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Chemotherapy-responsive hepatocellular carcinoma cell lines in organoid systems: Advances and clinical applications

Abstract

Hepatocellular Carcinoma (HCC) remains a challenging malignancy with poor prognosis despite therapeutic advances. Although immunotherapy has gained attention, chemotherapy continues to play a central role in systemic treatment, particularly in advanced or drug-resistant disease. Traditional preclinical models fail to capture the complexity of tumor heterogeneity and chemoresistance mechanisms. Recent progress in three-dimensional organoid culture, especially when derived from chemotherapy-sensitive or drug-resistant HCC cell lines, provides a valuable platform for preclinical testing. These organoid systems preserve tumor diversity, replicate microenvironmental influences, and allow screening of cytotoxic drugs and combination regimens. This review highlights the limitations of conventional HCC models, discusses the integration of chemotherapy-responsive and resistant cell lines into organoid systems, and emphasizes their translational value for guiding clinical chemotherapy strategies and personalized oncology care. Immunocompetent Hepatocellular Carcinoma Cell Lines in Organoid Systems: Implications for Chemotherapy Research and Clinical Case Applications chemotherapy discovery and personalized oncology.

Keywords: Chemotherapy; Immunocompetent cell lines; Organoids; Tumor microenvironment.

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Background and rationale for chemotherapy in HCC

Hepatocellular Carcinoma (HCC) is a leading cause of cancer-related mortality worldwide. Despite advances in systemic therapies, the prognosis for patients with advanced HCC remains poor. Chemo checkpoint inhibitors have emerged as a therapeutic option, yet response rates remain modest due to the complex Tumor Microenvironment (TME) [1]. A key limitation in the development of effective treatments is the lack of preclinical models that faithfully recapitulate chemo–tumor interactions [2]. Recent efforts have focused on utilizing immunocompetent HCC cell lines to generate organoid systems capable of supporting chemo co-cultures. These organoid systems provide a more accurate platform for assessing immunotherapeutic agents and predicting clinical outcomes [3].

The immunological landscape of hepatocellular carcinoma

HCC arises within a liver that is frequently inflamed due to chronic viral hepatitis, alcohol-related liver disease, or non-alcoholic steatohepatitis. This chronic inflammation fosters an immunosuppressive TME that is fundamentally distinct from other solid tumors. The liver's baseline immunotolerant nature—evolved to manage gut-derived antigens—becomes hijacked by tumor cells to facilitate chemo escape [4,5]. Key immunoregulatory cell types dominate the HCC TME, including regulatory T cells (Tregs), Tumor-Associated Macrophages (TAMs), and Myeloid-Derived Suppressor Cells (MDSCs), all of which secrete immunosuppressive cytokines such as IL10 and TGFβ [6,7]. Additionally, tumor cells frequently express chemo checkpoint ligands like PDL1 and CTLA4, which bind to receptors on Cytotoxic T cells ($CD8^+$), inducing exhaustion and apoptosis [8,9]. Another hallmark is the activation of the Wnt/-

catenin pathway, which reduces dendritic cell recruitment and impairs T cell priming, leading to an immunologically "cold" tumor [10,11]. These features collectively contribute to the poor response rates observed with chemotherapy in HCC. Therefore, preclinical models aiming to evaluate or optimize immunotherapeutic strategies must closely replicate this immunological landscape to yield translationally relevant data.

Limitations of traditional hepatocellular carcinoma models

Traditional models used in HCC research, such as two-dimensional (2D) cell cultures and xenograft models in immunodeficient mice, have significantly contributed to our understanding of tumor biology. However, these models are profoundly limited in their ability to replicate the complex tumor–chemo interactions that exist in human HCC. Cell lines like HepG2, Huh7, and PLC/PRF/5, though widely used, lack expression of key chemo molecules such as MHC class I and co-stimulatory signals, making them unsuitable for studying antigen presentation and T cell activation [12,13]. Additionally, they fail to maintain tumor heterogeneity, stromal interaction, or hypoxic gradients seen in vivo. Xenograft models using immunodeficient mice, such as NSG or nude mice, are unable to support human chemo cell functions, precluding the evaluation of immunotherapies like checkpoint inhibitors or CART cells [14,15]. Furthermore, Patient-Derived Xenografts (PDXs), while preserving histological architecture, lack autologous chemo context and are time- and resource-intensive [16]. These limitations collectively lead to poor predictability in clinical translation, as drugs that show efficacy in these systems often fail in human trials. Thus, there is a critical need for next-generation models that retain both tumor intrinsic properties and chemo competence to better reflect the clinical reality of HCC.

Immunocompetent hepatocellular carcinoma cell lines for preclinical immunology

To overcome the inherent limitations of traditional HCC models, researchers have increasingly turned to immunocompetent HCC cell lines for use in preclinical immuno-oncology studies. These models, particularly murine HCC cell lines such as Hepa1-6 and TIB-75, can be engrafted into syngeneic, immunocompetent mice (e.g., C57BL/6), preserving the host's functional chemo system [17,18]. This allows for in vivo studies of tumor-chemo interactions, including T cell infiltration, cytokine signaling, chemo checkpoint expression, and immunoediting processes [18,19]. These models have proven particularly useful for evaluating the efficacy of chemo checkpoint inhibitors such as anti-PD-1 and anti-CTLA-4 antibodies, as well as combination immunotherapies [19,20]. Moreover, advances in gene editing technologies like CRISPR/Cas9 have enabled precise manipulation of these cell lines to model key mutations, enhance tumor antigenicity, or introduce reporter systems for in vivo imaging [21]. The major chemo checkpoints in HCC and their associated resistance mechanisms are outlined in (Table 1). Importantly, these cell lines are now being adapted into in vitro 3D systems, including organoids, to further enhance their physiological relevance [22]. Such innovations represent a crucial step forward in creating more predictive and translatable platforms for chemotherapy research in HCC.

Table 1: Immune checkpoints and associated resistance mechanisms in HCC.

Checkpoint category	Checkpoint category	Resistance mechanisms identified
PD-1 / PD-L1	Suppresses activation of effector T lymphocytes	β-catenin pathway activation; loss of MHC-I expression
CTLA-4	Restricts initiation of T cell priming	Expansion of regulatory T cells (Tregs) within the tumor milieu
Other inhibitory receptors (LAG-3, TIM-3, etc.)	Provide compensatory immunosuppressive signaling	Upregulated expression during single-agent ICI therapy

Organoid technology and hepatocellular carcinoma modeling

Organoid technology has emerged as a transformative platform in cancer research, enabling 3D in vitro modeling of tumor biology with greater physiological relevance than traditional 2D cultures. In the context of HCC, organoids derived from patient tumors or HCC cell lines faithfully recapitulate many of the key histological, molecular, and genetic features of the primary tumors, including driver mutations such as those in TP53, CTNNB1, and AXIN1 [23,24]. These 3D models preserve cellular heterogeneity, clonal architecture, and intrinsic signaling pathways, making them highly valuable for studying tumor progression, drug response, and resistance mechanisms [24,25]. Unlike 2D cultures, which fail to mimic the spatial organization and microenvironmental interactions present in solid tumors, organoids allow for the maintenance of cell polarity, nutrient gradients, and hypoxic zones—conditions that influence gene expression and therapeutic susceptibility [25,26]. This is especially critical in HCC, where tumor biology is shaped by its complex microenvironment. HCC organoids can be established from a variety of clinical sources, including resected tissues, biopsies, and even circulating tumor cells, facilitating real-time patient-specific modeling [27]. These models have already been used successfully to evaluate the efficacy of chemotherapeutics, kinase inhibitors, and targeted therapies, with some studies demonstrating strong concordance between organoid response and patient outcome [27,28]. However, despite their advantages, conventional HCC organoids are largely epithelial and lack the non-tumor components of the TME, such as chemo cells, fibroblasts, and vasculature [29]. This absence limits their utility in chemotherapy research, where interactions between cancer and chemo cells—particularly T cells, macrophages, and dendritic cells—are essential for understanding therapeutic response [29,30]. Moreover, the immunosuppressive features characteristic of HCC, including expression of PD-L1, TGF-β signaling, and the presence of regulatory chemo subsets, are not recapitulated in organoid monocultures [30,31]. To address these shortcomings, efforts are now focused on developing organoid models derived from immunocompetent HCC cell lines that can support chemo co-cultures [31,32]. Such platforms enable the simulation of cytotoxic T cell infiltration, antigen presentation, chemo checkpoint regulation, and cytokine dynamics in a controlled and measurable environment [32]. When combined with technologies such as CRISPR-based gene editing, live-cell imaging, and single-cell RNA sequencing, these organoid systems provide unparalleled opportunities for mechanistic studies and drug screening [32,33]. In summary, organoid modeling of HCC represents a significant advancement in preclinical cancer research. By bridging the gap between reductionist 2D systems and complex in vivo models, organoids provide a flexible, patient-relevant, and scalable platform. Their adaptation for chemo-oncology applications, particularly through the use of immunogenic cell lines and co-culture systems, marks a critical evolution toward more predictive and translational models

in the fight against liver cancer. Furthermore, the use of immunogenic HCC cell lines—those capable of presenting antigens and eliciting T cell responses—is essential for creating physiologically relevant models for immuno-oncology [33,34]. These cell lines enable chemo cells to interact meaningfully with tumor cells in a way that reflects in vivo chemo surveillance and evasion. Without immunogenicity, co-cultured chemo cells remain inactive, resulting in misleading conclusions about therapeutic efficacy. In addition, development of sorafenib-resistant HCC cell lines and their subsequent integration into organoid systems has emerged as a crucial tool for studying acquired drug resistance [34]. Since sorafenib is one of the most widely used systemic treatments for HCC, understanding the mechanisms underlying its failure is critical for identifying second-line or combination therapies. Organoids established from these resistant cell lines can be used to screen alternative agents—including ICIs, multikinase inhibitors, or epigenetic drugs—in a patient-specific manner [34,35]. This approach not only facilitates more precise drug selection but also mirrors clinical treatment decision-making under conditions of therapeutic resistance.

Chemo-augmented organoid development

While conventional organoid models have greatly advanced our ability to study tumor biology in vitro, they fall short in modeling the complex chemo interactions that govern cancer progression and response to chemotherapy. This is especially true in HCC, where the TME is dominated by intricate immunosuppressive networks involving various chemo cell subsets. As a result, a new generation of models—chemo-augmented organoids—has emerged as a powerful solution to bridge this gap [36,37]. These advanced systems incorporate chemo cells into organoid cultures, enabling a more comprehensive simulation of in vivo tumor–chemo dynamics [38,39]. Chemo-augmented organoids are typically developed through co-culture techniques that introduce autologous or allogeneic chemo cells, such as Peripheral Blood Mononuclear Cells (PBMCs), Tumor-Infiltrating Lymphocytes (TILs), Natural Killer (NK) cells, or Dendritic Cells (DCs), into established organoid systems [37,39,40]. These models preserve the 3D architecture and molecular heterogeneity of the tumor while allowing for direct interactions with chemo cells, such as antigen recognition, cytokine production, and cytotoxicity. Notably, studies have shown that T cells introduced into such systems can maintain functional activity, undergo clonal expansion, and exert tumor-specific killing effects, as evidenced by granzyme B release and IFN-γ production [39-41]. One of the key advantages of chemo-augmented HCC organoids is their ability to simulate chemo checkpoint signaling. For instance, the upregulation of PD-L1 on tumor cells and PD-1 on co-cultured T cells can be monitored to assess the Impact of chemo Checkpoint Inhibitors (ICIs) in a controlled, patient-specific setting [42,43]. Furthermore, these models allow for real-time evaluation of T cell exhaustion markers (e.g., TIM-3, LAG-3), chemo evasion mechanisms, and the emergence of resistance following treatment. Such data are critical for understanding why only a subset of HCC patients responds to chemotherapy and for identifying predictive biomarkers [42,43].

Technical innovations have further improved the fidelity and utility of chemo-augmented organoid systems. Microfluidic devices ("organs-on-chips") now enable dynamic perfusion and mechanical stress, which can influence chemo cell behavior and drug diffusion [41,44]. Additionally, advances in 3D bioprinting and hydrogel matrix engineering have made it possible to spatially organize chemo and tumor compartments, replicating tumor niches and enabling more physiologically relevant studies [44,45]. Importantly, chemo-augmented organoids offer a unique opportunity for personalized medicine. Because these models can be derived from patient-specific tumors and autologous chemo cells, they can serve as ex vivo testbeds to predict individual responses to chemotherapy regimens before initiating treatment [42,46]. This has already been demonstrated in other cancers, such as colorectal and non-small cell lung cancer, and is now being actively explored in HCC. The integration of next-generation sequencing, single-cell RNA-seq, and spatial transcriptomics allows researchers to map chemo cell phenotypes and states within the organoid system, adding another layer of mechanistic insight [40,41,46]. In summary, chemo-augmented HCC organoids represent a major advancement in preclinical immuno-oncology. By providing a physiologically relevant platform that captures both tumor architecture and chemo complexity, they pave the way for improved drug discovery, biomarker development, and individualized treatment planning in hepatocellular carcinoma.

Tumor–chemo interactions in co-culture systems

Understanding tumor–chemo interactions is central to the development of effective cancer immunotherapies. In HCC, these interactions are particularly complex due to the liver's intrinsic chemo tolerance and the immunosuppressive nature of the HCC microenvironment. Co-culture systems—where tumor cells are grown alongside chemo cells—provide a valuable framework for dissecting these interactions in vitro. When combined with organoid technology, co-culture systems allow for high-fidelity modeling of cellular cross-talk, chemo evasion, and therapeutic response within a 3D context [47,48]. In chemo-augmented HCC organoids, chemo cells such as $CD8^+$ cytotoxic T lymphocytes, $CD4^+$ helper T cells, tumor-associated macrophages, or dendritic cells can be introduced to simulate the functional dynamics observed in patient tumors [48,49]. These co-cultures enable investigation into how tumor cells modulate chemo activity through checkpoint ligands like PD-L1 and CTLA-4 or via secretion of immunosuppressive cytokines such as TGF-β and IL-10 [49,50]. Conversely, researchers can observe how chemo cells respond to tumor antigens, proliferate, and engage in cytolytic activity. One particularly valuable insight gained from co-culture systems is the induction and progression of T cell exhaustion—a hallmark of chronic liver inflammation and advanced HCC. Organoids allow for temporal analysis of exhaustion marker expression, including PD-1, LAG-3, TIM-3, and TIGIT, on TILs [50,51]. Monitoring these markers provides key information about the immunological health of the T cell population and its potential responsiveness to chemo checkpoint blockade. Moreover, real-time imaging technologies have made it possible to visualize immunological synapse formation between T cells and tumor cells, providing functional readouts such as calcium flux, granzyme B release, and tumor cell apoptosis [51,52]. These systems are also instrumental in understanding mechanisms of chemo evasion. For instance, activation of oncogenic pathways such as Wnt/β-catenin has been shown to suppress chemokine production (e.g., CCL5, CXCL9), leading to impaired T cell recruitment. Co-culture organoid models can recapitulate such signaling changes and allow for perturbation studies using CRISPR/Cas9 or small-molecule inhibitors [52,53]. Additionally, myeloid cell co-cultures enable investigation into macrophage polarization (M1 vs. M2), antigen presentation by dendritic cells, and the role of MDSCs in modulating T cell responses [53].

Applications in chemotherapy screening

The clinical landscape of HCC has increasingly embraced chemotherapy, particularly ICIs, as a promising treatment modality. However, only a fraction of patients experiences durable responses, largely due to tumor heterogeneity and the complexity of chemo regulation within the TME. To improve response rates and identify optimal therapeutic combinations, preclinical screening platforms that closely reflect the chemo–tumor interface are urgently needed. In this context, immunocompetent organoid-based co-culture systems have emerged as powerful tools for chemotherapy screening, offering a high degree of physiological relevance, scalability, and personalization [54,55]. One of the primary applications of these models is the evaluation of ICIs targeting PD-1, PD-L1, and CTLA-4. By co-culturing HCC organoids with autologous or allogeneic T cells, researchers can assess chemo activation, exhaustion, and cytotoxicity in response to checkpoint blockade. Readouts such as IFN-γ release, granzyme B production, and tumor cell lysis provide quantifiable metrics for chemo efficacy [55,56]. Furthermore, chemo-augmented organoids allow real-time monitoring of resistance mechanisms, such as MHC downregulation, upregulation of alternative checkpoints (e.g., LAG-3, TIM-3), and immunosuppressive cytokine secretion, thus supporting iterative testing of combination therapies [56,57]. These platforms also facilitate the screening of engineered chemo cell therapies, including Chimeric Antigen Receptor (CAR) T cells and T Cell Receptor (TCR)-engineered cells. Immunocompetent organoids expressing tumor-associated antigens (e.g., GPC3, AFP) can be used to evaluate CAR-T cell specificity, activation, and persistence. In one study, CAR-T cells targeting GPC3 showed robust cytotoxicity in HCC organoid co-cultures, demonstrating the feasibility of using such models for efficacy and toxicity profiling prior to clinical translation [58].

High-throughput adaptations of organoid–chemo co-culture systems further extend their utility. Automation and miniaturization into multi-well formats enable simultaneous testing of hundreds of compounds or chemo effector cell populations. This is particularly valuable for combinatorial approaches, such as ICIs with kinase inhibitors or anti-angiogenic agents, which require detailed dose–response analysis [59]. These models also support functional genomics screening using CRISPR-Cas9 libraries to identify genes associated with chemo sensitivity or resistance [60]. Importantly, organoid-based platforms can be used to guide personalized treatment decisions. Patient-Derived Organoids (PDOs), when co-cultured with autologous chemo cells, reflect individual tumor–chemo dynamics, offering an ex vivo prediction of treatment outcomes. This personalized approach can inform the selection of ICIs, cellular therapies, or immunomodulatory agents on a case-by-case basis, reducing trial-and-error in clinical settings [60]. Representative applications of organoid–chemo co-culture systems in chemotherapy screening are summarized in Table 2. Moreover, these models are compatible with next-generation analytics, such as single-cell RNA sequencing, T cell receptor repertoire profiling, and cytokine multiplex assays. These tools enable a deep understanding of chemo engagement and provide biomarkers for clinical response prediction. Biomarkers identified through organoid platforms can be validated in larger patient cohorts, accelerating their incorporation into clinical practice [54]. In summary, immunocompetent HCC organoid models represent a next-generation platform for chemotherapy screening. They offer dynamic, patient-relevant systems for evaluating drug efficacy, studying resistance, and guiding personalized immuno-oncology. Their continued development and integration into drug pipelines hold great potential for improving therapeutic outcomes in liver cancer.

Table 2: Functional applications of HCC organoid systems in preclinical studies.

Application domain	Description (Rephrased)	Outcome measures
ICI evaluation	Liver cancer (HCC) organoids maintained with T cell co-cultures	IFN-γ secretion, granzyme B activity, tumor cell killing
Analysis of resistance pathways	Continuous monitoring of immune evasion in organoid systems	Reduced MHC expression, induction of alternative inhibitory receptors
Evaluation of adoptive cell therapies	CAR-T or TCR-engineered T cells combined with tumor-derived organoids	Cytotoxic potential, antigen specificity, long-term persistence
High-throughput drug screening	Miniaturized, multi-well automated organoid co-culture systems	Combination testing of ICIs with kinase inhibitors
Personalized medicine approaches	Patient-specific organoids integrated with autologous lymphocytes	Ex vivo prediction of therapeutic response tailored to the patient

Challenges and future directions

Despite the remarkable progress in the development of immunocompetent HCC organoid models, several challenges remain that hinder their widespread adoption and standardization in research and clinical translation. These limitations span technical, biological, and logistical domains, and addressing them is critical to fully realize the potential of these systems as predictive platforms for chemotherapy. One of the foremost technical challenges is maintaining chemo cell viability and function within the organoid co-culture over extended periods. Chemo cells, particularly T lymphocytes and dendritic cells, are sensitive to the culture conditions typically optimized for epithelial organoids. Differences in cytokine requirements, growth factors, and oxygen tension can lead to premature chemo cell exhaustion or apoptosis, limiting the duration and interpretability of co-culture experiments. Strategies to improve chemo cell integration, such as temporal cytokine pulsing or microfluidic perfusion systems, are actively being explored to mitigate this issue. Another significant barrier is the lack of stromal and vascular components in most current organoid systems. In vivo, the TME includes fibroblasts, endothelial cells, and extracellular matrix proteins, which contribute to chemo regulation, drug diffusion, and tissue remodeling. Their absence in conventional organoid systems results in an oversimplified model that fails to recapitulate key aspects of tumor biology, such as chemo cell trafficking, angiogenesis, and hypoxia-driven signaling. Recent advances in 3D bioprinting and scaffold engineering offer promising avenues to incorporate these components in a spatially organized manner, thus enhancing the physiological relevance of the models. Standardization and reproducibility also pose substantial hurdles. Organoid generation protocols vary widely between laboratories, leading to batch-to-batch variability and inconsistent outcomes. This issue becomes even more pronounced when chemo cells are introduced, as donor variability, cell activation status, and co-culture ratios can drastically affect experimental results. To address this, consortiums and guidelines are emerging to promote harmonized methodologies, quality control checkpoints, and open-access biobanks of validated organoid lines. From a translational perspective, another critical challenge lies in scaling these models for high-

throughput applications. Drug discovery and biomarker validation require systems that are not only biologically relevant but also compatible with automation and cost-effective screening. While miniaturized organoid formats and robotic handling systems are under development, further optimization is required to maintain model fidelity while enhancing throughput. Ethical and regulatory considerations are also emerging as organoid technologies approach clinical application. The use of patient-derived materials, particularly in personalized medicine, necessitates robust consent procedures, data privacy protections, and equitable access to these technologies. Furthermore, as organoid-based assays begin to influence clinical decision-making, validation against gold-standard outcomes in prospective trials will be essential for regulatory approval and clinical integration. Looking forward, integration of multi-omics approaches—such as single-cell RNA sequencing, proteomics, and epigenomics—into organoid systems will provide unprecedented insights into chemo-tumor interactions. Spatial transcriptomics, in particular, will allow for the mapping of chemo gradients and cellular niches within the 3D architecture. Artificial intelligence and machine learning are also poised to play a role in analyzing complex datasets and predicting treatment responses based on organoid behavior. In conclusion, while immunocompetent HCC organoid models represent a transformative leap in preclinical modeling, their full potential has yet to be realized. Continued innovation in bioengineering, culture optimization, and analytical integration will be key to overcoming current limitations. As these challenges are addressed, organoid systems are likely to become indispensable tools for chemotherapy development, precision medicine, and ultimately improving outcomes for patients with liver cancer.

Conclusion and future chemotherapy perspectives

The integration of immunocompetent HCC cell lines into three-dimensional organoid platforms marks a pivotal advancement in preclinical cancer modeling. Unlike conventional systems, these chemo-augmented organoids preserve key features of the tumor microenvironment, including dynamic interactions between tumor and chemo cells. This enables a more accurate evaluation of immunotherapeutic agents, including checkpoint inhibitors and cell-based therapies (Figure 1). Moreover, these models support the discovery of resistance mechanisms and predictive biomarkers, making them valuable for both drug development and personalized medicine. Although technical challenges remain, ongoing innovations in co-culture methods, microengineering, and omics integration are rapidly enhancing their fidelity and scalability. As such, immunocompetent HCC organoid systems represent a critical bridge between laboratory research and clinical application, offering new hope for improving therapeutic outcomes in patients with liver cancer.

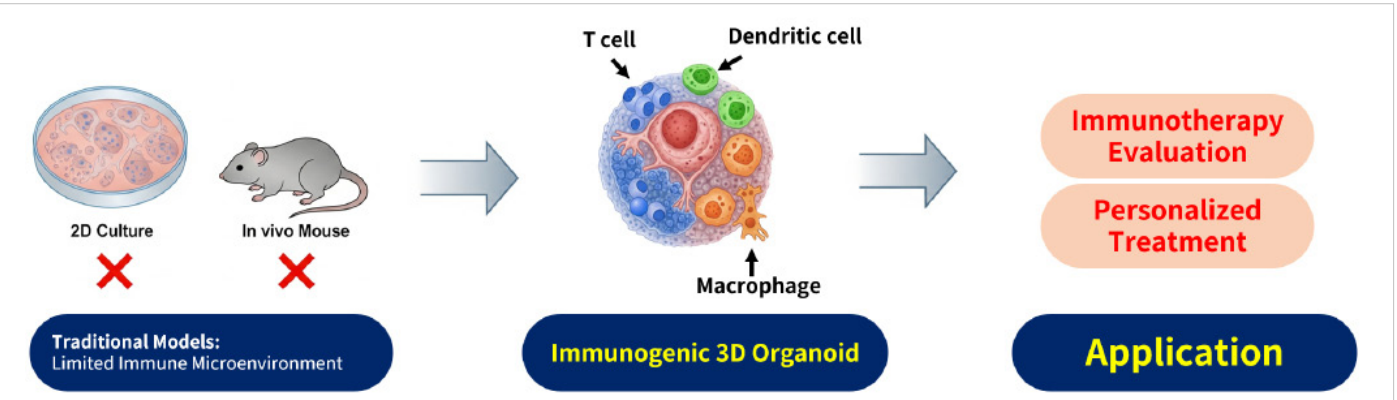

Figure 1: Comparison of traditional models with advanced 3D organoid models and their applications.
This diagram highlights the limitations of traditional 2D cell cultures and in vivo mouse models, which fail to replicate the complex tumor microenvironment. It introduces an advanced 3D organoid model that mimics tumor-immune interactions by co-culturing various immune cells. The figure outlines the applications of this model, including the evaluation of immunotherapeutic agents, elucidation of resistance mechanisms, guidance for personalized treatment strategies, and the discovery of predictive biomarkers.

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