Review

Ex Vivo Drug Testing of Patient-Derived Lung Organoids to Predict Treatment Responses for Personalized Medicine

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Simple Summary: Lung cancer leads to ~1.8 million deaths each year. Standardized therapy for lung cancer has improved patient survival but some tumors may relapse due to drug resistance. An effective and efficient screening of novel therapies is urgently needed when the drug resistance arises. 3D patient-derived organoid (PDO) models have emerged to meet that need. PDOs maintain major properties and reflect drug sensitivity of original lung tumors. With technical improvement, the PDO-based drug screening represents a promising technology of personalized medicine for lung cancer patients.

Abstract: Lung cancer is the leading cause of global cancer-related mortality resulting in ~1.8 million deaths annually. Systemic, molecular targeted, and immune therapies have provided significant improvements of survival outcomes for patients. However, drug resistance usually arises and there is an urgent need for novel therapy screening and personalized medicine. 3D patient-derived organoid (PDO) models have emerged as a more effective and efficient alternative for *ex vivo* drug screening than 2D cell culture and patient-derived xenograft (PDX) models. In this review, we performed an extensive search of lung cancer PDO-based *ex vivo* drug screening studies. Lung cancer PDOs were successfully established from fresh or biobanked sections/biopsy of lung tumors, PDXs, and pleural affusion. PDOs were subject to *ex vivo* drug screening with chemotherapy, targeted therapy and immune therapy agents. PDOs mainly recapitulated the genomic alterations, transcriptomic landscape and drug sensitivity of primary tumors. Although sample sizes of the previous studies were limited and some technical challenges remained, PDOs showed promise to screen novel therapy drugs. With the technical advance of high throughput, tumor-on-chip, combined microenvironment, and air-liquid interface (ALI), the drug screening using PDOs would serve better for precision care of lung cancer patients.

Keywords: lung cancer; organoid; drug screening; high throughput; translational; pre-clinical; clinical; tumor microenvironment; personalized medicine

1. Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide accounting for approximately 1.8 million deaths in 2020[1]. Non-small cell lung cancer (NSCLC) is the predominant

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histologic subtype, accounting for 85% of all lung cancer cases in the United States[2]. In comparison, small cell lung cancer (SCLC) represents 15% of lung cancer cases, occurs almost exclusively in smokers and has the most aggressive clinical course with survival outcomes of 2 to 4 months in untreated patients[3,4]. Systemic therapy is generally indicated for patients who present with advanced disease, including those who present with metastases. With the advent of molecular targeted therapies and immunotherapies, there have been significant improvements in survival outcomes for patients[5,6]. Although these newer therapies have durable responses, drug resistance often occurs with long-term use of these therapies[7]. For these reasons, there is an urgent need to identify novel therapies in patients with treatment-refractory lung cancer of different molecular subtypes who fail standard systemic treatments.

An advanced understanding of lung cancer biology has led to novel therapeutic strategies that target other signal transduction or angiogenesis pathways, as well as leverage the immune system in favor of an anti-tumor response[8-12]. Historically, cancer cell lines have been used to test novel anti-cancer therapies, but they do not accurately predict treatment responses for individual cancer patients due, in part, to interpatient variability and molecular tumor heterogeneity[13-16]. As a result, ~85% of therapeutic agents that show promising effects in preclinical models fail to demonstrate efficacy in patients[16-18]. Though patient-derived xenograft models can mitigate some limitations, poor success rate, intensive resource requirement, high experimental cost, long-term duration of study, limitations owing to statistical power and difficulties in high-throughput expansion have confined their application to preclinical drug screening and demanded the development of alternative models[15,19]. Patient-derived organoids (PDOs), also described as “human tissue in a dish”, can overcome some of these challenges[20-22]. Exponentially growing PDO models provide a platform closest to native organs for mapping and exploiting molecular mechanisms of cellular lineage differentiation, organ development, regeneration and diseases[23-28]. For malignant progression study, PDOs are 3-D cultures of cancer cells which can be established from a variety of tumor specimens including tissue resections, biopsies, body fluids, circulating tumor cells and pleural effusions from cancers [29-36]. Along with the revolutionary advance, lung organoids were capable of reconstruction of dynamic spatial architecture, molecular identity of cellular components in proximal/distal airways and pathological driver discovery in lung tissue and tumors [37-42].

From single stem cells in appropriate Matrigels and culture medium, organoids grow exponentially following unique morphological patterns[24,37,43]. In the presence of small molecule inhibitors or drugs, the growth perturbation efficacy of inhibitors could be assessed based on morphological changes, volume reduction or cell viability of organoids at the endpoints [23]. Thus, PDO models serve as a surrogate of tumors for genomic and transcriptomic profiling, biomarker identification, genetic manipulation and high-throughput drug sensitivity screening. Moreover, they are suitable for long-term expansion or cryopreservation as living organoid biobanks [44-46]. An important clinical aspect of PDOs is the potential for genomic and transcriptomic profiling and therapeutic screening as personalized assessment and precision medicine[47-51] (Figure 1). Therefore, PDOs of lung cancer can further our understanding of lung cancer pathophysiology and assess/predict drug efficacy for individual patients in the clinic. Here, we conduct a comprehensive literature review to explore the research to date surrounding ex vivo drug testing using organoids derived from lung cancer patients. We discuss the translational work, challenges and future applications of this promising technology.

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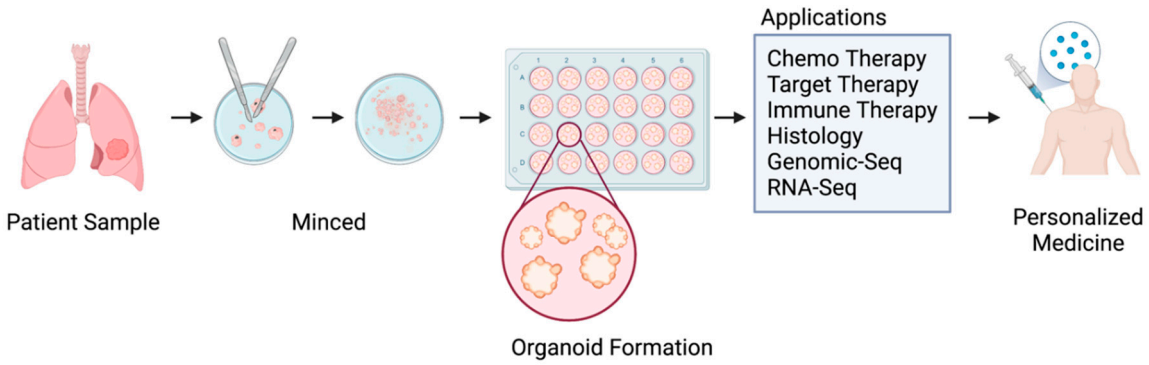

Figure 1. The illustration of applications of patient-derived lung cancer organoids for precision medicine.

2. Methods

This is a comprehensive review of the published literature focusing on research of *ex vivo* drug testing using organoids from lung cancer patients. The following databases were searched: PubMed, Cochrane Library and Scopus. All full-text, peer-reviewed publications published through March 2023, were included for consideration. The search was restricted to full-text, peer-reviewed articles and reports published in English. Abstract-only publications were excluded, as were any papers that failed to fully elucidate outcomes (editorials, etc.). The search strategy for this review was the result of prior research in the fields. In consultation with an experienced medical librarian, a search strategy was developed. The search terms included (but were not limited to): "patient derived organoids", "human derived organoids", and "drug screening". Further, appropriate MeSH terms and subject headings were also employed. A full search strategy was developed for Ovid Medline and is shown in the Table 1. Lastly, the reference list of identified articles (of the sources that were selected from the full-text review stage and included in the review) were searched for additional sources. The final search results were exported into EndNote and duplicates were removed by the librarian.

Identified titles and abstracts published through March 23, 2022, were screened independently by three reviewers Dr. Josephine Taverna and Dr. Maddison Williams. Disagreements were resolved by discussion until consensus was reached. If consensus could not be reached, a third expert reviewer (Dr. Ryan Williams) was consulted. This process was also used to screen the full text of articles. The reference lists of all included articles were then examined by Dr. Chun-Liang Chen, and additional articles were included in the review until a consensus among the research team was attained. The following information was then abstracted for each included study: primary author, date of publication, country of origin, cancer type, organoid model, methodology, results, conclusions, and limitations.

Table 1. Literature search strategy in Database(s): Ovid MEDLINE(R) ALL from 1946 to March 23, 2023.

#	Searches	Results
1	(((patient-derived adj3 organoid*) or patient derived) adj3 organoid*).ti,ab.	1187
2	exp Organoids/de [Drug Effects]	1031
3	(((human-derived adj3 organoid*) or human derived) adj3 organoid*).ti,ab.	35
4	1 or 3	1218
5	2 and 4	96
6	drug screening.mp. or exp Drug Evaluation, Preclinical/	297911
7	4 and 6	269
8	5 or 7	312

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3. Results

The previous studies of *ex vivo* drug testing based on patient-derived lung cancer organoids are summarized in Table 2 and organoids culture media with supplements in Table 3. Among the studies, PDOs (n=1-84) were successfully established from resected primary tumors or biopsy of NSCLC (adenocarcinoma, squamous, adenosquamous, and large cell carcinoma) and SCLC, patient-derived xenografts or pleural effusion. Genomic alternations and EGFR/KRAS/BRAF status of PDOs were revealed to be consistent with primary tumors by exome, RNA, targeted sequencing or whole genomic sequencing. The PDOs were subject to *ex vivo* screening with single or combined antineoplastic drugs (n=1-86), mainly EGFR inhibitors (e.g. Gefitinib, Erlotinib and Afatinib), chemotherapeutic agents (e.g. Cisplatin, Docetaxel and Gemcitabine), immune checkpoint inhibitors and other receptor kinase inhibitors. The contributions and significant discoveries for each study are elaborated separately further in four categories (high throughput, pre-clinical, clinical and mechanistic) as below.

3.1. Organoid technology for high-throughput drug screening

Historically, cancer cell lines have been widely used as preclinical models to evaluate antineoplastic agents. Despite its wide application, the sensitivity and specificity of drug candidates in 2D-cell culture have been widely variable. In order to better predict clinical responses, lung cancer organoid technology was developed for high-throughput drug screening. Li et al. created a living biobank of frozen NSCLC PDOs (n=10) and used high-throughput drug screening assay to demonstrate the anti-tumor effects of three natural compounds -- chelerythrine chloride, cantharidin and harmine[52]. Chen et al. evaluated drug sensitivity of 26 anti-cancer therapeutic agents in organoids derived from 12 patients with locally advanced NSCLC (Stage I-III) which included tumors that harbored EGFR and KRAS G12C mutations[50]. They found that PDOs retained the histological and genetic characteristics of the primary tumors with more than 80% concordance between tumors[50]. Furthermore, they demonstrated PDOs correlated with molecular profiles of primary lung tumors with mutations involving the top 20 NSCLC-related genes[50]. In a separate study, Li et al. tested 24 antineoplastic drugs in PDOs derived from 12 lung adenocarcinoma patients using a high throughput system and found that drug sensitivity also correlated with mutational profiles[53]. Tamura et al. demonstrated the feasibility of using a high throughput system for PDO drug screening from a large compound library comprised of 61 anti-cancer agents (targeted therapies and chemotherapies) [54]. In a similar fashion, Takahashi et al. used their high-throughput assay to evaluate 86 anti-cancer agents (molecular targeted drugs, immune checkpoint inhibitors and cytotoxic chemotherapies) in PDOs developed from lung tumors with squamous and adenosquamous histology[17]. In all these studies, the pharmacogenomic profiles of PDOs were highly comparable to that of tumor tissue indicating that organoid models can better predict individual therapeutic responses on a larger scale to inform therapeutic strategies over a reasonable time frame[55-57].

3.2. PDOs in Preclinical Studies

In addition to large-scale screening, PDOs are being employed for personalized drug screening for patients with treatment resistant lung cancer. Jung et al. observed that PDOs derived from SCLC display an inherent drug resistant phenotype to conventional chemotherapy with cisplatin and etoposide[58]. Gmeiner et al. demonstrated that dysregulated pyrimidine biosynthesis contributes to drug resistance in SCLC and they found that the novel fluoropyrimidine polymer (CF10) could overcome drug resistance to conventional chemotherapy using PDOs[59]. In another study, patient derived organoids from NSCLC patient demonstrated the efficacy of an oral potent tubulin destabilizing agent, S-40, in overcoming paclitaxel resistance[60]. These study models highlight the utilization of organoids for assessing drug resistant phenotypes which can be directly translated to the clinic for individual lung cancer patients who develop tumor progression on standard chemotherapy.

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The identification of oncogenic activation of tyrosine kinases in NSCLC tumors, most notably mutations in the epidermal growth factor receptor (EGFR) or rearrangements of the anaplastic lymphoma kinase (ALK) gene or c-ROS oncogene 1 (ROS1) gene, has led to the development of targeted agents. Matching a specific targeted drug to the identified driver mutation for an individual patient has resulted in significantly improved survival outcomes, compared with chemotherapy and/or immunotherapy [61,62]. In spite of dramatic tumor shrinkage initially seen in NSCLC patients with driver mutations, drug resistance to targeted therapy often develops. PDOs are currently being used to test small molecular inhibitors with the potential to overcome this secondary resistance. One study conducted by Saraon et al. evaluated the efficacy of EMI66 and other small molecules that inhibit mutant EGFR signaling and alters ER stress response pathway[63]. They found that EMI66 and other derivatives reduced viability of organoids derived from three PDOs with EGFR mutations, suggesting another bypass mechanism to target tumor growth[63]. Hu et al. found that PDOs with the EML4-ALK rearrangement mutation and EGFR activation mutations showed reduced viability and sensitivity to TKI inhibition, compared to wild-type[64]. They demonstrated that lung organoids with TKI sensitive L858R mutation and absent T790 mutation could develop drug resistance early, highlighting the potential role of PDOs to predict drug resistance better than the T790M molecular marker[64]. These collective studies suggests that lung organoids can be used as a treatment prediction model to allow clinicians to prioritize targeted and chemotherapies prospectively by comparing the responses of the individual PDOs to different drug combinations in real-time.

3.3. PDOs in clinical studies - an enhanced model for personalized drug screening

In a large clinical study, 84 organoids were established from patients with oncogene-driven lung adenocarcinoma and found that PDOs recapitulated PFS and ORR of patients receiving clinically approved targeted agents[61]. They found that combination treatment with dabrafenib and trametinib elicited both ex vivo and clinical responses in a NSCLC patient harboring an EGFR exon 19 deletion and a BRAF G464A mutation[61]. They also demonstrated pre-clinical and clinical efficacy of afatinib against PDOs that harbored the rare EGFR L747P mutation. Among ERBB2 targeted therapies (erlotinib, lapatinib, neratinib, and afatinib), poziotinib demonstrated the greatest potency in lung organoids with ERBB2 exon 20 insertions[61]. The preclinical studies led to a phase 2 study (ZENITH20-2 Trial) of poziotinib which yielded a response rate of 28% in 25 of 90 patients with treatment refractory HER2 exon 20 insertion NSCLC. Both the preclinical and clinical studies indicated robust and durable anti-tumor activity of poziotinib in NSCLC patients with ERBB2 exon 20 insertions[61]. Among the multi-kinase inhibitors (vandetanib, cabozantinib, and lenvatinib), pralsetinib demonstrated clinical efficacy in RET-rearranged lung tumors[61]. In a recent phase I/II clinical trial (ARROW trial), robust treatment responses were observed in patients with RET fusion-positive NSCLC (n=233)[65]. In 53 of 87 NSCLC patients previously treated with platinum-based chemotherapy, there was a 61% overall response to therapy including five patients with a complete response[65]. In 19 of 27 treatment naïve NSCLC, a 70% overall response was observed with a complete response in three patients[65]. Overall, these studies highlight the potential of organoid-based drug sensitivity testing to predict clinical outcomes better than molecular markers. This accuracy in treatment prediction offers clinicians the ability to prioritize targeted and chemotherapies prospectively by comparing the individual responses of the generated PDOs to different drugs.

About 2%–3% human epidermal growth factor receptor 2 (HER2, ERBB2) mutation have been identified as one of tumorigenic drivers and observed in of NSCLC[66]. Several HER2-targeted agents (afatinib, dacomitinib, neratinib and trastuzumab) have shown limited clinical activity in patients with HER2-mutant NSCLC[67-70]. Wang et al. recently studied the anti-tumor effects of pyrotinib, a pan-HER inhibitor, in NSCLC with HER2 exon 20 mutations in both preclinical and clinical models [49]. Pyrotinib showed significant growth inhibition of organoids relative to afatinib[49]. In PDX model, pyrotinib showed a superior antitumor effect compared with afatinib and trastuzumab emtansine (a humanized monoclonal antibody trastuzumab covalently linked to the cytotoxic agent DM1) [49]. Specifically, mice treated with pyrotinib had >50% reduction in their tumor volumes[49]. This anti-tumor drug effect was attributed to the inhibition of HER2 and its downstream signaling

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pathways, including ERK and Akt. They validated their preclinical findings in a phase 2 study of pyrotinib (400 mg oral daily dose) in patients with advanced stage III-IV NSCLC patients harboring HER2 mutations[71]. In this single arm study, 15 patients with HER2 mutations involving exon 20 (n=11), exon 19 (n=3), and exon 17 (n=1) had reduction in tumor burden with an objective response rate of 19.2%, median duration of 9.9 months and disease control rate of 74.4% suggesting[71]. Collectively study also demonstrated the applicability of lung organoid models to study patient populations with rare mutations.

The fibroblast growth factor/fibroblast growth factor receptor (FGF/FGFR) is a tyrosine kinase signaling pathway plays a critical role in oncogenesis (tumor proliferation, angiogenesis, migration, and survival) via gene amplification, activating mutations, or translocation in lung tumors[72,73]. In this way, the FGFR signaling pathway represents an important target for lung cancer. In vitro studies suggest that combination of MEK and PI3K inhibitors with FGFR inhibitors may be effective in FGFR-aberrant cancers [74]. To test this hypothesis in preclinical models, Shi et al. developed organoids from surgically resected lung tumors derived from 19 patients with lung adenocarcinoma and 15 patients with squamous cell lung carcinoma[74]. Additionally, they processed 16 lung adenocarcinoma PDX and 26 squamous cell lung cancer PDX tumors for organoid establishment[74]. They tested trametinib (MEK inhibitor) and the PI3K inhibitor BKM120 with BGJ398 (inhibits pFGFR and pAkt.) in their FGFR1-amplified organoid models[74]. Strong synergy was observed in the BGJ398 + trametinib combination (combination index < 0.5), whereas weaker synergy (combination index >0.5) was observed in the BGJ398 + BKM120 (PI3K inhibitor) combination[74]. Furthermore, PDOs retained the histologic and molecular features of their parental tumors[74]. The spectrum of mutations was highly concordant between the organoid and matched patient tumor and PDX tissue[74]. Mutational burden in the five long-term established PDOs was similar to parental patient/PDX tumors[74]. This indicates that their organoid culture conditions did not destabilize the cancer genome. Copy number variation (CNV) profiles of the parental tumors were largely preserved during PDO culture[74]. There have been early phase clinical studies in patients with lung cancer (and other solid tumors) evaluating FGFR inhibitors as monotherapy or in combination with existing therapies. As more clinical trials of both selective and non-selective FGFR inhibitors emerge[75], concentration on patient selection as it pertains to predicting response to therapy should be undertaken with patient derived organoids given the accuracy of this model system.

Insertion mutations in exon 20 of the epidermal growth factor receptor (EGFR) gene are the largest class of EGFR mutations in non-small cell lung cancer (NSCLC) for which there are no FDA approved targeted therapies[76]. Yun et al. tested amivantamab (an EGFR-MET bispecific antibody) in tumors derived from organoids and xenograft models harboring diverse Exon 20 insertion mutations and found that the drug showed anti-tumor activity and suppressed EGFR and MET signaling pathways[77]. Furthermore, they established their PDOs from malignant pleural effusions collected from two patients with NSCLC with Exon 20 insertion mutations[77]. In a first-in-human study, amivantamab produced robust tumor responses in two patients with EGFR Exon 20 insertion NSCLC, highlighting the importance of ex vivo drug testing to inform clinical trial design[77].

3.4. Mechanistic studies involving PDOs

PDOs also serve as a valuable model to study tumor evolution and the acquisition of secondary mutations associated with treatment resistance. Banda et al., treated lung tumor organoids with erlotinib, a first generation EGFR-tyrosine kinase inhibitor[78]. Upon subsequent passaging, the organoid cultures developed additional mutations in BRAF, EGFR, KRAS and PIK3CA genes which have been commonly reported in patients who develop resistance against EGFR inhibitors[78]. These observations were supported by a comprehensive mutation profiling analysis (multiplex testing) of tumor specimens collected from 153 NSCLC patients identified the co-existence of EGFR with KRAS (n=29), or BRAF (n=2) or PIK3CA somatic mutations (n=58)[79].

Proper identification of subclones and targeting individual tumor subpopulations with effective targeted therapies can lead to effective elimination of tumor cells and durable treatment responses in lung cancer patients. Taverna et al. used single-cell proteomic profiling to first identify continuous

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AXL and JAK1-STAT3 (bypass loop) signal activation in lung tumors derived from 11 treatment naïve lung adenocarcinoma patients who underwent tumor resection of their primary tumor[80]. Using single cell profiling, they were able to stratify tumor subpopulations based on their AXL and STAT3 signaling (bypass mechanism) [80]. They found that tumor subpopulations with high AXL/STAT3 expression concordantly expressed high expression of epithelial/ mesenchymal markers (hybrid epithelial-to-mesenchymal phenotype) and cancer stemness proteins, suggesting high tumorigenic and metastatic potential[80]. They subsequently stratified tumor based on high AXL/STAT3 and low AXL/STAT3 expression and found that patient derived organoids (PDO=3) with high AXL/STAT3 expression responded robustly to combination treatment with dubermatinib (AXL inhibitor) and ruxolitnib (JAK inhibitor) as compared with single agents[80]. Conversely, PDOs (n=2) that expressed low AXL/STAT3 expression did not respond to combination treatment and/or single agents[80]. In H2009 and A549 mouse models, they found that combination treatment with AXL and JAK inhibitor was synergistic and reduced tumor volumes significantly compared with single agent treatments (unpublished, manuscript under revision). In this study, they found that PDOs successfully captured the cellular heterogeneity of their primary lung tumor cells and could provide insights into potential combination therapy to overcome drug resistance.

Another major strength for PDOs is that they can be subjected to genetic manipulation, which can be very a very powerful tool understand oncogenic signaling in lung cancer. For example, Dost et al. used organoids model to study transcriptional hallmarks of oncogenic KRAS activation in lung epithelial progenitor cells[81]. They developed organoid systems from primary mouse and human induced pluripotent stem cell-derived lung epithelial cells to model early-stage lung adenocarcinoma, showing organoid approaches can be utilized for uncovering the early consequences of oncogenic KRAS expression.

4. Conclusions and future directions

Lung cancer PDOs have emerged as an effective alternative model for *ex vivo* drug screening. From the previous studies, lung cancer PDOs showed promise in mechanistic and pre-clinical studies for drugs targeting lung malignancy to achieve potential personalized medicine.

Whether lung organoids can accurately predict treatment responses in clinical trials remains an area of active research. PDOs have become widely used for testing anti-neoplastic agents for therapeutic efficacy in clinical trials. The ability to generate organoid models from individual patients enrolled in clinical trials allows investigation of patient-specific responses to therapeutic drugs in real-time[61]. One research team established a PDO biobank from patients with metastatic gastrointestinal cancer who were actively undergoing treatment in clinical trials and demonstrated that the *ex vivo* responses in organoid cultures closely mirrored clinical responses with 100% sensitivity, 93% specificity, 100% negative prediction accuracy and 88% positive prediction accuracy[82]. This study provides a strong rationale for designing co-clinical trials with organoid models to better predict drug responses. Some matched studies have also shown that PDOs accurately predicted therapeutic responses of patients and allow comparison of outcomes of different novel monotherapies or combination therapies[77,82] . Organoids have also been adopted to predict treatment response to radiotherapy and immunotherapy[83-85]. By simulating cancer behavior *ex vivo*, organoid technology can integrate molecular biology with the decision-making process of early-phase clinical trials to improve patient selection for particular therapeutic agents.

Based on our review of the literature, organoid models can be successfully established from pleural effusions which is scarce source of lung cancer cells[77,86,87]. Strikingly, organoids derived from pleural fluid aspirate can developed tumor-specific cellular and molecular characteristics and preserved tumor heterogeneity[86,87]. Since pleural effusion aspiration is fairly a non-invasive and routine procedure among lung cancer patients, generation of organoid models from these samples can allow effective disease modeling, monitor disease progression and treatment responses in real-time. Another advantage of using pleural effusion-derived cells is that they constitute both tumor cells and stromal cells such that tumor microenvironment and disease pathophysiology can be preserved and cellular interactions in response to disease progression or anti-cancer agents can be

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studied simultaneously[87,88]. Co-culturing tumor organoids with immune cells can mimic complex cellular microenvironment and tumor-intrinsic interactions [17]. For example, Takahashi et al. recently demonstrated that lung PDOs exposed to antibody-drug conjugates (trastuzumab, pertuzumab and trastuzumab emtansine) can effectively model complex interactions with immune cells (THP-1 effector cells) and reflect the antibody-dependent cellular cytotoxicity (ADCC) response of the drug[17]. The study also expanded on the use of anti-PD1 monoclonal antibodies (nivolumab and pembrolizumab) to evaluate efficacy of immune checkpoint inhibitors.

Although current methods of generating NSCLC PDOs are robust and straightforward, they are not without limitations. Indeed, some challenges still exist regarding establishing pure NSCLC PDOs. For example, Dijkstra et al. reported that that the majority of organoids derived from intrapulmonary lung cancers are overgrown by normal airway organoids, causing the overall establishment rate of pure lung cancer organoids to be only 17%[89]. Such low establishment rate of pure lung cancer organoids limits their clinical utility. Therefore, innovative methods are still needed to generate more robust and purer NSCLC PDOs.

Although organoid culture systems have several advantages over traditional 2D cell-based models, there are certain drawbacks that need to be overcome. For example, the most common organoid models consist of only the epithelial compartment and lack the stromal signature and further signals from the microenvironment[90]. The contribution of tumor stroma and complex tumor microenvironment in tumor progression has become well recognized and this has led to recent advances in the engineering of co-culture systems. For example, microfluidic organ-on-chips have been developed to model cell-cell and cell-extracellular matrix (ECM) interactions. These novel systems are designed to recapitulate organ-level functionality including physical forces that mimic in vivo cyclic strain and fluid shear stress. Moreover, the microfluidic nature of these systems and microsensors within the microchip sustains longer-term experiments and allows for the collection of real-time data such as barrier function and effluent collection to monitor byproducts as an indirect measure of tissue functionality[91]. As a part of rapidly advancing organ-on-chip field of research, tumor-on-chip technology has emerged as a promising tool to mimic the mechanical and biochemical properties of the tumor microenvironment. This includes factors such as oxygen and nutrient gradients, extracellular matrix stiffness, and cell-cell interactions. In this way, tumor-on-chips create a miniature model of lung tumors on a microfluidic chip allowing researchers to gain insights into how cancer cells directly engage with their environment and allows for the testing of novel therapeutics in a more efficient manner. In lung cancer, there have been several recent advances with tumor-on-chip technology. In a recent study, Liao et al. describe the development of a lung cancer-on-chip model consisting of both tumor and immune cells which recapitulates the complex interactions between these cell types ex vivo[92]. Jiang et al. employed an immunotherapeutic high-throughput observation chamber to test the efficacy of anti-PD-1 antibodies on cancer spheroid (MDA-MB-231) and T cell (Jurkat) cell-cell interactions[93]. The system detected T cell inhibition and activation by measuring IL-2 secretion and measuring tumor infiltration. However, not only cellular make-up of the tumor microenvironment but also mechanical determinants need to be recapitulated on-a-chip to faithfully monitor drug response. For example, Hassell et al. described microfluidic organ chip device complete with endothelium, epithelium and NSCLC cells suitable for studies on cancer growth, invasion, mechanotransduction and drug response. Interestingly, applying breathing-like mechanical motions to such alveolous chip bestowed cancer cells with resistance to a tyrosine kinase inhibitor drug rociletinib[94]. Mechanotransduction is undoubtedly important yet often overlooked aspect of tumorigenesis, tumor spread and drug response. Organ-on-chips studies should facilitate development of transduction-targeting “mechanotherapeutics”[95].

Summarizing, the future development of PDO on chip technology will revolutionize IO cancer treatment by allowing for personalized treatment reflecting individual patients’ drug responses in their unique tumor microenvironment. These model systems will be able to mimic multiple aspects of tumor microenvironments (tumor cells, stromal fibroblasts, endothelial cells, immune cells, mechanical determinants) and their individual responses (cytokine release, mechanotransduction) to immuno- and other therapies[95].

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Tumor-on-chip platform also provides a powerful tool to gain insights into the underlying mechanisms of metastasis. Metastasis is a complex process that involves multiple steps, including the invasion of tumor cells into surrounding tissue, colonization at the secondary site, and intravasation into the blood or lymphatic vessels. Recent studies validated that tumor-on-chip models can be used to simplify and study each of these steps. One example is a study by Zhang et al. where they use tumor-on-chip system to study the interaction of tumor cells with endothelial cells that line blood and lymphatic vessels and demonstrated that an anti-angiogenic drug can inhibit the intravasation and extravasation of breast cancer cells[96]. The microenvironment such as oxygen and nutrient availability is different at secondary site of metastasis and tumor cells must adapt to survive and grow in the new environment. In a recent report, researchers developed microfluidic chips to investigate the colonization of tumor cells at the secondary site during metastasis and showed that hypoxic condition at the secondary site promotes the growth and migration of prostate cancer cells.

Overall, tumor-on-chip technology has proven great promise in drug discovery and development and will likely advance to become more widely used in the future. More specifically, the progress with development of multi-organ-on-chips may allow researchers to test combination therapies that target multiple organs simultaneously and test how drugs affect different organs in the body[91,94,95,97-100]. This development is crucial to better understand the potential side effects of drugs. In addition, combining advancements in microfluidic technology and automation could enable high-throughput screening of large number of drugs using tumor-on-chip models. This could greatly accelerate the drug discovery process by reducing the need for trial-and-error approaches to treatment, and rapid testing of potential drug candidates in a patient specific manner. Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

There is a potential drawback of 3D organoid model in that these organoids form 3D structures with apical side of cells facing the lumen. This prevents apical surfaces of cells from air interface and direct exposure to inhaled substances as it occurs in their tissue of origin[46]. The technical downside may be alleviated by The Air-liquid interface (ALI) technique where lung organoids are dissociated and grown as a monolayer may be applied as an alternative to address this issue[101]. Contrary to traditional cell culture methods which grow cells in a liquid medium, in ALI technique, media may be removed from the apical side of cells to expose them directly to the air. This approach not only improves the differentiation process where lung epithelium forms a more mature and functional pseudo-stratified epithelium, it also allows recapitulating the complex physiological conditions of the lung tissue. The validity of ALI as a lung model has been shown by different studies comparing the functionality and genomic profile of harvested cells to *in vivo* condition[102-104]. Although ALI condition has been extensively used for culturing airway epithelial cells, it has not been explored enough for pharmacological assays such as permeability and transport studies. However, the ALI technique not only may improve insights on drug effects compared to the use of submerged cultures, but it also gives access to both apical and basolateral sides of the cells for convenient addition of any stimulus, as well as sample collection. With limited number of studies that used ALI to investigate the effects of smoking or environmental pollutants in lung cancer, the authors demonstrated that the exposure to these factors induces significant changes in cell growth, apoptosis, and inflammation[101,105].

Table 2. Summary of organoids as an enhanced model for personalized drug screening.

Cancer type	Organoid Model	PDX Model (n)	Compounds Tested	Technology/ Unique culture supplement	Limitations	Ref
Adenocarcinoma	Primary lung tumor spheroids (n=14) were cultured in 3D-Matrigel culture methods	N/A	Erlotinib	• Stem Pro hESC Supplement	The number of different drug and dose combinations that can be investigated at one time is limited.	[78]
Adenocarcinoma (NSCLC patients with tumors stage I–III, EGFR L858R, EGFR Ex20 ins, KRAS G12C)	1) Fresh tumor samples harvested for organoid culture. 2) Primary tumor samples and PDOs were analyzed via whole-exome sequencing and IHC. (n=7)	N/A	26 antineoplastic drugs tested (gefitinib, osimertinib, afatinib)		Small cohort sized used and will require further large-scale analyses to validate the findings.	[50]
Small cell lung cancer – refractory (tumors stage I–III, EGFR L858R, EGFR Ex20 ins, KRAS G12C)	PDOs (n=4) were developed from human SCLC PDX samples to test if TS inhibition could be a viable strategy for SCLC treatment.	PDXs were generated from SCLC tumor biopsy samples.	• Cisplatin • Thymidylate synthase inhibitors: CF10, 5-FU	• HyStem-HP hydrogel kits		[59]
1)Adenocarcinoma 2)Squamous cell carcinoma 3)Adenosquamous carcinoma 4)Large cell carcinoma 5)Small cell lung cancer	Surgically resected lung cancer tissues from 36 patients were embedded in Matrigel and submerged in MBM to create PDOs (n=80).	• PDXs were generated from tissue samples. • PDXs from 10 samples (43%)	• Docetaxel • Olaparib • Erlotinib • Crizotinib		Limitation of cancer organoid models is the lack of a cancer microenvironment.	[16]
NSCLC	PDOs derived from NSCLC were cultured in vitro (n=10)	N/A	• Chelerythrine chloride • Cantharidin • Harmine • Berberine • Betaine			[52]
Adenocarcinoma	Developed PDOs from human lung adenocarcinoma biopsy samples (n=12)	N/A	• 24 antineoplastic drugs tested		Small sample size limits the power to detect molecular markers of drug response.	[53]
Small cell lung cancer (n=1)	Tumor organoids were generated from primary lung cancer cells from patients with SCLC.	N/A	• Cisplatin • Etoposide	• Microfluidic-based lung cancer organoid culture platform for testing drug sensitivity.	Small sample sized used and will require further large-scale analyses to validate the findings.	[58]

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				• Patient-derived lung cancer organoids (PDOs) were cultured and expanded in Matrigel droplets in 24-well plates.		
Adenocarcinoma Metastatic lung cancer	Developed PDOs from pleural effusion of patients with lung adenocarcinoma (n=2)	N/A	Cisplatin + pemetrexed; carboplatin + pemetrexed; crizotinib	• 3D hydrogel-based model	Small sample size. Will require further large-scale analyses to validate the findings.	[86]
Non-small cell lung cancer	PDO (n=1)	N/A	S-40 (oral potent tubulin destabilizing agent)	No detail description of organoid culture	Small sample size	[60]
1)Squamous cell carcinoma 2)Adenosquamous carcinoma	PDOs developed from human tumor lung cancer surgical specimens, (n=3)	N/A	86 antineoplastic drugs tested, including molecular targeted drugs, immune checkpoint inhibitors, and cytotoxic chemotherapy	• High throughput 96- and 384-well screening • Organoids were cultured in FBIM001 medium. • Organoids culture condition as previous describe [54]	Few and limited PDO examples were selected for each type drug screen.	[17]
1)Lung adenocarcinoma 2)Squamous cell	PDOs developed from 19 surgically resected lung adenocarcinomas (LUAD) and 15 lung squamous cell carcinomas (LUSC),	• LPTO and PDXO were used to denote organoid models derived from lung primary patient tumor and PDX, respectively. • 16 LUAD PDXs, and 26 LUSC PDXs were processed for organoid establishment.	• Study evaluated the efficacy of clinically approved EGFR-targeted therapy in NSCLC in four short- term organoid models. • BGJ398 (FGFR inhibitor) • Trametinib (MEK inhibitor) • Selumetinib (MEK inhibitor) • Afatinib (EGFR inhibitor)			[74]
Lung adenocarcinoma	Three primary lung cancer organoid models: • XDO-137 (EGFR ex19del) • PDXO-4000 (EGFR ex19del) • XDO-344 (wild-type EGFR)	N/A	• EMI66 and other derivatives.	Organoids culture condition previously described [74]		[63]
1) Squamous cell carcinoma 2) Adenocarcinoma 3) small cell lung cancer	PDOs (n=21) generated from 16 adenocarcinoma and 4 squamous cell carcinoma, two small cell lung cancer organoids	On-chip drug responses of the PDX derived organoids (n=3) were consistent with the in vivo PDX results	• Pemetrexed-cisplatin • Gemcitabine+cisplatin • Docetaxel+cisplatin • Paclitaxel-cisplatin • Afatinib • Erlotinib • Gefitinib • Osimertinib • Crizotinib	• Organoids were cultured with Matrigel in integrated superhydrophobic microwell array chip (InSMAR-chip) • Perform drug testing within a week.	Well-designed pilot study would improve sensitivity and specificity of the assay.	[64]

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Lung adenocarcinoma.	Organoids (n=84) were established from patients with advanced lung adenocarcinoma.		• Six EGFR inhibitors • Two EGFR/Her2 inhibitors • Vandetanib • Lenvatinib • Cabozantinib • Pralsetinib • Poziotinib	Organoids culture medium previously described [106]	This was a retrospective study which cannot provide real-time information for clinical decision making.	[61]
Lung adenocarcinoma	PDOs (n=5) were developed from fresh lung tumors obtained from treatment naïve patients.	N/A	• Dubermatinib (TP-0903) • Ruxolitinib • TP0903 + ruxolitinib	Single cell CyTOF analysis of tumor tissues	Not for high throughput screening with a higher cost	[80]
Adenocarcinoma HER2 mutant	PDO (n=1) was developed from human lung tumor specimens,	Human lung tumor fragments were subcutaneously implanted in mice for generating PDX	• Pyrotinib • Afatinib • T-DM1		One PDO and small size of clinical trial patient cohort	[49]
NSCLC	PDOs (n=2) were developed from malignant pleural effusions of patients with NSCLC with Exon20ins mutations.	Human lung tissue specimens were implanted subcutaneously in mice	Amivantamab	Organoids culture medium previously described [106]		[77]
EGFR-Mutant NSCLC	Patient surgical resection or tumor biopsy specimens (n=3).	N/A	Osimertinib	Organoids culture medium previously described [106]	• The presence of the driver oncogene and secondary mutations are needed. • no tumor microenvironment included	[107]

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Table 3. Summary of organoids culture medium.

Ref	Matrigel	Base medium	Bovine serum albumin	N 2	B 27	EGF (ng/mL)	bFGF (ng/mL)	FGF-10 (ng/mL)	FGF-4 (ng/mL)	Y-27632 (ROCK inhibitor)	GlutaMax (L-glutamine alternative)	HEPES (mM)	R-spondin 1 (ng/mL)	Noggin (ng/mL)	Nicotinamide(mM)	Prostaglandin E2	SB202190	N-acetylcysteine	A83-01	Note
[78]	Yes	DMEM/F12	25%				Yes			10 μM	Yes									Stem Pro hESC Supplement, 0.1mM 2-mercaptoethanol
[50]	Yes	DMEM/F12		Yes	Yes	50				10 μM										
[59]	N/A																			HyStem-HP hydrogel kits
[16]	Yes	DMEM/F12		Yes	Yes	50	20			10 μM										
[52]	Yes	Advanced DMEM/F12	0.01%	Yes	Yes	50	1	20					250	100	10	1μM	10 μM	1 mM	500 nM	2mM L-glutamine, 100 ng/ml Wnt3a, 10 nM gastrin 1
[53]	Yes	Advanced DMEM/F12		Yes	Yes			20		10 mM	Yes		500	100	10		10 mM	1.25 mM	500 nM	25 ng/mL FGF-7
[58]	Yes	DMEM/F12		Yes	Yes	50	20			10 μM										
[86]	hydrogel cultures	RPMI 1640																		5% fetal bovine serum
[60]	No detailed description																			
[17]																				FBIM001 medium [54]
[74]	Yes	RPMI 1640		Yes	Yes	50		100	100	10 μM	Yes	10		100					500 nM	250 nM CHIR 99021, 100 nM SAG
[63]	Yes	RPMI 1640		Yes	Yes	50		100	100	10 μM	Yes	10		100					500 nM	250 nM CHIR 99021, 100 nM SAG
[64]	Yes	DMEM/F12		Yes	Yes	50				10 μM	Yes	10			5		3 μM	1 mM	5 μM	10 μM Forskolin, 3 nM Dexamethasone
[61]	Yes	Advanced DMEM/F12			Yes			100		5 μM	Yes	10	500	100	5		500 nM	1.25 mM	500 nM	25 ng/mL FGF-7
[80]	Yes	Advanced DMEM/F12		Yes	Yes	50	10	10		10 μM			500	100	4	1μM	5 μM		500 nM	20 ng/ml HGF
[49]	Yes	Advanced DMEM/F12			Yes	50		10			Yes	10	Yes*	Yes *	10		10 μM	1.25 mM	500 nM	R-spondin and Noggin form condition medium, 1 ng/ml FGF2, Dihydrotestosterone (DHT)
[77]	Yes	Advanced DMEM/F12			Yes			100		5 μM	Yes	10	500	100	5		500 nM	1.25 mM	500 nM	25 ng/mL FGF-7
[107]	Yes	Advanced DMEM/F12			Yes			100		5 μM	Yes	10	500	100	5		500 nM	1.25 mM	500 nM	25 ng/mL FGF-7

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