SPANDIDOS PUBLICATIONS

ONCOLOGY REPORTS 55: 49, 2026

Recapitulating lung cancer metastasis *in vitro*: Advances in organoid models and challenges in clinical translation (Review)

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Received May 28, 2025; Accepted November 26, 2025

DOI: 10.3892/or.2026.9054

Abstract. Lung cancer remains a significant global health challenge, with metastatic progression being the leading driver of mortality. Organoid technology provides a tractable, physiologically relevant platform to model key aspects of lung cancer metastasis *in vitro*. The present review summarized methodologies for constructing and interrogating these models, covering tissue sources, culture modalities, gene editing and *in vivo* transplantation; applications in studying metastatic mechanisms, drug screening and capturing intra- and intertumoral heterogeneity are also highlighted. Persistent challenges include standardizing derivation and culture conditions, improving preservation of tumor-microenvironmental interactions, expanding immune-competent and vascularized models, and addressing scalability, cost, and regulatory and ethical considerations for clinical translation. Future directions include integrating multi-omics approaches and spatial profiling, leveraging artificial intelligence for image and response analytics, advancing immune-organoid models and establishing shared standards, reference materials and reporting guidelines to enhance reproducibility and clinical impact.

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Key words: lung cancer, organoids, metastasis model, tumor microenvironment, precision medicine, tumor heterogeneity

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1. Introduction

Metastasis accounts for up to 90% of cancer-related deaths, driven by complex interplay between malignant cells and the tumor microenvironment (1-4). It is a progressive, multistep process in which cancer cells disseminate from the primary tumor, invade surrounding tissue, survive in circulation and colonize distant organs (5,6). A deeper understanding of how cancer cells deviate from normal programs of growth, motility and stromal-immune crosstalk is essential for identifying potential targets and optimal time windows for detection, prevention and treatment of the metastatic diseases.

Lung cancer is the most common and most fatal cancer worldwide, with 2.5 million new cases (12.4% of all global cancers) and 1.8 million deaths (18.7% of global cancer deaths) in 2022 (7); it remains the leading cause of cancer mortality largely due to the high incidence of metastasis (8,9). Non-small cell lung cancer (NSCLC) constitutes 80-85% of all lung cancer cases (10). While surgical resection benefits early-stage NSCLC, a large proportion of patients present with advanced disease, rendering surgery unfeasible. These patients often experience poor survival and diminished quality of life, with limited survival gains from conventional treatments such as chemotherapy or radiotherapy. Prolonged exposure to radiotherapy or chemotherapy frequently induces resistance (11). In clinical practice, multiple therapeutic strategies have been developed to overcome drug resistance and for patients with lung cancer with targetable driver gene mutations, such as

EGFR or ALK, the treatment paradigm has evolved. While starting with first-generation inhibitors (such as gefitinib and crizotinib) was practiced previously, current first-line standards often involve newer agents (such as osimertinib and alectinib). Switching to next-generation inhibitors upon resistance remains the standard strategy (12). For instance, osimertinib effectively treats *EGFR* T790M-mediated resistance after first-generation EGFR-tyrosine kinase inhibitors (13,14). Combination approaches that leverage non-overlapping mechanisms, such as pairing targeted therapy with chemotherapy or anti-angiogenic agents, or using dual-target inhibitors, which aim to suppress parallel pathways, thereby delaying the emergence of resistant clones (15). For patients progressing after targeted therapy or chemotherapy, immune checkpoint inhibitors [such as programmed cell death protein 1 (PD-1)/programmed death-ligand (PD-L1) antibodies] offer an orthogonal strategy independent of traditional cytotoxic pathways (16,17). These agents act by reinvigorating anti-tumor T cells, with enhanced efficacy in subsets characterized by high tumor mutation burden (TMB) or PD-L1 expression (18). These precision treatments have improved outcomes, yet benefit remains heterogeneous and often transient.

Given these advances, a focus of current clinical research is identifying patients most likely to benefit from these therapies early in the treatment course. However, relying solely on biomarkers such as PD-L1 and TMB has notable limitations (19). Among advanced NSCLC with PD-L1 expression ≥50%, response rates to single-agent immunotherapy are ~44% (20). Similarly, for TMB ≥10 mutations per megabase, response rates remain <30% (21). While combining immunotherapy with chemotherapy increases objective response rates to 50-70%, high-dose chemotherapy can significantly damage key effector subsets, including $CD8^+$ T cells, potentially shortening the durability of response relative to immunotherapy alone (22,23). These gaps underscore the need for functional, patient-specific models that more faithfully recapitulate tumor-immune-stromal interactions for drug testing and response prediction.

Accurate modeling of the tumor microenvironment is crucial for effective drug screening, particularly for immunotherapies. Traditional models, such as cell lines and xenografts, have considerable constraints. Extended passaging of cell lines (>10-15 passages) leads to loss of the original intratumoral heterogeneity, and xenografts may not faithfully recapitulate human stromal and immune contexts due to interspecies differences (24,25). Most patient-derived organoid (PDO) models similarly lack critical microenvironment elements, such as vasculature and immune components (26). Even in vascularized organoids, the absence of perfusion often results in vascular regression. To address this, researchers have developed approaches combining vascularized organoids with immune co-cultures (27). Reconstructing the tumor immune microenvironment by co-culturing tumor-infiltrating lymphocytes (TILs) with tumor cells shows promise for more accurately predicting responses to immune checkpoint inhibitors.

Lung cancer organoid-based metastasis models utilize three-dimensional (3D) culture systems that preserve genomic diversity and phenotypic heterogeneity of patient tumors. These models can incorporate key microenvironmental components, including stromal and immune cells, which enables simulation of critical steps of the metastatic cascade and provides a powerful platform for investigating metastasis-driving mechanisms.

A key aspect of developing physiologically relevant lung cancer organoids is the choice of supporting scaffold. Early models predominantly relied on commercially available basement membrane extracts (such as Matrigel) (28). While instrumental for initial success, these matrices suffered from ill-defined composition, batch-to-batch variability and non-physiological mechanical properties. Consequently, there is a growing emphasis on defined and tunable scaffolds, such as synthetic polyethylene glycol (PEG) hydrogels (29) and decellularized extracellular matrix (dECM) hydrogels (30), to provide more precise control over biochemical cues and biophysical properties, improving reproducibility and enabling hypothesis-driven testing of matrix-regulated metastatic behaviors.

The present review summarized recent advances in lung cancer organoid metastasis models, with emphasis on their use in elucidating metastatic mechanisms, optimizing drug screening and resolving tumor heterogeneity. Methodological considerations, persistent technical challenges, barriers to clinical translation and ethical considerations were discussed. The future directions described includes interdisciplinary collaborations involving multi-omics and spatial profiling, artificial intelligence (AI)-assisted analytics, immune-competent and vascularized platforms, and standardization effort. The present review aimed to provide methodological guidance for basic research and a conceptual foundation for developing precision treatment strategies.

Literature search methodology. To comprehensively review advances in this field, a systematic literature search of the PubMed database (https://pubmed.ncbi.nlm.nih.gov/) was conducted for publications from 2009 to 2025, prioritizing studies from the past 5 years, using the terms 'lung cancer organoids', 'metastasis model', 'patient-derived organoids', 'tumor microenvironment' and 'drug screening', alone and in combination, to identify original research articles, high-quality reviews and key methodological papers. Emerging lung cancer organoid-based metastasis models recapitulate critical steps of the metastatic cascade, yielding mechanistic insights and informing therapeutic discovery.

2. Definitions and stem cell origin of organoids

Stem cells are central to life sciences research and are defined by two core properties: Self-renewal and multipotent differentiation. Self-renewal is the capacity to maintain a stable stem cell pool through mitotic division, generating daughter cells with the same genetic characteristics (31). Multipotent differentiation is the ability to give rise to diverse cell types under specific environmental conditions and signaling cues, thereby contributing to tissue formation (32). Organoids are 3D *in vitro* constructs derived from stem cells that recapitulate the cellular composition, spatial organization and selected functions of their native organs. Organoids provide essential platforms for studying developmental biology and disease pathogenesis (33).

Organoids derived from stem cells. Since the pioneering development of intestinal organoids by Sato *et al* (34) in 2009, stem cell-derived organoid technology has rapidly expanded across multiple tissues, including the brain, liver and diverse tumor types, offering new opportunities for drug screening and disease modeling. Brain organoids serve as a prominent example, as they are constructed through the systematic differentiation of neural stem cells and precise cellular interactions, ultimately forming highly complex neural networks. These networks not only exhibit functions similar to those of the natural brain but also provide essential biological markers for investigating brain development and neurodegenerative diseases (35). Leveraging this advantage, researchers have systematically studied the pathogenesis, pathological evolution and potential therapeutic targets of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease using *in vitro* models that closely mimic *in vivo* physiological conditions (36,37).

PDOs. In oncology research, tumor organoids derived from patient samples often retain the phenotypic characteristics, genomic heterogeneity and mutational landscape of the original tumor. Tumor organoids derived from patient samples preserve the architectural, molecular and genetic features of the primary tumor, including structure, function, mutation spectrum and gene expression profile. These organoids are effectively used for disease characterization, mechanism exploration, high-throughput drug screening and evaluation, discovery of innovative therapeutic targets and potential compounds and the development of personalized treatment strategies based on individual tumor characteristics (38).

The construction of lung cancer organoid metastasis models begins with the acquisition of suitable tissue samples. Primary lung cancer tissues, obtained from surgical resections or biopsies are commonly used (39,40). To investigate metastatic mechanisms, tissues from metastatic lesions are particularly valuable, including samples from brain, bone or liver metastases, which directly reflect the characteristics of tumor cells within site-specific metastatic microenvironments. For example, a previous study successfully established organoid models from surgical specimens of brain metastases in patients with lung cancer, providing essential tools for exploring the mechanisms of brain metastasis (41).

Malignant pleural effusions (MPEs), including pleural effusion and ascitic fluids, represent an important source for organoid construction. MPEs are rich in tumor cells, and their collection is relatively noninvasive (42). Mazzocchi *et al* (43) successfully developed 3D lung cancer organoids from pleural effusion. These organoids, composed of tumor and stromal cells embedded in a hydrogel that mimics the ECM, exhibited alveolar-like structures and *in vivo*-like drug responses. The study confirmed that pleural effusion-derived organoids are suitable for investigating lung adenocarcinoma (LUAD) and that 3D cultures outperform two-dimensional (2D) cultures in disease modeling and drug screening. In another study, Wang *et al* (44) established lung cancer organoids from both tumor tissue and MPE. It was found that MPE significantly enhanced organoid formation, reduced culture time by >50% and altered drug sensitivity profiles, resulting in increased resistance to certain drugs. Mechanistically, although MPE did not alter the genomic composition of the organoids, it influenced stem cell distribution and cell architecture, including expansion of the extracellular space. Gene expression analyses revealed significant enrichment of pathways related to the ECM and mitochondrial function.

Distinct challenges in developing lung cancer organoids. The development of lung cancer organoids presents unique challenges that are not typically encountered in other organoid systems. A primary obstacle is the need for multiple, subtype-specific culture protocols to account for the substantial histological and genetic diversity between NSCLC and small cell lung cancer (SCLC). Furthermore, the high clinical mortality associated with metastasis necessitates modeling organ-specific metastatic niches, such as those in the brain and bone. In addition, given the focus on guiding precision therapies, particularly immunotherapy, there is a pressing need to develop sophisticated, immune-competent co-culture models. This combination of factors makes lung cancer organoids a highly specialized and complex platform.

Advantages of organoids as drug screening platforms. Organoids provide physiologically relevant platforms for drug screening and sensitivity testing by closely replicating the *in vivo* architecture and functional characteristics of human tissues. Furthermore, organoids retain tissue-specific features and preserve critical cellular interactions, and capture tumor heterogeneity more accurately. This makes them especially valuable for personalized treatment strategies (45). Drug responses can be evaluated by treating patient-derived tumor organoids (PDTOs) with a range of therapeutic agents and measuring efficacy through multiple analytical readouts. In oncology, organoid-based models are widely used to assess cancer drug sensitivity and to predict therapeutic outcomes, covering a variety of agents, including targeted therapies, immune monotherapies, combination immunotherapies and anti-angiogenic drugs (46,47). For example, clinical studies have employed organoid drug sensitivity assays to anticipate chemotherapy efficacy (48,49).

3. Modeling key steps of the metastatic cascade in organoids

Lung cancer organoid models are powerful tools for deconstructing the complex, multistep process of metastasis into discrete, investigable mechanisms. By engineering specific culture conditions, these models can replicate key biological events, including ECM remodeling, epithelial-mesenchymal transition (EMT) and tumor-immune interactions, which collectively drive metastatic progression.

Simulating metastatic microenvironment conditions. To mimic the *in vivo* tumor microenvironment, lung cancer organoids are cultured in 3D matrices such as Matrigel, which provide both structural support and biochemical cues (50). Cultures are maintained in a base medium (such as advanced DMEM/F12) supplemented with growth factors and nutrients essential for organoid survival and expansion (51,52). Furthermore, media are tailored to histological subtypes. NSCLC organoids typically require the addition of EGF, FGF, R-spondin1 and Noggin, whereas SCLC organoids often need additional factors to maintain their neuroendocrine characteristics (53) Further optimization is necessary to model organ-specific

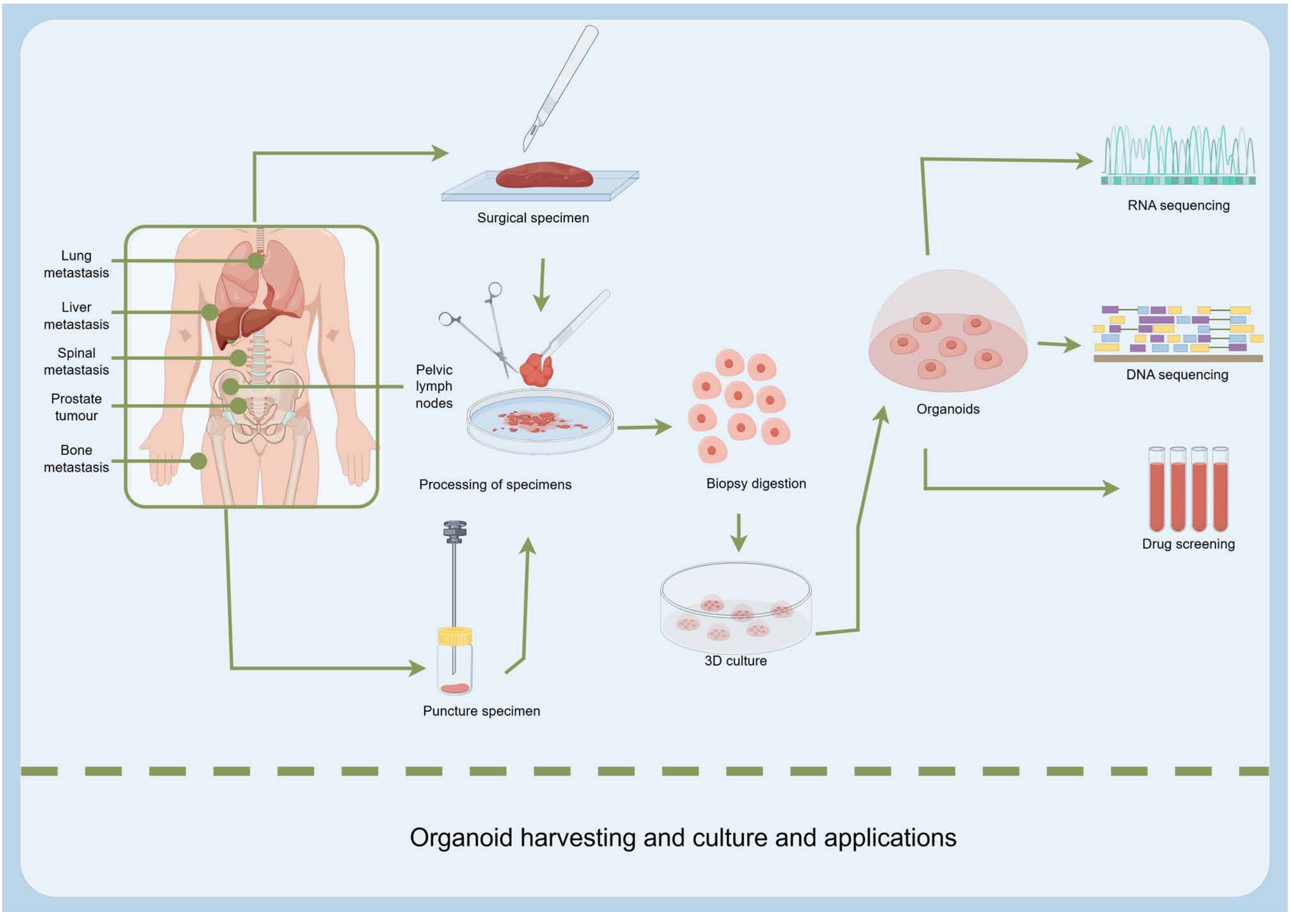

Figure 1. Co-culture of a patient-derived lung cancer organoid with autologous immune cells from peripheral blood. Created by figdraw.com.

metastasis more accurately. For brain metastasis models, neural-supportive medium components such as Neurobasal™ (Gibco; Thermo Fisher Scientific, Inc.) are incorporated into the medium (54). Culture conditions, including oxygen tension, temperature and humidity, are tightly controlled. Hypoxia, in particular, can preserve cancer stem-like phenotypes and upregulate metastasis-associated programs, providing a more physiologically relevant context for studying tumor invasion, spread and therapy response (55,56).

Modeling ECM remodeling. The 3D scaffold not only supports organoid growth but also serves as a modifiable ECM surrogate. Tumor organoids dynamically remodel their surrounding matrix to facilitate invasion, which can be captured by live imaging using fluorescent ECM components (57,58). Parallel biochemical analyses of conditioned media reveals elevated levels of matrix metalloproteinases and other ECM-modifying enzymes, providing quantitative readouts of invasive potential (59).

Inducing EMT. EMT is a key transcriptional reprogramming event that confers migratory and invasive capacities. In organoid systems, EMT can be reproducibly elicited by microenvironmental stimuli. Exposure to hypoxia or exogenous transforming growth factor-β (TGF-β) drives molecular and morphological shifts, including downregulation of epithelial markers (such as E-cadherin), upregulation of mesenchymal markers (such as vimentin) and morphological transitions from smooth, spherical structures to irregular, invasive protrusions. This phenotypic change, often mediated by effectors, directly enhances tumor cell migration and invasion, thereby modeling early metastatic steps (60).

Reconstituting tumor-immune interactions. Immune-organoid co-culture systems are crucial for elucidating the dynamic interactions between tumor cells and the immune microenvironment, particularly the mechanisms by which cancer cells evade immune surveillance during metastasis. Lung cancer organoids can be directly co-cultured with autologous immune components, such as TILs or peripheral blood mononuclear cells (Fig. 1) (61), to recapitulate patient-specific immune responses. This setup enables real-time assessment of immune cell cytotoxicity and tumor cell lysis, and it captures adaptive immune evasion mechanisms, including the upregulation of checkpoint molecules such as PD-L1 on organoid cells. These co-culture models also provide a physiologically relevant platform for evaluating immunotherapies, including immune checkpoint inhibitors such as anti-PD-1/PD-L1 antibodies, by quantifying treatment-induced organoid death (62).

4. Construction strategies and key technologies for lung cancer organoid metastasis models

Leveraging organoids for lung cancer metastasis research requires a multifaceted approach. Key elements include

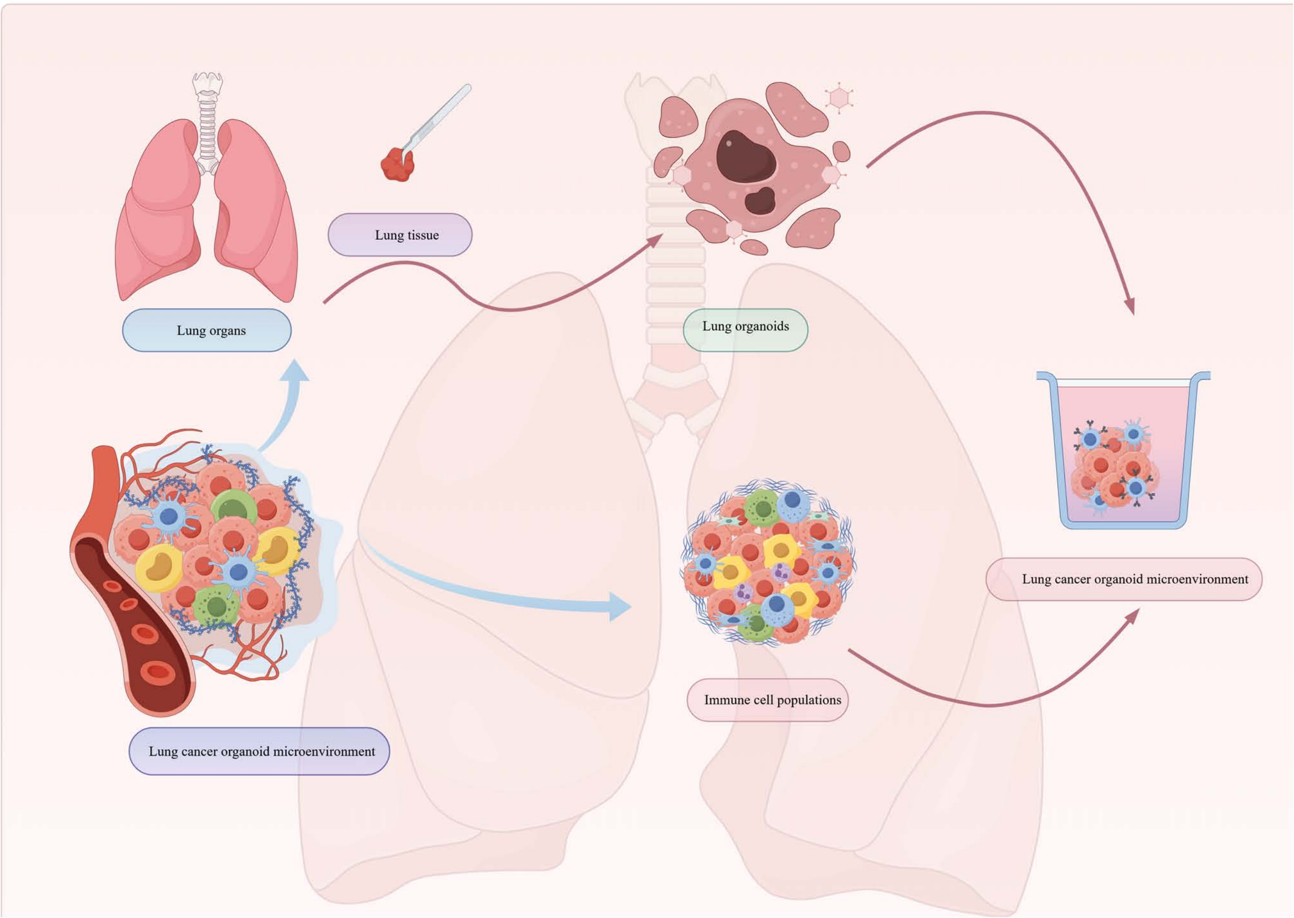

Figure 2. Pipeline for establishing lung cancer organoid models and their downstream applications. Created by figdraw.com.

developing subtype-tailored culture protocols, creating customized models through gene editing, recapitulating metastatic steps via *in vivo* transplantation, and directly capturing disseminating cells using circulating tumor cell (CTC)-derived organoids (Fig. 2; Table I).

Specific culture requirements for primary lung cancer subtypes. The successful establishment of lung cancer organoids critically depends on tailoring culture conditions to each subtype, reflecting their distinct cellular origins and oncogenic drivers.

i) LUAD organoids. LUAD often originates from alveolar type II (AT2) cells and thus requires culture conditions that sustain AT2 stemness and proliferative capacity. The base medium typically includes essential mediators such as EGF, FGF-7/10, Noggin and R-spondin 1 to promote proliferation and maintain an AT2-like state. This protocol was further optimized by inhibiting the BMP signaling pathway and supplementing with ligands such as heregulin-β1 to better mimic the alveolar niche, as demonstrated in feeder-free systems that enable long-term expansion and modeling of LUAD (63,64).

ii) Lung squamous cell carcinoma (LUSC) organoids. LUSC, which arises from bronchial basal cells, often relies more on ECM-derived physical and biochemical cues than exogenous growth factors. A robust LUSC model was established using a base medium without the addition of growth factors such as R-spondin, EGF or BMP-4. The success of this system hinged on a tailored ECM composed of Matrigel and collagen I, as well as the strategic initiation of cultures from pre-formed cell spheroids. This approach reproducibly generated organoids that self-organize into a characteristic hierarchical structure with a p63-positive basal cell layer and inward differentiation, providing a distinctive model for studying LUSC biology (65).

iii) SCLC organoids and metastatic modeling. The high-grade neuroendocrine phenotype of SCLC necessitates specialized culture conditions. A base media typically includes B27 and N2 supplements, key mitogens (bFGF, EGF and insulin), hydrocortisone and ROCK inhibitors to prevent anoikis and support suspension growth. To model metastasis, particularly brain metastasis, advanced platforms co-culture SCLC cells with human cerebral organoids in a Matrigel-based 3D environment. When combined with modulation of key pathways such as Notch and longitudinal monitoring using live-cell imaging, this platform enables real-time assessment of invasion, colonization and subtype plasticity within the brain microenvironment (66,67).

Application of gene editing technologies. Patient-derived lung cancer organoids faithfully replicate the pathological and genomic characteristics of their source tumors, preserving key driver alterations, while maintaining malignant cell phenotypes (68). Using gene editing technologies such as

Table I. Comparative analysis of primary strategies for establishing lung cancer organoid metastasis models[a].

Strategy	Core advantages	Limitations	Relative cost	Translational readiness	Primary application scenarios
PDOs	Preserves genomic heterogeneity, phenotype, and drug response of he toriginal tumor. Short establishment time (weeks), suitable for high-throughput screening. Provides a platform for personalized medicine.	Suboptimal overall culture success rate (particularly for SCLC and rare subtypes). Difficult to fully recapitulate the complete tumor microenvironment (for example, vasculature and immune components). Lack of standardized protocols.	Medium-high	Intermediate (used in retrospective studies guiding clinical therapy, requires prospective trial validation)	Disease modeling, drug sensitivity testing, personalized therapy exploration, tumor heterogeneity research
Gene-edited organoids	Enables precise study of causal roles of specific genes in metastasis. Allows creation of customized models driven by specific driver mutations. Useful for gene knockout, knock in, and correcting resistance mutations	Ethical and safety considerations. Technically complex, requires specialized platform. Single-gene editing may not mimic the complex polygenic background of human cancer.	High	Low (primarily a powerful basic research tool; direct clinical translation path is long)	Mechanism studies, target validation, gene function research, overcoming drug resistance
In vivo organoid transplantation	Provides the most physiologically relevant *in vivo* microenvironment; models the entire metastatic cascade. The 'gold standard' for validating the tumorigenicity and metastatic potential of organoids. Evaluates drug efficacy within a whole body physiological context.	Time-consuming (months). Immunodeficient mouse models lack a human immune system, limiting immunotherapy research.	High	Intermediate (core component of preclinical research but species differences are a translational bottleneck)	Validation of *in vitro* findings, studying the metastatic cascade, preclinical drug efficacy evaluation.
CTC-derived organoids (liquid biopsy).	Minimally invasive sample acquisition Directly derived from metastatic cell populations, representing real time tumor status. Potential to capture cell subpopulations with high metastatic potential.	Extreme rarity of CTCs in blood, high technical challenge. Very low culture success rate (for example, ~58% in prostate cancer) Requires tumor-specific culture conditions	Very high	Low (the technology itself is immature, but has great future translational potential as an extension of liquid biopsy)	Metastasis mechanism research, dynamic therapy monitoring, personalized therapy for metastatic disease

[a] Summary of the core advantages, limitations and practical considerations of the four main modeling approaches discussed in the present review. Key comparative dimensions include relative cost, translational readiness (an estimate of the model's proximity to clinical application) and primary research scenarios for which each strategy is best suited. PDOs, patient-derived organoids; CTCs, circulating tumor cells; SCLC, small cell lung cancer.

the CRISPR/Cas9 system (69), lung cancer organoids can be genetically modified to create metastasis models driven by specific gene mutations. By knocking out or inserting key genes associated with lung cancer metastasis, researchers can study gene function and its impact on the metastatic process.

For example, the CRISPR/Cas9 system has been used to knock out the *SOX9* gene in human embryonic stem cells prior to directed differentiation into lung organoids. While *SOX9* deletion did not affect epithelial cell differentiation, it impaired proliferation and increased apoptosis. Upon transplantation under the renal capsule of immunodeficient mice or orthotopically into bleomycin-injured mouse lungs, *SOX9* inactivation was found to affect the expression of specific cell markers and cell maturation. Techniques such as immunofluorescence and histological analysis revealed these changes, which provided direct evidence for the role of *SOX9* in human lung epithelial development (70).

Application of functionalized hydrogel scaffolds in organoid culture. Gmeiner *et al* (71) (2020) successfully established small cell lung cancer PDX-derived organoid models using HyStem-HP hydrogel scaffolds and demonstrated that the novel drug CF10 could overcome tumor chemoresistance. Functionalized hydrogels are defined scaffolds with well-defined compositions and precisely tunable properties. As a result, they are increasingly supplanting animal-derived matrices such as Matrigel, whose undefined composition and batch variability limit reproducibility and mechanistic control. Current advancements are primarily focused on two main directions. The first involves synthetic hydrogels, such as PEG and polyisocyanopeptides, which provide a highly controllable microenvironment for organoids through the grafting of cell-adhesive peptides (such as RGD and GFOGER), and through fine-tuning of mechanical properties (such as stiffness and viscoelasticity). This approach enables precise investigation of the role of mechanical signals in organoid growth, EMT and invasion (72). The other direction focuses on engineered natural polymers, such as organ-specific dECM, peptide-functionalized nanocellulose and alginate (73). These materials retain tissue-specific biological signals and enhance functionality through chemical modifications, effectively promoting structural maturity and functional complexity of organoids (such as vascularization). The establishment of these functionalized hydrogel scaffold systems has significantly improved the reliability and utility of organoids in standardized modeling, disease mechanism research, drug screening and clinical translation (74-76).

In vivo transplantation and comparative analysis of preclinical models. The metastatic cascade unfolds within a complex physiological milieu that is difficult to fully recapitulate *in vitro*. Consequently, the *in vivo* transplantation of organoids serves as a critical intermediary, validating their tumorigenic and metastatic capacities in a more physiologically relevant framework for investigation. For example, orthotopic transplantation of lung cancer organoids into the lungs of immunodeficient mice can reproduce the native tumor microenvironment, enabling comprehensive observation of the metastatic continuum from local invasion to distant colonization (77).

Organoid transplantation for metastasis research. The utility of organoid transplantation has been extensively demonstrated across multiple cancer types. In colorectal cancer, organoid libraries retain stable pathological and genetic characteristics. GFP-labeled organoids derived from patient-derived xenografts (PDXs), when transplanted into immunodeficient mice, facilitate visualization and quantification of micrometastatic lesions (such as hepatic metastases following splenic injection) (78). Comparable strategies have been employed in models of lung, breast (79), pancreatic (80) and brain (81) cancer, effectively recapitulating tumor dynamics and supporting preclinical evaluation of therapeutic efficacy.

By constructing humanized lung cancer PDX models through engraftment of human hematopoietic stem cells into immunodeficient mice to reconstitute a human immune system, researchers can effectively evaluate the efficacy of PD-1/PD-L1 inhibitors such as pembrolizumab and nivolumab (82).

A comparative perspective on preclinical models. While organoid transplantation is a versatile tool in metastasis research, it is one of several available preclinical models. A comparative evaluation of these mainstream platforms underscores their distinct advantages and optimal applications, with PDOs occupying a unique and increasingly important niche in translational studies.

i) PDOs vs. PDXs. Comparative studies have highlighted the distinct advantages of PDOs over PDXs in several key aspects. PDOs can often be established within weeks, which is significantly faster than the months typically required for PDXs. PDOs are more cost-effective and offer greater scalability, making them suitable for high-throughput *in vitro* studies (83). A critical limitation of conventional PDX models, which are grown in immunodeficient mice, is the absence of a functional human immune system, restricting their application in immunotherapy research. Humanized PDX models address this issue to some extent, but still face challenges such as incomplete immune reconstitution. Additionally, murine stromal cells gradually replace the human tumor microenvironment in PDXs, potentially altering key aspects of tumor biology. Although PDOs also face challenges in fully replicating the tumor microenvironment, they offer greater flexibility for incorporating human immune components through co-culture systems (84).

ii) Characteristics and limitations of spheroids. Spheroids are simple 3D cell aggregates that are valuable for high-throughput drug screening and for studying basic nutrient gradients. However, they generally lack the complex and architecturally accurate tissue morphology, cellular diversity (including stromal components) and long-term functional differentiation that characterize PDOs derived from stem or progenitor cells. These shortcomings limit their ability to mimic the complexity of native tissues (85,86).

iii) Comparative analysis of PDOs and lung-on-a-chip models. Organ-on-a-chip platforms are highly effective at engineering precise physiological microenvironments, incorporating fluid flow, mechanical forces and multi-cellular interactions to model dynamic processes such as vascular perfusion and immune cell trafficking. Their limitation often stems from the use of established cell lines, which may not fully represent the patient-specific genomic heterogeneity.

PDOs complement these systems by offering a genetically accurate, patient-derived tissue source, which can be integrated into chip-based devices to create more personalized and representative models (87).

iv) Comparative analysis of PDOs and genetically engineered mouse models (GEMMs). GEMMs are an optimal model for studying tumorigenesis and metastasis within an intact, immunocompetent organism and in the context of developing tissues. However, their primary limitations include long timelines, high costs and inherent species differences, which can hinder the direct translation of findings to human patients. PDOs offer a more rapid, human-based platform for personalized screening and genetic manipulation, providing a valuable complement to GEMMs (88).

v) Integrating preclinical models: Synergistic applications. These models are not mutually exclusive, and they are highly complementary. Insights gained from high-throughput drug screening in PDOs can be validated within the more physiologically intact contexts of PDXs or GEMMs. Conversely, PDOs can be integrated into organ-on-a-chip devices to create more complex, human-relevant organoid-on-a-chip systems. The choice of model depends on the specific research question, with PDOs offering a balanced platform that preserves human tumor heterogeneity while enabling scalable *in vitro* experimentation.

vi) Advances in patient-derived models for studying metabolism-immunity interplay in lung cancer. PDOs and patient-derived organotypic tissue cultures (PD-OTCs) have recently demonstrated notable value in recapitulating the lung cancer tumor microenvironment and elucidating its functional dynamics, particularly the interactions between metabolism and immunity. Using stable isotope-resolved metabolomics, Fan *et al* (89) found that PD-OTCs more accurately reproduce complex metabolic reprogramming features observed in patients, including enhanced glycolysis, nucleotide synthesis and anaplerotic reactions, compared with PDX models of NSCLC. Building on this platform, the study directly uncovered key regulatory mechanisms along the metabolism-immunity axis. It demonstrated that immunomodulators (such as β-glucan) and checkpoint inhibitors can reprogram the metabolic state of immune cells, effectively reversing immunosuppression by shifting TAMs from the M2 anti-inflammatory phenotype to the M1 pro-inflammatory phenotype. These experimental findings provide empirical support for the theoretical framework proposed in the study by Huang *et al* (90), which suggested that metabolic networks within the tumor microenvironment coordinate immune responses and drive immune evasion. In summary, PDOs and PD-OTCs have emerged as key bridges connecting the theory of metabolic reprogramming with the practice of immunotherapy, establishing a methodological foundation for developing individualized combination strategies that target the metabolism-immunity axis.

CTC-derived organoids. By contrast, CTC-derived organoids are established from tumor cells shed into the bloodstream from primary or metastatic sites, thereby capturing dynamic and comprehensive tumor-related information. Culturing organoids from sources such as intestinal tissues is relatively straightforward, as these models can be stably cultured and passaged in defined media and matrix gels using well-established protocols. However, culturing CTC organoids poses greater technical challenges. CTCs are extremely rare in peripheral blood, typically on the order of 1 to 10 cells/ml, making their isolation and enrichment particularly demanding. CTCs derived from different tumor types require tumor-specific culture conditions, further contributing to low success rates (91,92). In prostate cancer, for example, the reported success rate of culturing CTCs is ~5.8% (93).

Regarding practical applications, general organoids are widely used to study organ development, disease modeling and drug testing. CTC organoids are particularly valuable for investigating tumor metastasis, personalized cancer therapy and drug resistance mechanisms. In terms of cellular characteristics, organoids derived from normal tissue stem cells typically exhibit stable morphology and function, while those derived tumor tissues retain some malignant features of the original tumor cells. CTC organoids display a high degree of heterogeneity that reflects the diversity of tumor cells during the metastatic process, and they may exhibit stronger metastatic potential (94).

CTC organoids derived from blood samples of patients with SCLC can simulate the tumor microenvironment and support drug response testing. By preserving tumor cell heterogeneity, CTC organoids can improve prediction of drug efficacy relative to traditional preclinical models. This enables clinicians to identify effective therapeutic agents tailored to individual patients, prioritize drugs with higher sensitivity, avoid those with potential resistance and reduce adverse effects associated with ineffective treatments. Ultimately, this approach can improve clinical outcomes and enhance patient life quality. Beyond their utility in guiding personalized therapy, CTC organoids serve as a powerful tool for investigating the biological features of SCLC, including the mechanisms of tumor initiation, progression and metastasis (94,95). They also offer insights into the development of drug resistance and inform strategies to overcome it. From a translational perspective, molecular analysis of CTC organoids can facilitate the discovery of novel therapeutic targets, assist in evaluating the efficacy and safety of emerging drug candidates and support early-stage clinical trials due to their operational simplicity, cost-effectiveness and relatively short culture time (96).

5. Application of lung cancer organoid metastasis models in research

Mechanistic studies of metastasis. Lung cancer organoid-based metastasis models provide an effective platform for in-depth exploration of metastatic mechanisms. Research on metastasis-related genes and signaling pathways has revealed that numerous genes serve critical roles in lung cancer metastasis. For example, deletion of the histone methyltransferase gene *KMT2C* promotes distant metastasis in SCLC through *DNMT3A*-mediated epigenetic reprogramming. In lung cancer organoid models, *KMT2C* knockout markedly increases organoid invasiveness and metastatic potential. Further analyses have revealed associated alterations in histone modification and DNA methylation patterns, which regulate downstream genes involved in metastatic progression (97).

These models are also instrumental for studying the impact of the tumor microenvironment on metastasis. Cellular components such as tumor-associated macrophages

(TAMs) and fibroblasts interact with tumor cells and modulate their metastatic behavior. Co-culture experiments involving TAMs and lung cancer organoids have demonstrated that macrophages secrete cytokines that enhance organoid invasion and migration. This pro-metastatic effect has been further validated *in vivo*, where the presence of TAMs significantly promoted metastatic spread following transplantation (98).

Drug screening and efficacy evaluation. Lung cancer organoid metastasis models are valuable for drug screening and efficacy evaluation. They support high-throughput screening of anti-metastatic drugs by evaluating treatment-induced changes in cancer cell migration, invasion and expression of metastasis-associated markers. Lung cancer organoid metastasis models are also well suited for assessing the synergistic effects of combination therapies, thereby informing optimization of clinical treatment strategies. In addition, correlating genetic characteristics and molecular marker expression in patient-derived lung cancer organoids with clinical metastasis data may facilitate the development of predictive models for metastatic risk. For example, elevated expression of specific genes or proteins could indicate increased metastatic risk and poor prognosis, aiding early intervention and improved outcomes (99).

Metastasis is a major determinant of prognosis in lung cancer. A previous study established lung cancer organoids from malignant serous effusions that retained key tumor characteristics and showed high genomic concordance with the original tumors. The Lung Cancer Organoids-Drug Sensitivity Test (LCO-DST) demonstrated strong predictive performance, with a sensitivity of 84.0%, specificity of 82.8% and accuracy of 83.3% for distinguishing drug-sensitive from drug-resistant patients. This test effectively predicted responses to targeted therapies and chemotherapy. Notably, lung cancer organoids exhibit both stability and inter-patient heterogeneity. Organoids derived at different time points from the same patient maintained consistent genomic features, while organoids from different patients showed distinct morphologies and drug sensitivities. These findings highlight the potential of LCO-DST for predicting the effectiveness of combination therapies in advanced lung cancer (100).

Using lung cancer brain metastasis organoid models as an example, researchers have evaluated the impact of various therapeutic agents on the growth and invasion of brain metastases. Treating lung cancer brain metastases (LCBM) remains a significant clinical challenge, underscoring the importance of drug screening. Traditional cancer cell lines and PDX models have notable limitations; cell lines often lack *in vivo* tumor heterogeneity and PDX models face interspecies incompatibilities and low establishment efficiency (101). By contrast, LCBM organoids recapitulate the molecular and phenotypic features of metastatic tumors. They can be used to screen targeted therapies based on specific genetic alterations such as *EGFR* or *ALK* mutations, to identify potential radiosensitizers and evaluate the combined radio-chemotherapy efficacy. With access to large-scale drug libraries, organoid models enable high-throughput drug screening, providing strong support for LCBM drug development and clinical treatment selection (101).

Research on tumor heterogeneity. Tumor heterogeneity is a critical factor in the development, therapeutic resistance and metastasis of lung cancer. The dynamic evolution of tumor heterogeneity in space (between primary and metastatic sites and within different tumor regions) and in time (before and after treatment or at different disease stages), poses significant challenges for constructing effective metastasis models (102-104). Compared with traditional cell lines and GEMMs, lung cancer organoids more accurately preserve genomic diversity, phenotypic variations and key components of the tumor microenvironment, including co-culture systems that incorporate stromal and immune cells from the original tumors (105).

Single-cell sequencing studies (106-109) have demonstrated that lung cancer organoids retain subclonal structures and transcriptomic heterogeneity comparable to those of the primary tumors. These organoids are also enriched for invasive front cell populations, particularly cells undergoing EMT, which serve crucial roles in metastasis (110). However, replicating the dynamic evolution of heterogeneity within organoid-based metastasis models remains technically challenging. As the metastatic cascade involves selection of heterogeneous CTCs, current organoid systems are limited in their ability to mimic circulatory dynamics, including fluid shear stress and trans-endothelial migration. To address these limitations, integration with organ-on-a-chip technologies will be necessary to achieve more physiologically relevant spatiotemporal reconstruction (111-113).

LCBMs are a common and severe complication in advanced diseases. Brain metastases exhibit higher median absolute deviation and higher mutation allele tumor heterogeneity scores than primary tumors, indicating greater genomic diversity (114). In addition, single-cell RNA sequencing has revealed marked differences in gene expression between tumor cells from brain metastases and those from primary sites, highlighting cell-level heterogeneity that may influence disease progression and therapeutic response (115). In the future, the integration of spatial transcriptomics, live-cell imaging and AI-driven algorithms for quantifying heterogeneity will enable lung cancer organoid models to capture dynamic tumor evolution at single-cell resolution. These advances will support early prediction and precise intervention for metastatic risk.

6. Challenges and future directions in lung cancer organoid metastasis models

Clinical translation of lung cancer organoid metastasis models faces several intertwined technical, translational and ethical challenges. Overcoming these obstacles is essential for advancing the field, and future development will depend on integrating innovative technologies and establishing standardized practices. Addressing these challenges will enable more effective and personalized approaches to lung cancer research and treatment.

Current challenges. i) Technical hurdles in model construction. The success rate of organoid culture remains suboptimal for certain lung cancer subtypes, such SCLC and sarcomatoid carcinoma, due to their limited stemness and strong dependence on the ECM (116). A major bottleneck is the widespread use of ill-defined matrices such as Matrigel, which are affected by batch-to-batch variability, undefined composition and non-physiological mechanical properties. Although dECM provides a more native biochemical environment, donor-dependent variability complicates standardization (117-119).

Reconstructing the tumor microenvironment also presents significant challenges. Co-culture systems that integrate immune cells are hindered by poor immune cell infiltration into dense hydrogel domes and by the difficulty of sourcing authentic TILs. These limitations impede accurate modeling of complex tumor microenvironment interactions that are crucial for studying tumor-immune dynamics and metastasis (120). In addition, current models are limited in ability to replicate full cellular diversity and complex stromal interactions, including functional vasculature, which limits the ability to model tumor growth, metastatic dissemination and dynamic interactions between tumor cells and their microenvironment.

ii) Barriers to clinical translation and ethical considerations. From a clinical perspective, the absence of standardized protocols for model construction, culture and assessment severely limits reproducibility and hinders large-scale application. High costs associated with tissue acquisition, organoid culture and molecular analyses present significant economic barriers. Additionally, there is a lack of large-scale, multi-center clinical validation to confirm the predictive accuracy of these models for drug response and personalized therapy, which impedes the broader clinical adoption (121).

Ethically, the collection and use of patient-derived tissues requires rigorous informed consent processes. The application of advanced techniques such as gene editing introduces additional ethical complexities, requiring clear regulatory frameworks for safety assessment and intellectual property management.

Integrated future directions. To address these challenges, the field is moving towards a multi-faceted strategy that leverages cutting-edge technologies and promotes standardization.

i) Advancing model fidelity through technology integration. Integrating multi-omics technologies, including genomics, transcriptomics, proteomics and metabolomics, with organoid models will provide a comprehensive molecular view of metastasis. This approach may reveal novel drivers, elucidate dysregulated metabolic pathways and uncover complex molecular interactions within the tumor microenvironment. Combining these high-throughput techniques with organoid models will deepen mechanistic insights and accelerate the development of more targeted and effective therapies (122).

A key goal in cancer research is the development of sophisticated immune-organoid models. By co-culturing lung cancer organoids with autologous immune cells, including T cells and natural killer cells, or by utilizing humanized mouse models, researchers can better replicate crucial tumor-immune interactions. These models provide a powerful platform for evaluating immunotherapy efficacy and for studying mechanisms of immune evasion. Understanding how tumors interact with immune cells in a more physiologically relevant context can accelerate the discovery of novel therapeutic strategies and improve the precision of cancer immunotherapy (123-128).

The synergy between organoid models and liquid biopsy is also highly promising. Analysis of circulating tumor DNA (ctDNA) from patient plasma or from organoid culture supernatants, provides a non-invasive method to track tumor evolution in real-time. This approach can guide drug selection in organoid models, enabling personalized and adaptive treatment strategies. By incorporating ctDNA analysis, researchers can continuously monitor treatment response and identify emerging resistance mechanisms, creating a closed-loop feedback system that enhances the precision and flexibility of oncology therapies. This combination has the potential to transform personalized cancer care by aligning treatments with the evolving molecular landscape of each tumor (91,129-131). Single-cell RNA sequencing is another pivotal tool; it can resolve cellular heterogeneity within organoids and their tumor microenvironment, validate model fidelity and identify rare cell populations critical to metastasis research (106).

ii) Leveraging AI and standardization. The application of AI and machine learning is poised to transform the field. AI algorithms can analyze high-content imaging data from organoids to automate quantification of metastatic phenotypes, including invasion and migration, which streamlines the screening process (132-134). Furthermore, AI can integrate multi-omics and drug response data to build predictive models for metastatic risk and therapy optimization, thereby guiding personalized treatment decisions. These advances must be supported by rigorous standardization. Harmonized protocols for tissue processing, culture conditions and quality control are essential to ensure reproducibility, enable multi-center collaborations and facilitate clinical adoption. In parallel, optimizing workflows and reducing costs are important for improving accessibility.

Conclusion. Lung cancer organoid metastasis models have emerged as pivotal platforms for deciphering metastatic mechanisms and advancing precision therapy. Despite persistent challenges in standardization and tumor microenvironment recapitulation, future investigations should prioritize the following directions: Integrating multi-omics with spatial transcriptomics to systematically resolve spatiotemporal dynamics of metastasis; developing immunocompetent organoid co-culture systems to deepen understanding of tumor-immune interactions; employing chemically defined functionalized hydrogels for precise modulation of the tumor microenvironment; and combining artificial intelligence with liquid biopsy to establish dynamic drug response prediction models. Through interdisciplinary integration and standardized framework development, these organoid models will accelerate anti-metastatic drug discovery and ultimately facilitate the clinical translation of precision medicine for lung cancer.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Xi'an Science and Technology Program (grant no. 2024JH-ZCLGG-0040), the Wu Jieping Medical Foundation Clinical Research

Special Grant Fund (grant no. 320.6750.2023-17-23), the Technology Program (grant no. 2024YXYJ0133), The Xi'an Science and Technology Program (grant no. 24YXYJ0179), Fundamental Research Funds for the Central Universities ('Free Exploration' Teacher Project) of Xi'an Jiaotong University (grant no. xzy012025151), the Beijing KC Medical Development Foundation Research Project (grant no. KC2023-JX-0288-PM92) and the China Health and Medical Development Foundation (grant no. chmdf2024-xrzx09-20).

Availability of data and materials

Not applicable.

Authors' contributions

JJ conceived the study. JJ, GMD, QG, ZYZ, XYL, FSH, SNL, JQM, JB, HW and ZZ wrote and reviewed the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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