to anlotinib. The Jin et al. study[62] characterized the crucial role of epithelial-mesenchymal interactions mediated by lactate/BDNF/TrkB signaling in GC anlotinib resistance.

Another role of organoid models is in the preclinical validation of TKI efficacy. Wang et al.[63] demonstrated the efficacy of pyrotinib in a study of HER2 exon 20 insertions in non-small cell lung cancer patients using PDOs and PDX models. Lee et al.[64] developed a fourth-generation EGFR TKI and evaluated antitumor activity in patient-derived cell, organoid, and xenograft models with EGFR mutations.

Additionally, several studies have reported that organoid models can predict a patient's response to drugs. Hu et al.[37] demonstrated that hundreds of lung cancer organoids, which can be generated from the majority of samples in the first generation, are sufficient to produce clinically significant drug responses, including EGFR TKIs, within 1 week. Kim et al.[65] developed a clinical response model for targeted therapy in patients with advanced lung adenocarcinoma, which is capable of predicting clinical responses to single and combination targeted therapies. Lee et al.[66] developed a PD-1-based HTS and CODRP assay to predict and analyze the efficacy of promising anticancer drugs in lung cancer patients with potential applications in precision medicine platforms. Similar studies are emerging[67], highlighting the role of organoid models in predicting the clinical efficacy of drugs.

Monoclonal antibodies (mAbs)

mAbs have emerged as a pivotal therapeutic strategy in oncology due to high specificity and an ability to precisely target tumor-associated antigens. However, conventional preclinical models, such as 2D cell cultures and animal xenografts, often fail to recapitulate the complexity of the TME and the heterogeneity present in human cancers. This limitation contributes to discrepancies in the clinical translation of mAb-based therapies. Organoid models offer a promising platform to bridge this gap by better mimicking human tumor biology and enhancing the translational relevance of preclinical studies.

Organoid models closely recapitulate the *in vivo* TME and have become valuable tools for evaluating the efficacy of mAbs in preclinical studies. For example, Chen et al.[68] reported that CD146 activates the PI3K/AKT signaling pathway by preventing DCBLD2 degradation, thereby promoting the malignant progression of breast phyllodes tumors. Using a malignant phyllodes tumor organoid model, Chen et al.[68] demonstrated that the anti-CD146 mAb, AA98, significantly inhibited tumor growth, underscoring the potential of organoids in precision medicine. Similarly, Wang et al.[69] used a patient-derived neuroendocrine prostate cancer (NEPC) organoid model to test the humanized anti-NRP2 mAb (aNRP2-10), which blocked VEGF binding to NRP2, reduced PD-L1 expression, and activated antitumor immune responses. In another study, Abril-Fornaguera et al.[70] found that combining the mAb, xentuzumab (targeting IGF1/2), with cisplatin produced synergistic antitumor effects in hepatoblastoma (HB) organoids and mouse models with elevated IGF2 expression. Additional studies using organoid models of GC and triple-negative breast cancer (TNBC) have further validated the therapeutic efficacy of novel mAbs, emphasizing the broad potential for clinical application[71,72].

Organoid models have also demonstrated significant value in elucidating mechanisms of drug resistance. Zheng et al.[73] used a CRC organoid model to reveal the key role of Lgr4-Wnt signaling in tumor resistance and developed a mAb targeting LGR4 (LGR4-mAb) to overcome this resistance. The organoid model accurately simulated the clinical drug resistance phenotype and confirmed that LGR4-mAb significantly enhanced the efficacy of chemotherapy by promoting ferroptosis, thereby facilitating the development of precision therapeutic strategies. Lin et al.[74] used a prostate organoid model and mAb against NUDT21 R15me1 to reveal the key role of NUDT21 demethylation in enzalutamide resistance of prostate cancer. Notably, drug distribution and monitoring can also be effectively achieved by integrating organoid models with advanced analytic technologies. For example, Liu et al.[75] developed a matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI)-based method to visualize the distribution of the mAb cetuximab in 3D colon cancer spheroids and PDOs, providing a powerful tool for antibody drug development and clinical monitoring.

Although organoid models cannot fully replicate the complexity of the *in vivo* TME, organoid models have demonstrated indispensable value in evaluating therapeutic efficacy, investigating mechanisms, and optimizing strategies for mAbs. Validation through organoid models supports the efficient clinical translation of novel mAb therapies.

Predicting immunotherapy efficacy using PDOs

Immunotherapy has revolutionized cancer treatment by harnessing the immune system to combat malignancies. However, immune-related mechanisms are often suppressed or evaded during cancer progression. To counteract this suppression and evasion, strategies like non-specific (using cytokines to boost overall immunity) and specific immunotherapy (targeting tumor antigens) have been developed. Other approaches directly supply immune components like mAbs or engineered T cells to attack cancer cells. These mechanisms have been detailed in prior reviews.

Currently, U.S. Food & Drug Administration (FDA)-approved immune checkpoint inhibitors (ICIs) targeting PD-1, PD-L1, and CTLA-4 have become the standard of care for the treatment of various tumors[76,77]. However, despite the clinical success of immunotherapy, especially with approaches like ICIs and chimeric antigen receptor T (CAR-T) cell therapy, predicting patient responses remains a major challenge. Traditional preclinical models, especially murine systems, often fail to recapitulate the complexity of human immune-tumor interactions due to interspecies differences in immunology, limiting the translational relevance.

In this context, PDOs have emerged as a powerful and physiologically relevant platform for immunotherapy research. Organoids retain the genetic, histologic, and functional characteristics of the original tumors, and when co-cultured with autologous immune cells or engineered immune components,

organoids enable precise modeling of tumor-immune dynamics. This makes organoids a valuable platform for evaluating CAR-T cell therapy, investigating mechanisms of immunotherapy response and resistance, and comparing the efficacy of different immunotherapeutic strategies. By bridging the gap between traditional preclinical studies and patient-specific clinical decision-making, organoid models also represent a critical advance in the development of personalized immunotherapy.

Evaluating CAR-T therapy

CAR-T therapy has gained significant attention in recent years. The principle behind this approach is to introduce antibodies that bind to receptors on tumor cells onto the surface of T cells, thereby creating effective tumor-killing cells. The progress of CAR-T therapy for solid tumors has been slow, primarily due to the immunosuppressive TME, which leads to T cell exhaustion. Organoids provide a platform for validating various improvements to CAR-T therapy. PDOs, which closely mimic the characteristics of the TME, are widely used to assess the efficacy of CAR-T therapies across different cancer types. For example, breast cancer microarray models integrating PDOs have been developed to evaluate the efficacy and safety of ROR1-targeted CAR-T cells. These models can simulate the TME, monitor CAR-T cell infiltration, cytotoxicity, and cytokine release, and facilitate the investigation of cytokine release syndrome and the refinement of therapeutic intervention strategies[78]. Jacob et al.[28] established a patient-derived GBO model that reproduces tumor histologic architecture, cellular heterogeneity, gene expression profiles, and mutational signatures, making the GBO model a valuable tool for personalized evaluation of EGFR-targeted CAR-T therapy. Similar applications have also been conducted in liver cancer[79], bladder cancer[80], and clear cell renal carcinoma (ccRCC)[81]. It is worth noting that Logun et al.[82] reported that organoids can serve as real-time efficacy predictors in glioblastoma, highlighting a new role for organoids in CAR-T therapy.

The development of multi-target CAR-T cells and combinatorial antigen screening strategies in recent years has shown promise in enhancing CAR-T cell efficacy with organoid models playing a crucial role in experimental validation. Wehrli et al.[83] developed a CAR-T cell targeting pancreatic cancer by using anti-FAP and anti-CD3 antibodies to target the extracellular matrix. Wehrli et al.[83]validated the ability of the CAR-T cell to target the tumor and the extracellular matrix in PDO models. Qiao et al.[84] enhanced the anti tumor activity of CAR-T cells targeting EGFR and B7H3 by knocking down six inhibitory membrane proteins and confirmed the efficacy using a cholangiocarcinoma organoid model. Additionally, CAR-T cells co-targeting CD44 and CD133 were evaluated in a GBO model, demonstrating effective anti tumor activity[85].

In addition to multi-target strategies, gene editing and the co-expression of functional molecules have also been used to enhance CAR-T cell efficacy with organoid models serving as robust platforms for validation. Li et al.[86] reported a CAR-T cell expressing the anti-B7H3 antibody, which demonstrated antitumor effects in pulmonary cancer organoids. Additionally, by co-expressing CCL2, the CAR-T cell enhanced permeability through the blood-brain barrier, thereby improving the therapeutic effect on brain metastases. Liang et al.[87] engineered T cells to co-express SMAD7 and a HER2-targeted CAR, inhibiting TGF-β signaling. Liang et al.[87]demonstrated enhanced CAR-T cell infiltration, sustained activation, and reduced cytokine release in patient-derived ovarian cancer organoids, improving antitumor efficacy and safety in solid tumor models. Li et al.[88] developed a novel CRISPR-Cas9 gene editing platform by combining modified Cas9 protein with phosphorothioate oligonucleotides (PSs). This technology, which was evaluated in patient-derived tumor organoid models, enhanced the viability, expansion, and antitumor efficacy of CAR-T cells targeting PARG or CIC-DUX4. The dual-specificity of the PS-Cas9/gRNA complex also reduced off-target effects and increased editing precision. Overall, organoid models closely mimic the *in vivo* environment and represent powerful tools for evaluating the efficacy of emerging CAR-T cell therapies in preclinical research.

Organoid models have numerous important applications beyond evaluating the efficacy of various CAR-T cell types. Organoids can be used to differentiate to produce CAR-T cells. Wang et al.[89] used artificial thymic organoids (ATOs) to differentiate T-cell-derived iPSCs into CD19-targeted CAR-T cells for the treatment of B-cell malignancies. These CAR-T cells exhibited a more homogeneous T-cell receptor (TCR) repertoire and lower CAR expression compared to conventional methods, thereby reducing T cell exhaustion and enhancing therapeutic efficacy. In the context of personalized treatment, Zhu et al.[90] screened heterogeneously expressed antigens (e.g., B7-H3, IL-13Rα2, HER2, and GD2) to construct a "targeting library," selecting personalized combinations through multi-antigen immunohistochemistry (IHC) analysis of patient tumors. Patient-derived GBO models can be used

for personalized screening, expanding the utility of organoids in therapeutic evaluation. Moreover, organoid models derived from pancreatic islets and other tissues have been used to evaluate the protective potential of T cells engineered with synthetic Notch (synNotch) receptors, which helps prevent off-target effects in CAR-T cell therapies and provides a promising approach to reducing treatment-associated toxicity[91].

Studies suggest that organoid models also have strong potential for validating CAR-T therapy and the function as real-time efficacy predictors should be given serious consideration given the widespread use in preclinical efficacy validation.

Studying immunotherapy response mechanisms

Tumors are not merely aggregates of malignant cells but rather highly organized and complex ecosystems. The immunologic components within tumors, collectively referred to as the TIME, have a critical role in tumor development, recurrence, and metastasis.

CTLA-4, a member of the immunoglobulin superfamily expressed on activated T cells, transmits inhibitory signals to regulate T cell activity. PDO models offer unique and irreplaceable advantages in visualizing dynamic changes in the immune microenvironment associated with the three major immunotherapy approaches. These models provide a physiologically relevant platform for studying immune interactions, facilitating the development and optimization of immunotherapeutic strategies.

Küçükköse et al.[92] established a model of spontaneous multi-organ metastasis in CRC by transplanting PDOs and analyzed the therapeutic effects of CTLA-4 and PD-1 immune checkpoint inhibitors using this model. Küçükköse et al.[92]also suggested that B cells may play a key role in anti tumor immunity. Ou et al.[93] used melanoma organoid models to investigate the effects of PD-1 therapy on gamma delta T cells. The study showed that ICIs increased gamma delta T cell infiltration in the 3D models and led to the complete elimination of melanoma cells in all four 3D models. An et al.[94] constructed a GC organoid model with a mutated Smad4 gene to study the immunosuppressive microenvironment. The findings demonstrated that CTLA-4 and PD-L1 therapies can effectively treat GC resistant to immune checkpoint blockade (ICB) monotherapy. Wan et al.[95] utilized a co-culture model of organoids and immune cells to demonstrate the therapeutic value of simultaneously blocking PD-1 and PD-L1 in high-grade serous ovarian cancer, highlighting the key role of NK cells in this process. Hamdan et al.[96] developed a novel oncolytic adenovirus expressing an enhanced cross-immunization IgG-Fc PD-L1 inhibitor, which can activate multiple immune responses and has demonstrated an ability to enhance tumor killing in organoid models. Shiri et al.[97] used organoid models to demonstrate that the absence of IL-10 reduces liver metastasis, suggesting that IL-10 promotes anti tumor immune suppression by upregulating PD-L1.

The above examples highlight the significant advantages of patient-derived organoid models in elucidating the TME response to immunotherapy. The primary strength lies in the ability to visualize dynamic changes in various cell types, providing valuable insights into immune interactions and treatment efficacy.

Studying resistance mechanisms

One of the major challenges in immunotherapy is drug resistance observed across multiple cancer types. A comprehensive investigation of the underlying resistance mechanisms is crucial for overcoming this issue, which has long hindered clinical success and treatment efficacy. Using PDOs to study resistance mechanisms offers a distinct advantage because patients can be clearly identified as either resistant or non-resistant.

Sun et al.[98] identified the innate immune kinase TANK-binding kinase 1 (TBK1) as a candidate immune evasion gene. Inhibition of TBK1, when combined with PD-1 blockade, has been shown to be effective in PDOs. Koikawa et al.[99] conducted similar studies using various models, including PDOs, in pancreatic cancer. Koikawa et al.[99]demonstrated that targeting Pin1 with a combination of anti-PD-1 and gemcitabine induces complete eradication or sustained remission of aggressive PDAC. Inflammation is a common complication associated with many types of tumors. Sui et al.[100] demonstrated in PDO models that high neutrophil infiltration and inflammatory responses are linked to poor therapeutic efficacy of ICIs in CRC. The organoid models, co-cultured with tumor-infiltrating lymphocytes (TILs) or peripheral blood mononuclear cells (PBMCs), showed that neutrophil-dominated inflammation correlates with an immunosuppressive TME and reduced sensitivity to PD-1 blockade. These findings highlight the utility of organoid models in predicting treatment outcomes in CRC.

Similar high-quality studies have been conducted in most solid tumors, including ovarian cancer[95], diffuse GC[71], lung cancer[6], and CRC[101]. Although numerous studies have demonstrated the value of PDOs in studying drug resistance

mechanisms, it is standard practice to validate findings using other models in addition to organoid models.

Evaluate the efficacy of new drugs and combination therapy strategies

PDO models are also widely used to evaluate the efficacy of new drugs and combination therapy strategies.

Although the clinical application of CTLA-4 ICIs is still limited, PDO models have proven valuable in evaluating efficacy in individual studies. Ou et al.[27] used MPDO models to evaluate the efficacy of PD-1 therapy, demonstrating that these organoids are a clinically relevant preclinical model for assessing melanoma treatment efficacy. Similar to CTLA-4 immune checkpoint inhibitor therapy (ICB), organoids also show promising potential for predicting the efficiency of PD-1 ICI therapy. Tian et al.[102] conducted a single-arm phase II clinical trial on CRC using PDO models to investigate the immune microenvironment in patients with better clinical outcomes. The study found that immune program induction in organoids correlates with the degree of MAPK inhibition. Similarly, in a multicenter phase II clinical trial for esophageal squamous cell carcinoma, investigators designed a translational sub-study to assess PD-1 and PD-L1 at different time points, establish organ cultures, and perform microbiome analysis to predict treatment response. However, no reports have been published on the findings[103]. In a preclinical study targeting HER2-positive GC, researchers developed a novel bispecific antibody that targets both PD-1 and HER2, demonstrating antitumor efficacy using PDOs and PDX tumor-bearing mouse models[104].

Observe immune responses and evaluate biomarker efficacy

Our understanding of microenvironmental changes in patients receiving immunotherapy remains limited due to the limitations of traditional models. PDO models offer a valuable *ex vivo* platform to observe immune responses and screen and evaluate biomarkers of efficacy at different time points.

Voabil developed an *ex vivo* organoid model and observed that the ability of immune cells to reactivate off-body could predict clinical responses. Researchers constructed patient-derived tumor organoids by embedding the tumor tissue in an artificial extracellular matrix to retain immune cells within the model, thereby preserving the TIME for functional studies. Perturbation analysis identified tumor-resident T cells as a key component of this immune response. Sun et al.[105] developed a human mucosal melanoma organoid platform co-cultured with activated PBMCs and TILs, which validated the ability to explore selective immunotherapy combinations. Liu et al.[106] developed a single-cell RNA sequencing platform specifically for pulmonary cancer organoids, which preserves the temporal heterogeneity of tumor tissue. The Liu et al. study[106] validated the key role of $CD8^+$ T cells in mediating the anti tumor effects of anti-PD-1 therapy. Lung squamous cell carcinoma organoid models have also been reported, demonstrating the value in evaluating the efficacy of immunotherapy[107]. Identifying biomarkers for immunotherapy is essential for accurately predicting treatment outcomes. Gao et al.[101] utilized PDO models co-cultured with TILs to demonstrate that elevated levels of *Fusobacterium nucleatum* are positively correlated with the efficacy of PD-L1 blockade immunotherapy in CRC.

In summary, PDOs have emerged as an effective research platform due to the unique ability to capture dynamic changes in the TME while preserving immune cell activity. PDOs are particularly valuable for investigating immunotherapy response mechanisms, drug resistance, novel and combination therapies, as well as for evaluating treatment efficacy and biomarker validity. However, organoids still face limitations in modeling tumor-immune responses compared to animal models. Animal systems remain dominant due to their intact immune environment, multi-organ interactions, and capacity to study systemic drug effects and long-term dynamics. Organoids, while promising for personalized screening, lack physiologic immune complexity, vascularization, and systemic connectivity. Future advances should focus on developing high-fidelity organoid models that more accurately retain the characteristics of tumors and the TME, including immune and stromal cell components, to further enhance the translational potential. **Figure 2** shows how PDO predict targeted and immunotherapy efficacy. **Table 2** summarizes the important applications of organoid models in targeted therapy and immunotherapy.

A novel direction: the application in cancer vaccine

Cancer vaccines are biological agents designed to stimulate the human immune system to recognize and eliminate cancer cells. Therapeutic vaccines achieve this by utilizing tumor-specific

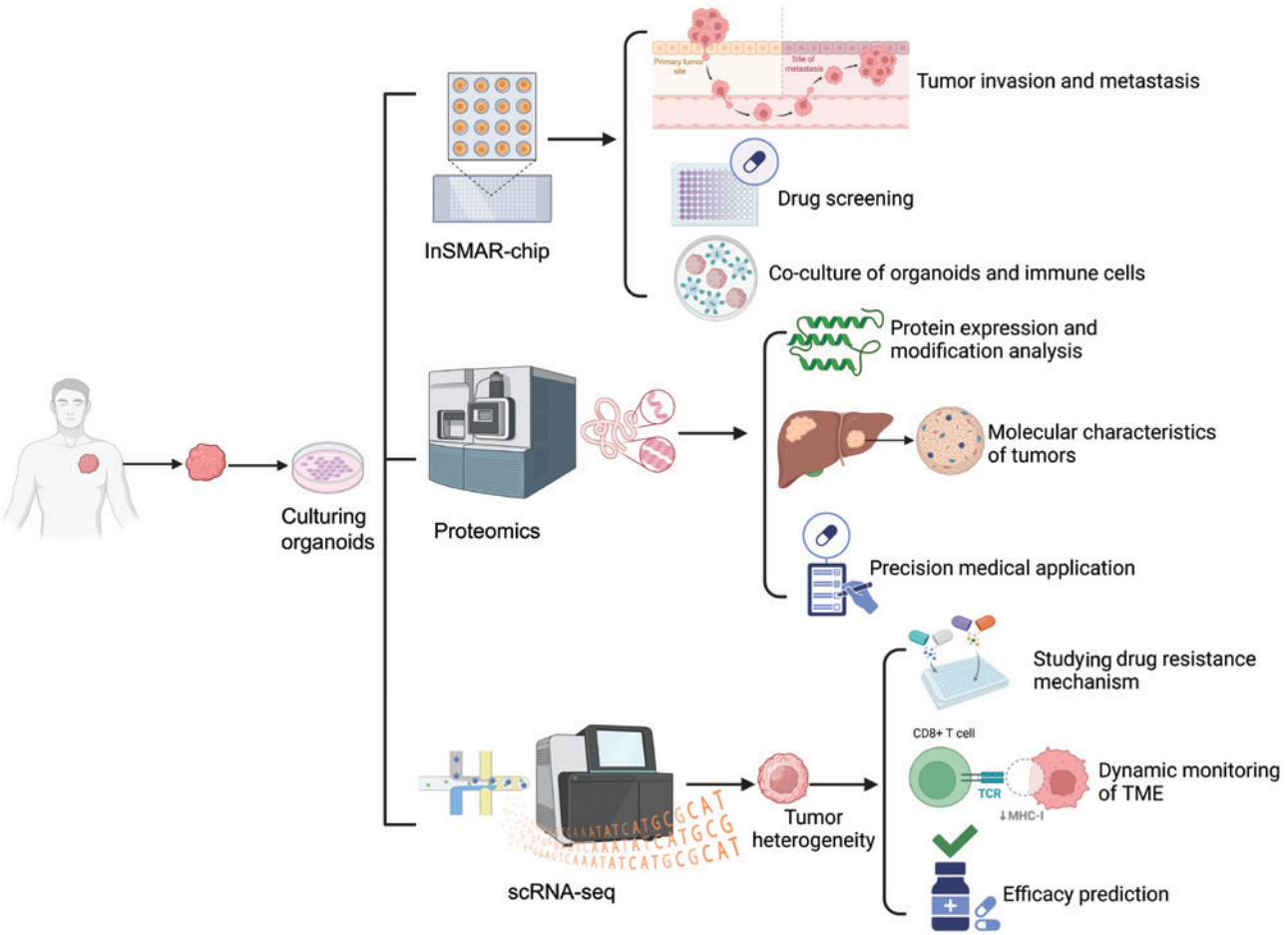


Figure 2 Integration of tumor organoids with microfluidics and multi-omics platforms for dynamic tumor characterization and therapy prediction. This figure presents a multi-dimensional approach to the analysis of tumor organoids derived from cancer patients, combining microfluidic engineering, proteomics, and single-cell transcriptomics to dissect tumor behavior and therapeutic response. Organoids are first established from resected or biopsied tumor tissues, then subjected to analysis as follows: (1) InSMAR-chip platforms, which enable dynamic assays of tumor invasion, metastasis, and drug sensitivity in a microfluidic environment, including immune cell co-culture for evaluation of immunotherapeutic interactions; (2) proteomics to assess protein expression, signaling pathway activation, and post-translational modifications, offering insights into tumor biology and potential therapeutic vulnerabilities; and (3) scRNA-seq, which captures tumor heterogeneity, maps the cellular composition of the tumor and TME, and enables prediction of the therapeutic efficacy and immune response. These integrated organoid-based technologies facilitate precision medicine by supporting functional annotation of tumor-specific features, real-time response monitoring, and discovery of resistance mechanisms. Created with BioRender.com.

antigens to train immune cells, primarily activating cytotoxic T lymphocytes (CTLs) through antigen-presenting cells, such as dendritic cells (DCs). This process induces targeted cancer cell destruction and establishes long-term immune memory.

Recent advances in mRNA vaccine technology have enabled the development of personalized cancer vaccines, allowing for the customization of neoantigens based on individual tumor mutation profiles. These vaccines have been applied to various solid tumors, including renal cancer, melanoma, lung cancer, and pancreatic cancer, marking a significant step toward precision oncology[108].

PDOs have been extensively utilized in the research, development, validation, and application of tumor vaccines. First, in response to the limited availability of clinical samples, PDOs enable the expansion of patient-derived tissues while preserving the molecular characteristics of the original tumor. This finding facilitates the creation of biobanks for neoantigen prediction and significantly broadens the applicability

Table 2 Important applications of organoid models in targeted therapy and immunotherapy

Type of therapy	Type of cancer	Application of PDO	Reference
TKIs	Hepatocellular carcinoma	Studying the resistance mechanisms of TKIs	59
TKIs	Lung adenocarcinoma	Studying the resistance mechanisms of TKIs	60
TKIs	Lung adenocarcinoma	Studying the resistance mechanisms of TKIs	61
TKIs	Gastric cancer	Studying the resistance mechanisms of TKIs	62
TKIs	Non-small cell lung cancer	Preclinical validation of TKI efficacy	63
TKIs	Non-small cell lung cancer	Preclinical validation of TKI efficacy	64
TKIs	Lung cancer	Predicting patients' responses to drugs	37
TKIs	Lung adenocarcinoma	Predicting patients' responses to drugs	65
mAbs	Breast phyllodes tumor	Evaluating the efficacy of mAbs	68
mAbs	Neuroendocrine prostate cancer	Evaluating the efficacy of mAbs	69
mAbs	Hepatoblastoma	Evaluating the efficacy of mAbs	70
mAbs	Colorectal cancer	Elucidating mechanisms of drug resistance	73
mAbs	Prostate cancer	Elucidating mechanisms of drug resistance	74
mAbs	Colon cancer	Drug distribution and monitoring	75
CAR-T	Breast cancer	Evaluating the efficacy of CAR-T therapy	78
CAR-T	Glioblastoma	Evaluating the efficacy of CAR-T therapy	28
CAR-T	Pancreatic cancer	Evaluating the efficacy of CAR-T therapy	83
CAR-T	Cholangiocarcinoma	Evaluating the efficacy of CAR-T therapy	84
CAR-T	Lung cancer	Evaluating the efficacy of CAR-T therapy	86
CAR-T	Ovarian cancer	Evaluating the efficacy of CAR-T therapy	87
CAR-T	B-cell malignancy	Differentiating to produce CAR-T cells	89
CAR-T	Glioblastoma	Personalized screening	90
ICIs	Colorectal cancer	Studying immunotherapy response mechanisms	92
ICIs	Melanoma	Studying immunotherapy response mechanisms	93
ICIs	Gastric cancer	Studying immunotherapy response mechanisms	94
ICIs	Ovarian cancer	Studying immunotherapy response mechanisms	95
ICIs	Pancreatic ductal adenocarcinoma	Studying resistance mechanisms of ICIs	99
ICIs	Colorectal cancer	Studying resistance mechanisms of ICIs	100
ICIs	Melanoma	Evaluating the efficacy of ICIs	27
ICIs	Gastric cancer	Evaluating the efficacy of ICIs	104
ICIs	Colorectal cancer	Evaluating biomarkers' efficacy	101

of personalized tumor vaccines. Second, co-culture systems combining PDOs with immune cells provide a robust platform for preclinical evaluation of tumor vaccines and serve as *in vitro* models for identifying responsive patient populations.

Collectively, the ability of PDOs to maintain parental molecular features and support sample amplification confers distinct advantages. Nevertheless, several challenges remain. Most notable of these challenges is the potential loss of molecular

fidelity during serial passaging, which must be addressed to fully realize the potential of PDOs in tumor vaccine development.

Organoids for antigen discovery and neoantigen identification

Advances in mass spectrometry and next-generation sequencing (NGS) technologies have significantly refined the process of extracting novel tumor antigens from patients' tumor tissues, thereby establishing a robust foundation for the development of personalized antigen peptide vaccines. Meeting the requirements for genomic identification can be difficult given the challenges and limited availability of clinical tumor tissue samples. Developing tissue-derived organoid models to expand the available tissue quantity presents a promising approach to overcome this limitation. Multiple preclinical studies have demonstrated the effectiveness and stability of this approach, showing that the organoid model preserves essential tissue characteristics.

In a recent study on liver and biliary tract tumors Wang et al.[109] utilized organoids and tumor tissues to identify novel antigens. The analysis compared 2,449 mutations from tumor tissues, yielding 9,203 predicted neoantigen peptides with 2,637 mutations from matched organoids, resulting in 9,991 predicted neoantigen peptides. The results showed that organoids retained most of the genetic characteristics, HLA alleles, and a comparable neoantigen profile to the original tumors. The selected antigen peptides were validated within the organoid model, demonstrating effective tumor killing and enhanced response when combined with ICIs. This study highlights the dual role of organoid models in neoantigen prediction and vaccine efficacy assessment. Demmers et al.[110] conducted a study to screen HLA class I antigen peptides in CRC organoid models. The findings revealed that 3D spatial signal transduction in organoid cultures provides a more accurate representation of intestinal (patho)physiology compared to traditional 2D *ex vivo* cultures. The study allowed for a direct assessment of protein and ligand specificity in CRC cells by analyzing normal colon organoids from the same patient in parallel, leveraging a largely identical genetic background. Parikh et al.[111] evaluated the use of organoid models in screening for novel antigens in common epithelial cancers. The results demonstrated that organoid models exhibit high genetic fidelity, supporting the utility of organoid models as effective tools for novel antigen screening.

In addition to serving as a tool for post-expansion screening, organoid models have also been applied to validate new antigens identified through screening, providing a crucial step in confirming the therapeutic potential. Ren et al.[112] utilized circular RNA to deliver novel antigens and validated the approach in organoid models. The results demonstrated selective tumor cell killing in tumor-derived organoids, while no significant cytotoxic effect was observed in normal tissues, highlighting the specificity and potential of this method for targeted cancer therapy. Newey et al.[113] tested the detection of novel antigens in CRC organoids derived from patients and validated the alterations in these antigens using organoid models. Newey et al.[113]demonstrated the potential of organoids in identifying and confirming the presence of novel antigens, which could inform future therapeutic strategies. Similarly, Liu et al.[114] used an organoid model to validate the efficacy of novel antigen peptides in inducing antitumor killing effects. The findings highlighted the potential of organoid models in assessing the therapeutic effectiveness of new antigen-based strategies.

New organoid models are being developed continuously to meet the ever-changing needs of vaccine validation with a focus on intestinal tumors. Song et al.[115] developed an organoid and metastatic *in situ* mouse model of mismatch repair-deficient CRC. This model holds potential for future use in validating the efficacy of tumor vaccines, providing a relevant platform for testing vaccine responses in a more clinically representative context. Accompanying detection methods for organoids, such as sequencing techniques based on organoid platforms, are gaining widespread recognition. Wu et al.[116] developed a sequencing technology tailored for CRC organoids, demonstrating the continuous advancements in related technologies for these models. This progress enhances the precision and applicability of organoid-based research in clinical and preclinical settings.

In conclusion, PDO models hold substantial promise for screening and validation of neoantigens. However, careful consideration must be given to the potential loss of neoantigen expression during serial passaging. Determining the optimal passage number and timing is essential to ensure the reliability and accuracy of neoantigen identification.

Preclinical testing of tumor vaccines with organoids

In previous studies, PDX models have been the predominant platform for preclinical research on tumor vaccines. While

PDOs have shown significant potential in neoantigen screening and the evaluation of validity in co-culture systems, PDOs are currently used primarily as complementary tools to PDX models. This approach is largely due to the complex mechanisms underlying immunotherapy, which are more comprehensively represented in the *in vivo* context of PDX models or PDO-transplanted mouse models. However, it is noteworthy that the success rate of establishing PDX models is generally lower than PDO models. As a result, reliance on the PDX model alone may lead to inefficient use of limited and valuable clinical specimens. In contrast, PDOs and PDO-transplanted models offer a more efficient approach to utilizing these samples. This finding may explain why many studies adopt a combined strategy, using both PDX and PDO models to maximize the reliability and translational value of preclinical tumor vaccine research.

Retain genetic heterogeneity and support personalized prediction

GBOs, as a method to expand limited tumor tissue, have been shown to faithfully reproduce tumor phenotypes and closely resemble the transcriptomic, proteomic, and metabolomic characteristics of the parental tissues[117]. This has paved the way for screening novel antigens at the organoid level for brain gliomas and suggests the feasibility of using organoids as validation tools. However, preclinical validation at the organoid level has not yet been conducted. Additionally, the complex TME of gliomas cannot be fully replicated, which remains a significant obstacle to the broader application of organoid models[118]. Notably, glioma vaccines have shown promising efficacy and safety in clinical practice, highlighting the potential as a viable immunotherapeutic approach for glioma treatment. GB tumor vaccines, including mRNA, peptide, and DC vaccines, are widely studied as potential therapeutic options[118,119]. Personalized antigen peptide vaccines have been studied in glioblastoma and have undergone clinical trials. The personalized peptide vaccine was integrated into standard care for GBM patients in the phase I GAPVAC trial. The trial used two treatment phases to extend the therapeutic window. Patients were randomly assigned to two groups. One group received the APVAC 1 vaccine before standard treatment, while the other group received the APVAC 2 vaccine at least 1 week after completing the standard treatment cycle. The trial included 15 patients with a median overall survival (OS) of 29.0 months and progression-free survival (PFS) of 14.2 months. *In vitro* and sequencing experiments showed that APVAC 1 induced persistent $CD8^+$ T cell immunity lasting several months. In contrast, APVAC 2 induced a multifunctional $CD4^+$ T cell response with some responses accompanied by $CD8^+$ T cell activity. These findings highlight the potential roles of HLA-I and HLA-II in antitumor immunity and support the promising application of personalized antigen peptide vaccines[120]. In the future, glioma organoids capable of rapid large-scale expansion while retaining parental cell characteristics and maintaining heterogeneity are expected to be integrated into glioma vaccine development. These advanced models will likely have a crucial role in optimizing treatment strategies and enhancing therapeutic efficacy.

Preclinical studies on the use of organoids in CRC vaccine development primarily focus on the selection of novel antigens but there are currently no studies evaluating the effectiveness of these vaccines in organoid models. As mentioned earlier, several studies have utilized CRC-like organoids as effective models for developing new methods to screen novel antigens, although these studies have not specifically targeted CRC itself[110,112,115].

Assessment of vaccine efficacy and drug resistance

As previously noted, the current standard practice for evaluating the efficacy of tumor therapeutics, including vaccines, still relies heavily on animal models. Immunogenicity testing of vaccine antigens is typically conducted using ELISpot assays with patient-matched PBMCs. Despite the growing utility of the PDO model, several limitations remain in the context of vaccine validation. These limitations include the inability to fully replicate the native TME and the need to co-culture PDOs with TILs or PBMCs to observe immune responses. In contrast, the PDX model allows for a more comprehensive analysis of tumor-immune interactions through integrated multi-omics approaches. Nevertheless, the PDO model has a crucial complementary role in assessing vaccine efficacy and drug resistance. The advantages in early-stage screening and drug sensitivity profiling underscore the value as a precursor to PDX studies and as a tool with strong translational potential for personalized medicine.

Qin et al.[121] utilized organoid models to explore the role of MAGEA, a melanoma-associated antigen subtype, in drug-resistant PDAC. The study provided insights into the mechanisms underlying therapeutic resistance in pancreatic cancer. Furthermore, Qin et al.[121]developed a novel DNA vaccine targeting MAGEA and demonstrated its efficacy in preclinical animal models, offering a promising approach for

overcoming resistance and improving therapeutic outcomes. Huang et al.[122] developed an engineered exosome (HELA-Exos) as a DC-primed vaccine carrier. This exosome significantly enhances the efficiency of DC antigen presentation *in vivo* and *in vitro*, while also increasing the number of $CD8^+$ T cells. Notably, TNBC organoids were used during the validation process and DC activation and $CD8^+$ T cell activation were observed, demonstrating the potential of HELA-Exos in immunotherapy. Despite the lack of further research into the application of organoid models in breast cancer vaccines, the potential value of organoid models in validating the effectiveness of vaccines was demonstrated. Shang et al.[123] reported a nanovaccine immunization strategy utilizing autologous DCs. This approach involved PDOs or cancer cell lysates, which were pulsed with cationic nanoparticles (cNP) to load immunogenic DC-derived microvesicles (cNP-cancer cell @ MVDC). The study demonstrated improved survival rates in *in situ* models of pancreatic and lung cancer. These findings highlight the dual role of organoid models in cancer vaccine research, not only as a platform for validating efficacy and expanding tumor tissues but also as a valuable tool for advancing future research. A summary of the roles and applications of organoids in the development of various tumor vaccines is shown in **Table 3**. **Figure 3** shows how PDOs predict targeted and immunotherapy efficacy.

The unique pathogenesis and drug resistance mechanisms underlying some cancers are fundamental to cancer heterogeneity. For example, the relationship between GC and *Helicobacter pylori* (*H. pylori*) infection highlights the role of microbial factors in tumor development[124]. Further investigation into these mechanisms is essential for advancing our understanding and improving targeted therapeutic strategies. Accordingly, current research on vaccines in the context of GC primarily focuses on the mechanisms of *H. pylori* with particular attention to the role of cytokines in the anti-*H. pylori* immune response[125]. This emphasis underscores the potential for further exploration and development in this promising area of study. Notably, some studies have utilized organoid models to examine the impact of *H. pylori* on PD-L1 expression[126], suggesting that organoids hold significant potential for advancing research on immunotherapy for GC.

Challenges and future prospects of organoid technology in clinical applications

Organoid technology has a crucial role in cancer research by enabling researchers to simulate and study the tumor growth environment in the laboratory. This allows for a deeper understanding of cancer heterogeneity and complexity, while also addressing ethical concerns associated with animal models. Organoids serve as an excellent platform for discovering new anti cancer targets and identifying potential neoantigens for tumor vaccines. The integration of organoid technology with microfluidic chips and multi-omics techniques has opened new frontiers in tumor research. Moreover, by generating organoids from patients' tumor cells, researchers can predict patient-specific responses to immunotherapy at high throughput, which is essential for personalized immunotherapy and vaccine development, thereby advancing the field of precision medicine.

Currently, organoid research faces several challenges. Among the most pressing is the need for simpler and more convenient cultivation methods that ensure organoids accurately mimic the physiological status and functions of human organs. Maintaining organoid stability during passaging and translating research findings into clinical applications also remain key obstacles. Moving forward, it is crucial to continually refine and optimize cultivation techniques, striving for more straightforward methods and standardized protocols. Additionally, establishing a robust quality control system is

Table 3 Functions and applications of organoids in the development of cancer vaccines

Type of cancer	Type of vaccine	Antigen	Role of organoid	Reference
Colorectal cancer	Peptide	Personalized neoantigen	Pre-clincal and antigen selected	110
Colorectal cancer	CircRNA	Personalized neoantigen	Functional validation	112
Pancreatic cancer	DNA	Multi-MAGEA antigen	Functional validation	121
Breast cancer	DC vaccine	Personalized neoantigen	Functional validation	122
Pancreatic cancer and lung cancer	$cNP_{cancer\ cell}@MV_{DC}$	Personalized neoantigen	Vaccine preparation	123

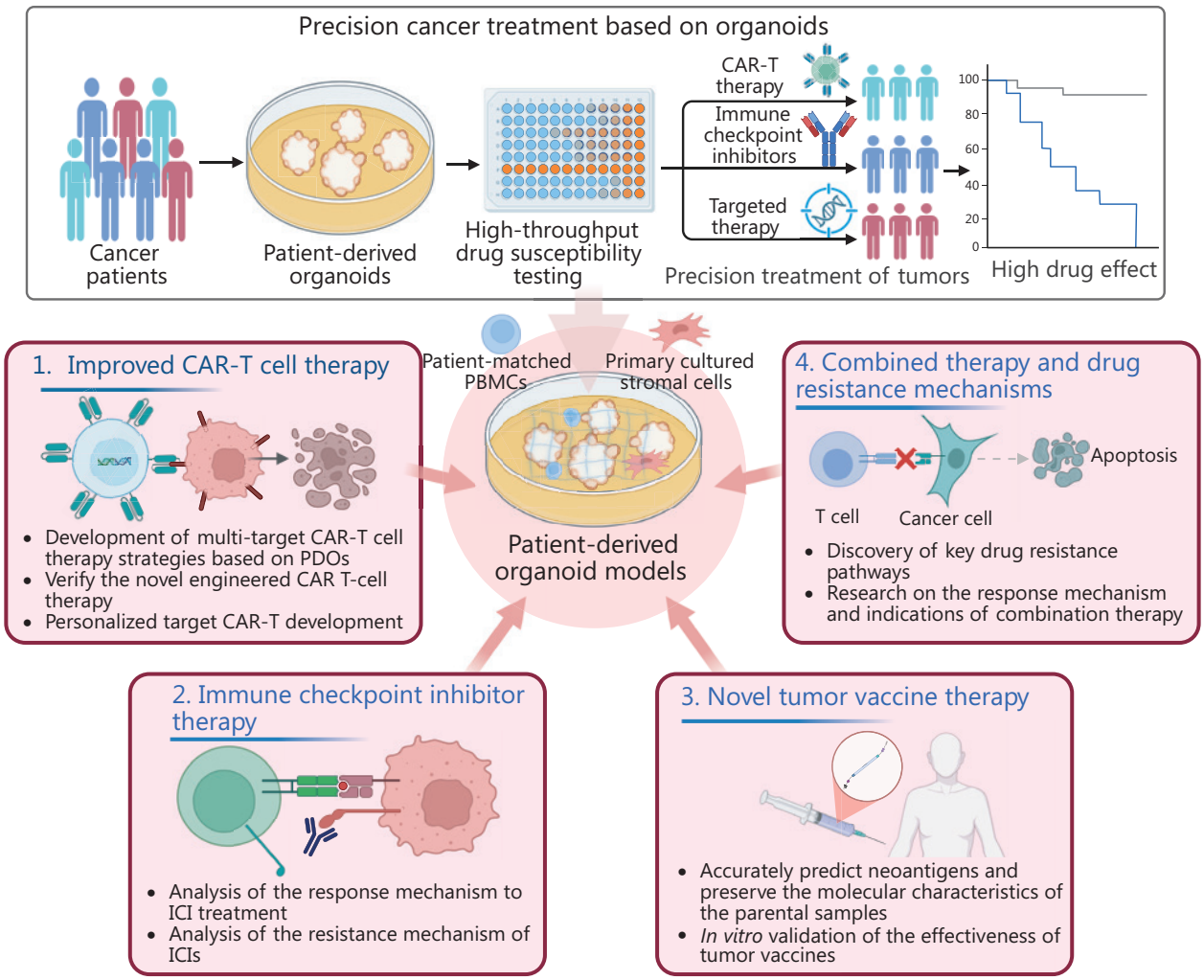


Figure 3 Applications of patient-derived organoid models in cancer immunotherapy and precision treatment strategies. The figure outlines the diverse applications of patient-derived organoids (PDOs) in advancing precision oncology. Tumor tissues from cancer patients are processed to establish organoid cultures that retain patient-specific molecular and phenotypic features. These PDOs serve as a platform for the following: (1) improved chimeric antigen receptor (CAR)-T cell therapy, enabling the development of multi-target CAR constructs, validation of novel engineered CAR-T strategies, and personalization of cell therapy through co-culture with autologous immune cells; (2) analysis of immune checkpoint inhibitor (ICI) therapy, providing insights into response dynamics and mechanisms of resistance to agents targeting PD-1/PD-L1 and CTLA-4; (3) development of tumor vaccines by predicting and validating tumor-specific neoantigens derived from the parental tumor with *in vitro* confirmation of immunogenicity and efficacy; and (4) investigation of combination therapies and drug resistance mechanisms through functional assays, co-culture systems, and apoptosis evaluation. This integrated organoid-immunotherapy pipeline allows for individualized therapeutic optimization, which enhances the efficacy and precision of clinical treatment strategies. Created with BioRender.com.

essential. This system should include regular genomic and phenotypic analyses to monitor the stability and reproducibility of organoids, allowing for the early detection and correction of deviations.

The future development potential of organoids is vast. The latest development occurred on April 10, 2025 when FDA officially announced that animal testing will be gradually phased out. Indeed, animal testing is an experimental tool that has been in use for nearly a century. Rather, the adoption of emerging alternative technologies, such as organoids and organ-on-a-chip for drug safety testing, will be encouraged by incorporating such data into the fast-track approval mechanism. Moreover, the FDA believes that in the safety evaluation of mAbs, organoid or organ-on-a-chip technology can provide

more human-relevant predictive data, offering more clinically relevant preliminary assessment data for drug development. In the future, multi-organ "human-on-a-chip" systems will enable comprehensive drug efficacy evaluation. For example, by connecting liver, tumor tissue, and immune modules, the tumor-killing effects and organ toxicity of anticancer mAbs can be simultaneously studied in a single microphysiologic system. Similarly, the Center for Drug Evaluation (CDE) of the National Medical Products Administration, building on gene therapy, further classified organoids as cell therapy products in 2024, explicitly encouraging the use of "models closer to human physiological conditions" (including organoids) to replace some animal testing, which is aligned with international regulatory trends.

With rapid advances in bioengineering, materials science, and computational modeling, organoids are expected to more accurately simulate the complex structure and function of the TME, providing invaluable support for tumor models, drug development, and personalized medicine. In the future the integration of organoid technology with various cutting-edge technologies will be crucial in advancing precision medicine, high-throughput drug screening, and vaccine development, driving significant progress in the healthcare field.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

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