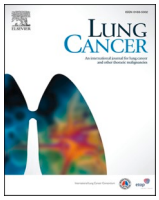
Lung Cancer 190 (2024) 107533

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan



Review article

# *Ex vivo* drug testing of patient-derived lung organoids to predict treatment responses for personalized medicine

Josephine A. Taverna [a,b,c,*,1], Chia-Nung Hung [a,1], Madison Williams [b,c,d], Ryan Williams [b,c,d], Meizhen Chen [a], Samaneh Kamali [e], Vaishnavi Sambandam [e], Cheryl Hsiang-Ling Chiu [a], Pawel A. Osmulski [a], Maria E. Gaczynska [a], Daniel T. DeArmond [c,d,f], Christine Gaspard [g], Maria Mancini [e], Meena Kusi [h], Abhishek N. Pandya [c], Lina Song [a], Lingtao Jin [a], Paolo Schiavini [e], Chun-Liang Chen [a,b,i,*]

[a] *Department of Molecular Medicine, University of Texas Health Science Center, San Antonio, TX, USA*
[b] *Mays Cancer Center, University of Texas Health Science Center, San Antonio, TX, USA*
[c] *Department of Medicine, Division of Hematology and Oncology, University of Texas Health Science Center, San Antonio, TX, USA*
[d] *Department of General Oncology, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA*
[e] *Champions Oncology, Inc., Hackensack, NJ, USA*
[f] *Department of Cardiothoracic Surgery, University of Texas Health Science Center, San Antonio, Texas and Department of Laboratory Medicine, Baptist Health System, San Antonio, TX, USA*
[g] *Dolph Briscoe, Jr. Library, University of Texas Health Science Center, San Antonio, TX, USA*
[h] *Deciphera Pharmaceuticals, LLC., Waltham, MA, USA*
[i] *School of Nursing, University of Texas Health Science Center, San Antonio, TX, USA*

ARTICLE INFO

*Keywords:*
Lung cancer
Organoid
Drug screening
High throughput
Translational
Pre-clinical
Clinical
Tumor microenvironment
Personalized medicine

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 bio-banked sections and/or biopsies, pleural effusions and PDX mouse models. PDOs were subject to *ex vivo* drug screening with chemotherapy, targeted therapy and/or immunotherapy. PDOs consistently recapitulated the genomic alterations and drug sensitivity of primary tumors. Although sample sizes of the previous studies were limited and some technical challenges remain, PDOs showed great promise in the screening of novel therapy drugs. With the technical advances of high throughput, tumor-on-chip, and combined microenvironment, the drug screening process using PDOs will enhance precision care of lung cancer patients.

## 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 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]. Immune checkpoint inhibitors (ICI) have revolutionized treatment paradigms and have been broadly integrated into the first line setting of non-oncogene driven NSCLC and small cell lung cancer therapy in combination with chemotherapy. ICI monotherapy has been shown to be effective in selected patients with NSCLC

* Corresponding authors at: Department of Molecular Medicine, University of Texas Health Science Center, San Antonio, TX, USA.
*E-mail addresses:* TavernaJ@uthscsa.edu (J.A. Taverna), chenc4@uthscsa.edu (C.-L. Chen).
[1] These authors contributed equally.

https://doi.org/10.1016/j.lungcan.2024.107533
Received 11 January 2024; Received in revised form 6 March 2024; Accepted 7 March 2024
Available online 14 March 2024

with high PD-L1 expression (>50 %) or high tumor mutational burden (TMB > 10). With the advent of molecular targeted therapies, there have also been significant improvements in survival outcomes for patients with oncogene driven NSCLC [5,6]. Although these systemic therapies have durable responses, drug resistance often occurs with long-term use of these therapies due to tumor intrinsic factors and complex tumor immune microenvironments that sustain tumor growth and metastasis [7–9]. 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 tumor 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 [8,10–13]. Historically, cancer cell lines have been used to test novel anti-cancer therapies, but they do not accurately predict treatment responses due, in part, to interpatient variability and molecular tumor heterogeneity [14–17]. Patient-derived xenograft (PDX) mouse models can predict better treatment responses but they are costly and difficulties in high-throughput expansion have confined their application to preclinical drug screening [16,18]. Patient derived organoids (PDOs) are human tissue derived tumors that can overcome some of these challenges [19–21]. PDOs (3-D cultures of cancer cells) can be established from different tumor specimens collected from lobectomies, wedge resections, core needle biopsies, body fluids (ascites, pleural fluid, cerebral spinal fluid), peripheral blood (circulating tumor cells) [22–29,36]. Lung organoids from tumor tissue from tumor resections can best capture the spatial architecture and molecular signatures [30–35]. Lung organoids grow exponentially from single stem cells embedded in matrigels/culture mediums and display unique morphological patterns [30,35,37]. PDO models can demonstrate test drug efficacy of anti-neoplastic drugs based on morphological changes, tumor volume reduction or reduction in cell viability [38]. Thus, PDO models can serve as a surrogate tumor 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 [39–41]. An important clinical aspect of PDOs is the potential for genomic and transcriptomic profiling and therapeutic screening for precision medicine [42–46] (Fig. 1). In this way, PDOs of lung cancer can further our understanding of lung cancer pathophysiology and 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 most promising technology.

## 2. Methods

This is a comprehensive review of the published literature focusing on *ex vivo* drug testing using organoids from lung cancer patients. PubMed, Cochrane Library and Scopus databases were searched. 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 (i.e., editorials). The search strategy for this review was the result of prior research in the fields. 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 Table 1.

**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/ | 297,911 |
| 7 | 4 and 6 | 269 |
| 8 | 5 or 7 | 312 |

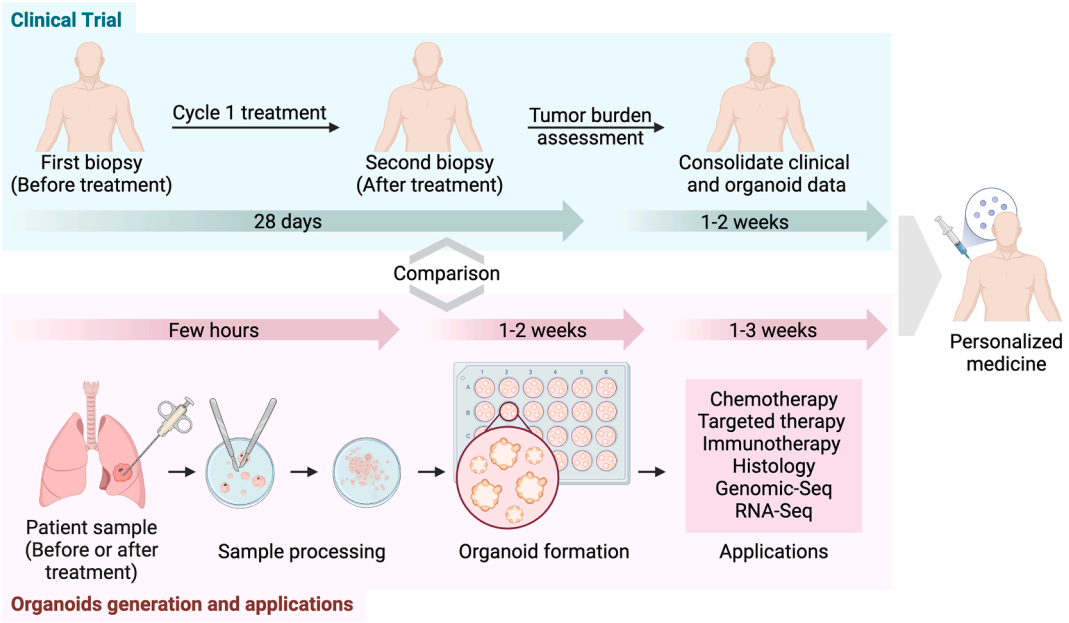



**Fig. 1.** The illustration of applications of patient-derived lung cancer organoids for precision medicine. Created with BioRender.com.

## 3. Results

### 3.1. Organoid technology for high-throughput drug screening

Historically, cancer cell lines have been widely used as preclinical models to evaluate the efficacy of antineoplastic drugs. Despite its wide application, the sensitivity and specificity of drug candidates in 2D-cell culture have been widely variable. Lung cancer organoid technology was developed for high-throughput drug screening to better predict clinical responses to drug treatment. In one study, 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 natural compounds (chelerythrine chloride, cantharidin and harmine) [47]. In a second study, Chen et al. evaluated drug sensitivity of 26 anti-cancer therapeutic agents in organoids derived from 12 patients with locally advanced NSCLC, including tumors that harbored EGFR and KRAS G12C mutations [45]. They found that PDOs retained the histological and genetic characteristics of the primary tumors with more than 80 % concordance [45]. Furthermore, they showed that PDOs correlated with molecular profiles of primary lung tumors with mutations involving the top 20 NSCLC-related genes [45]. In a separate study, Li et al. tested 24 antineoplastic drugs in PDOs derived from 12 patients with NSCLC using a high throughput system and found drug efficacy correlated with mutational profiles [48]. 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 that included targeted therapies and chemotherapy [49]. Takahashi et al. used their high-throughput assay to evaluate 86 anti-cancer agents (targeted agents, immune checkpoint inhibitors and cytotoxic chemotherapies) in PDOs developed from lung tumors with squamous and adenosquamous histologies [50]. In all these studies, the pharmacogenomic profiles of PDOs were highly comparable to that of tumor tissue indicating that organoid models can predict therapeutic responses on a larger scale to inform therapeutic strategies [17,51,52].

### 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 [53]. Gmeiner et al. found that dysregulated pyrimidine biosynthesis contributes to drug resistance in SCLC and they demonstrated that the novel fluoropyrimidine polymer (CF10) could overcome drug resistance to conventional chemotherapy using a PDO model system [54]. In another study, PDOs derived from a NSCLC patient demonstrated the efficacy of an oral potent tubulin destabilizing agent, S-40, in overcoming paclitaxel resistance [55]. 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.

The identification of oncogenic activation of tyrosine kinases in NSCLC tumors, most notably epidermal growth factor receptor (EGFR) mutations or anaplastic lymphoma kinase (ALK) gene or c-ROS oncogene 1 (ROS1) gene rearrangements, 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 [51,56]. Despite 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 [57]. They found that EMI66 and other derivatives reduced viability of organoids derived from three PDOs with EGFR mutations, suggesting a bypass mechanism to target EGFR driven tumor growth [57]. 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 [58]. 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 [58]. These collective studies suggests that lung organoids can be used as a treatment prediction model to allow clinicians to prioritize targeted by comparing the responses of the individual PDOs to different drug combinations in real-time. These previous studies of *ex vivo* drug testing based on patient-derived lung cancer organoids are summarized in Table 2 and organoids culture medium are summarized in Table 3.

### 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 could predict treatment in patients receiving clinically approved targeted agents (EGFR inhibitors, ERBB2 targeted therapies, multikinase inhibitors) [51]. They found that combination treatment with dabrafenib and trametinib demonstrated favorable *ex vivo* and clinical responses in a NSCLC patient harboring both an EGFR exon 19 deletion and a BRAF G464A mutation [51]. 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 [51]. The preclinical studies mirrored the phase 2 study (ZENITH20-2 Trial) of poziotinib in patients with advanced or metastatic NSCLC (n = 90 patients) [59]. With a median follow-up of 9.0 months, this study yielded an overall response rate (ORR) of 28 %, median progression free survival (PFS) of 5.5 months, median duration of response (DOR) of 5.1 months [59]. Most importantly, clinical benefit was seen regardless of prior lines of therapy, presence of brain metastasis and type of HER2 mutation [59]. Both the preclinical and clinical studies indicated robust and durable anti-tumor activity of poziotinib in NSCLC patients with ERBB2 exon 20 insertions [51]. Among the multi-kinase inhibitors (vandetanib, cabozantinib, and lenvatinib), pralsetinib demonstrated clinical efficacy in RET-rearranged lung tumors *ex vivo* and *in vivo* [51]. In a recent phase I/II clinical trial (ARROW trial), robust treatment responses were observed in patients with RET fusion-positive NSCLC (n = 233) and similar drug efficacy were recapitulated in respective PDOs [60]. In 53 of 87 NSCLC patients previously treated with platinum-based chemotherapy, there was a 61 % ORR to therapy including five patients with a complete response [60]. In 19 of 27 treatment naïve NSCLC, a 70 % overall response was observed with a complete response in three patients [60]. 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 [61]. Several HER2-targeted agents (afatinib, dacomitinib, neratinib and trastuzumab) have not shown long term responses in patients with HER2-mutant NSCLC [62–65]. 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 [44]. Pyrotinib showed significant growth inhibition of organoids relative to afatinib [44]. In PDX model, the pan-HER inhibitor reduced tumor volumes showed a superior antitumor effect compared

**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. | [74] |
| Adenocarcinoma (NSCLC patients with tumors stage I–III, EGFR L858R, EGFR Ex20 ins, KRAS G12C) | (1) Fresh tumor samples harvested for organoid culture.<br>(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. | [45] |
| 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<br>• Thymidylate synthase inhibitors: CF10, 5-FU | • HyStem-HP hydrogel kits | | [54] |
| (1) Adenocarcinoma<br>(2) Squamous cell carcinoma<br>(3) Adenosquamous carcinoma<br>(4) Large cell carcinoma<br>(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.<br>• PDXs from 10 samples (43 %) | • Docetaxel<br>• Olaparib<br>• Erlotinib<br>• Crizotinib | | Limitation of cancer organoid models is the lack of a cancer microenvironment. | [17] |
| NSCLC | PDOs derived from NSCLC were cultured *in vitro* (n = 10) | N/A | • Chelerythrine chloride<br>• Cantharidin<br>• Harmine<br>• Berberine<br>• Betaine | | | [47] |
| 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. | [48] |
| Small cell lung cancer (n = 1) | Tumor organoids were generated from primary lung cancer cells from patients with SCLC. | N/A | • Cisplatin<br>• Etoposide | • Microfluidic-based lung cancer organoid culture platform for testing drug sensitivity.<br>• Patient-derived lung cancer organoids (PDOs) were cultured and expanded in Matrigel droplets in 24-well plates. | Small sample sized used and will require further large-scale analyses to validate the findings. | [53] |
| Adenocarcinoma<br>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. | [82] |
| 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 | [55] |
| (1) Squamous cell carcinoma<br>(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<br>• Organoids were cultured in FBIM001 medium. Organoids culture condition as previous describe [49] | Few and limited PDO examples were selected for each type drug screen. | [50] |
| (1) Lung adenocarcinoma<br>(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.<br>• 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.<br>• BGJ398 (FGFR inhibitor)<br>• Trametinib (MEK inhibitor) | | | [69] |

(*continued on next page*)

**Table 2** (*continued*)

| Cancer type | Organoid Model | PDX Model (n) | Compounds Tested | Technology/ Unique culture supplement | Limitations | Ref |
|---|---|---|---|---|---|---|
| | | | • Selumetinib (MEK inhibitor)<br>• Afatinib (EGFR inhibitor) | | | |
| Lung adenocarcinoma | Three primary lung cancer organoid models:<br>• XDO-137 (EGFR ex19del)<br>• PDXO-4000 (EGFR ex19del)<br>• XDO-344 (wild-type EGFR) | N/A | • EMI66 and other derivatives. | Organoids culture condition previously described [69] | | [57] |
| (1) Squamous cell carcinoma<br>(2) Adenocarcinoma<br>(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<br>• Gemcitabine + cisplatin<br>• Docetaxel + cisplatin<br>• Paclitaxel-cisplatin<br>• Afatinib<br>• Erlotinib<br>• Gefitinib<br>• Osimertinib<br>• Crizotinib | • Organoids were cultured with Matrigel in integrated superhydrophobic microwell array chip (InSMAR-chip)<br>• Perform drug testing within a week. | Well-designed pilot study would improve sensitivity and specificity of the assay. | [58] |
| Lung adenocarcinoma. | Organoids (n = 84) were established from patients with advanced lung adenocarcinoma. | | • Six EGFR inhibitors<br>• Two EGFR/Her2 inhibitors<br>• Vandetanib<br>• Lenvatinib<br>• Cabozantinib<br>• Pralsetinib<br>• Poziotinib | Organoids culture medium previously described [91] | This was a retrospective study. | [51] |
| Lung adenocarcinoma | PDOs (n = 5) were developed from fresh lung tumors obtained from treatment naïve patients. | N/A | • Dubermatinib (TP-0903)<br>• Ruxolitinib<br>• TP0903 + ruxolitinib | Single cell CyTOF analysis of tumor tissues | Not for high throughput screening with a higher cost | [76] |
| 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<br>• Afatinib<br>• T-DM1 | | One PDO and small size of clinical trial patient cohort | [44] |
| 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 [91] | | [72] |
| EGFR-Mutant NSCLC | Patient surgical resection or tumor biopsy specimens (n = 3). | N/A | Osimertinib | Organoids culture medium previously described [91] | • The presence of the driver oncogene and secondary mutations are needed.<br>• no tumor microenvironment included | [92] |

with afatinib and trastuzumab emtansine (humanized monoclonal antibody trastuzumab covalently linked to cytotoxic agent DM1) [44]. Specifically, mice treated with pyrotinib had > 50 % reduction in tumor volumes [44]. This anti-tumor drug effect was attributed to the inhibition of HER2 and its downstream signaling pathways, including ERK and Akt. Preclinical findings were validated in a phase 2 study of pyrotinib (400 mg oral daily dose) in patients *HER2*-amplified NSCLC (n = 27) [66]. In this single arm study, pyrotinib demonstrated a median Progression free survival (PFS) and overall survival (OS) reported as 6.3 months and 12.5 months, respectively [66]. Furthermore, patients administered pyrotinib as first-line treatment achieved a median PFS of 12.4 months [66]. Approximately 30 % of the patients who had progressed on EGFR tyrosine kinase inhibitor responded to pyrotinib. Strikingly, patients with brain metastases had an overall response rate (ORR) of 40 %. Collectively, the preclinical and clinical study demonstrates 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 that plays a critical role in oncogenesis (tumor proliferation, angiogenesis, migration, and survival) via gene amplification, activating mutations, or gene translocation in lung tumors [67,68]. 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 [69]. 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 [69]. Additionally, they processed 16 lung adenocarcinoma PDX and 26 squamous cell lung cancer PDX tumors for organoid establishment [69]. They tested trametinib (MEK inhibitor) and the PI3K inhibitor BKM120 with BGJ398 (inhibits pFGFR and pAkt) in their FGFR1-amplified organoid models [69]. Strong synergy was observed in the BGJ398 + trametinib combination (combination index < 0.5), whereas weaker synergy

**Table 3**
Summary of organoids culture medium.

| Ref | Base medium | Matrigel | N2 | B27 | EGF (ng/mL) | FGF-10 (ng/mL) | Y-27632 (μM) | GlutaMax (L-glutamine alternative) | R-spondin 1 (ng/mL) | Noggin (ng/mL) | Nicotinaminde (mM) | SB202190 (μM) | N-acetylcysteine (mM) | A83-01 | Specific components |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| [76] | Advanced DMEM/F12 | Yes | Yes | Yes | 50 | 10 | 10 | | 500 | 100 | 4 | 5 | | 500 nM | 20 ng/ml HGF, 1 μM Prostaglandin E2, 20 ng/mL bFGF |
| [47] | Advanced DMEM/F12 | Yes | Yes | Yes | 50 | 20 | | | 250 | 100 | 10 | 10 | 1 | 500 nM | 2 mM L-glutamine, 100 ng/ml Wnt3a, 10 nM gastrin 1, 0.01 % BSA, 1 μM Prostaglandin E2, 1 ng/mL bFGF |
| [48] | Advanced DMEM/F12 | Yes | Yes | Yes | | 20 | 10 mM | Yes | 500 | 100 | 10 | 10 mM | 1.25 | 500 nM | 25 ng/mL FGF-7 |
| [44] | Advanced DMEM/F12 | Yes | | Yes | 50 | 10 | | Yes | Yes* | Yes* | 10 | 10 | 1.25 | 500 nM | R-spondin and Noggin form condition medium, 1 ng/ml FGF2, Dihydrotestosterone (DHT), 10 mM HEPES |
| [51,72,92] | Advanced DMEM/F12 | Yes | | Yes | | 100 | 5 | Yes | 500 | 100 | 5 | 0.5 | 1.25 | 500 nM | 25 ng/mL FGF-7, 10 mM HEPES |
| [45] | DMEM/F12 | Yes | Yes | Yes | 50 | | 10 | | | | | | | | |
| [17,53] | DMEM/F12 | Yes | Yes | Yes | 50 | | 10 | | | | | | | | 20 ng/mL bFGF |
| [58] | DMEM/F12 | Yes | Yes | Yes | 50 | | 10 | Yes | | | 5 | 3 | 1 | 5 μM | 10 μM Forskolin, 3 nM Dexamethasone, 10 mM HEPES |
| [74] | DMEM/F12 | Yes | | | | | 10 | Yes | | | | | | | Stem Pro hESC Supplement, 0.1 mM 2-mercaptoethanol, 25 % BSA, bFGF |
| [69,57] | RPMI 1640 | Yes | Yes | Yes | 50 | 100 | 10 | Yes | | 100 | | | | 500 nM | 250 nM CHIR 99021, 100 nM SAG, 100 ng/mL FGF4, 10 mM HEPES |
| [82] | RPMI 1640 | | | | | | | | | | | | | | Hydrogel cultures, 5 % fetal bovine serum |
| [54] | | N/A | | | | | | | | | | | | | HyStem-HP hydrogel kits |
| [50] | | | | | | | | | | | | | | | FBIM001 medium [49] |
| [55] | No detailed description | | | | | | | | | | | | | | |

(combination index > 0.5) was observed in the BGJ398 + BKM120 (PI3K inhibitor) combination [69]. Furthermore, PDOs retained the histologic and molecular features of their parental tumors [69]. The spectrum of mutations was highly concordant between the organoid and matched patient tumor and PDX tissue [69]. Mutational burden in the five long-term established PDOs were similar in parental tumor and PDX tumors [69]. This indicates that organoid culture conditions did not destabilize the cancer genome. Copy number variation (CNV) profiles of the parental tumors were largely preserved during PDO culture [69]. 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 [70], 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 NSCLC for which there are no FDA approved targeted therapies [71]. 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 [72]. Furthermore, they established their PDOs from malignant pleural effusions collected from two patients with NSCLC with Exon 20 insertion mutations [72]. In the Phase I (CHRYSALIS) study, amivantamab produced robust and

**Table 4**
Clinical trials related to organoid studies.

| NCT | Study description | Drug mechanism of action | Results | Adverse events | Conclusion | Ref |
|---|---|---|---|---|---|---|
| NCT03318939 | ZENITH20, a multicenter, multicohort, open-label phase II study, evaluated poziotinib in patients with advanced or metastatic NSCLC. In cohort 2, patients received poziotinib (16 mg) po once daily. | Poziotinib is an irreversible pan Her2 inhibitor with activity agains HER1, Her2, and Her4. Pozitinib has been shown to inhibit kinase activity of EGFR with exon 20 insertion mutations. | • ORR was 27.8 % (95 % CI, 18.9 to 38.2)<br>• DCR was 70.0 % (95 % CI, 59.4 to 79.2)<br>• Median PFS was 5.5 months (95 % CI, 3.9 to 5.8)<br>• Median DOR was 5.1 months (95 % CI, 4.2 to 5.5) | • TRAE (grade 3)<br>• Rash (48.9 %)<br>• Diarrhea (25.6 %)<br>• Stomatitis (24.4 %) | Poziotinib shows antitumor activity in patients with HER2 exon 20 insertion NSCLC. | [51,59] |
| NCT03037385 | ARROW is a multi-cohort, open-label, phase 1/2 study done at 71 sites in 13 countries. Patients aged 18 years or older with locally advanced or metastatic solid tumours, including RET fusion-positive NSCLC. In phase 2, patients received pralsetinib 400 mg po once daily. | Pralsetinib is highly-selective RET Inhibitor | • ORR was 61 % (95 % CI, 50–71) | • TRAE (grade 3, 48 %)<br>• Neutropenia (18 %)<br>• Hypertension (11 %)<br>• Anaemia (10 %) | Pralsetinib is a well-tolerated, promising, once-daily oral treatment option for patients with RET fusion-positive NSCLC. | [51,60] |
| ChiCTR1800020262 | A prospective, multicenter, single-arm trial, patients with advanced NSCLC with *HER2* amplification. Patients administered pyrotinib 400 mg po daily. | Oral irreversible pan-HER receptor tyrosine kinase inhibitor that target HER1, HER2, and HER4 | • 6-month PFS rate was 51.9 % (95 % CI, 34.0–69.3)<br>• Median PFS was 6.3 months (95 % CI, 3.0–9.6 months)<br>• Median OS was 12.5 months (95 % CI, 8.2–16.8 months)<br>• ORR of 22.2 % (95 % CI, 10.6 %-40.8 %) | • TRAE (grade 3, 22.2 %)<br>• Diarrhea (7.4 %)<br>• Vomiting (7.4 %)<br>• Alanine aminotransferase (ALT) increase (3.7 %)<br>• Rash (3.7 %) | Pyrotinib demanstrates antitumor efficacy in *HER2*-amplified NSCLC patients with a manageable safety profile. | [44,66] |
| NCT02609776 | CHRYSALIS is a phase I, open-label, dose-escalation, and dose-expansion study, which included a population with *EGFR* Exon20ins NSCLC. Recommended phase II dose of 1,050 mg amivantamab (1,400 mg, ≥ 80 kg) given once weekly for the first 4 weeks and then once every 2 weeks starting at week 5. | EGFR-MET bispecific antibody | • ORR was 40 % (95 % CI, 29 to 51)<br>• Median DOR of 11.1 months (95 % CI, 6.9 to not reached)<br>• Median PFS was 8.3 months (95 % CI, 6.5 to 10.9). | • TRAE (grade 3)<br>• Hypokalemia (5 %)<br>• Rash (4 %)<br>• pulmonary embolism (4 %)<br>• Diarrhea (4 %)<br>• Neutropenia (4 %) | Amivantamab yielded robust and durable responses with tolerable safety in patients with EGFR Exon20ins mutations after progression on platinum-based chemotherapy. | [72,73] |

*Non-small cell lung cancer (NSCLC), overall response rate (ORR), disease control rate (DCR), progression-free survival (PFS), overall survival (OS), duration of response (DOR), Rearranged during transfection (RET) gene, treatment-related adverse events (TRAE).

durable tumor responses in patients with EGFR Exon 20 insertion NSCLC (n = 81) who progressed on platinum chemotherapy. The ORR was 40 % (including three complete responses) with a median DOR of 11.1 months and median PFS was 8.3 months [73]. This study highlights the importance of ex vivo drug testing to inform clinical trial design [72]. Clinical trials have been summarized in Table 4.

### 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 [74]. 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 to EGFR inhibitors [74]. 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) [75].

Therapeutic targeting of tumor subpopulations based on their single cell expression of oncogenic markers can lead to robust treatment responses in PDO and PDX mouse models. Taverna et al. used mass cytometry by time-of-flight (CyTOF) to identify continuous AXL and JAK/STAT3 signal activation in lung adenocarcinoma tumors derived from 11 treatment naïve patients [76]. Using single cell profiling, they could stratify tumor subpopulations based on AXL and STAT3 signaling [76]. They found that tumor subpopulations with high AXL/STAT3 expression displayed hybrid epithelial-to-mesenchymal phenotype and cancer stemness, suggesting high tumorigenic and metastatic potential [76]. They subsequently demonstrated that PDO with high AXL/STAT3 expression responded robustly to combination treatment with dubermatinib (AXL inhibitor) and ruxolitnib (JAK inhibitor) as compared with single agents [76]. Conversely, PDOs with low AXL/STAT3 expression did not respond to combination treatment and/or single agents [76]. In a separate study, they created PDX mouse models derived from PDOs expressing high AXL and JAK/STAT3. The PDX mouse models with high AXL/STAT3 expression demonstrated reduced tumor volumes following combination treatment with AXL and JAK inhibitors, when compared to single agent (unpublished data). These studies highlight that PDOs can capture the oncogenic signaling of primary lung tumor cell populations and could help determine treatment sensitivity to targeted therapies (bench to bedside).

Another major strength for PDOs is that they can be subjected to genetic manipulation, which can be a powerful tool for understanding 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 [77]. 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. PDOs have become widely used for testing antineoplastic 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 [51]. 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 [78]. This study provides a strong rationale for designing co-clinical trials with organoid models to better predict drug responses. Organoids have also been adopted to predict treatment response to radiotherapy and immunotherapy [79–81]. By simulating cancer behavior *ex vivo*, organoid technology can integrate molecular biology with treatment decisions to improve patient selection for particular therapeutic agents.

Based on our review of the literature, we found that organoid models can be established from pleural effusions which is scarce source of lung cancer cells [72,82,83]. Strikingly, organoids derived from pleural fluid aspirate can developed tumor-specific cellular and molecular characteristics and preserved tumor heterogeneity [82,83]. 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 studied simultaneously [83,84]. Co-culturing tumor organoids with other immune cells can mimic complex cellular microenvironment and tumor-intrinsic interactions [50]. 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 THP-1 effector cells and reflect the antibody-dependent cellular cytotoxicity (ADCC) response of the drug [50]. The study also expanded on the use of anti-PD1 monoclonal antibodies to evaluate efficacy of immune checkpoint inhibitors.

Although organoid culture systems have several advantages over traditional 2D cell-based models, there are certain challenges that need to be overcome. First, establishing organoids from lung cancer is a demanding endeavor. The overall organoid establishment rate from lung cancer patients is limited and variable in different lung cancer types [85,86]. Moreover, the outgrowth of normal airway organoids could replace tumor organoids after long-term culture [41,85]. The variable successful rates and the overgrowth of normal airway organoids would impede the implementation of organoids for precision medicine and personalized drug screening in clinical trials. The organoid media, culture procedures, and tumor sources could be the potential factors leading to these challenges. Therefore, characterization of organoids via immunohistochemistry or immunofluorescence to evaluate the malignancy of organoids would be necessary before *ex vivo* drug testing. Moreover, obtaining an adequate amount of tumor tissues and fine-tuning the organoid media can potentially promote tumor growth and hinder the growth of normal airway organoids [85].

Second, the most common organoid models lack stromal components and paracrine signals unique to the tumor microenvironment [87]. To overcome this limitation, 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 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 [88]. Tumor-on-chip technology can mimic the mechanical and biochemical properties of the tumor microenvironment (oxygen and nutrient gradients, extracellular matrix stiffness, and cell–cell interactions). By creating a miniature model of lung tumors on a microfluidic chip, this technology allows researchers to study how cancer cells directly engage with their environment and allows for the testing of novel therapeutics mechanistically.

Tumor-on-chip platform also provides 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, intravasation into the blood or lymphatic vessels and colonization

at the secondary site. 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 [89]. In another study, researchers developed microfluidic chips to study microenvironmental conditions of metastatic tumor niches and demonstrated that hypoxic conditions at the secondary site promoted tumor growth and migration of prostate cancer cells.

In conclusion, the future development of PDO on chip technology will revolutionize drug discovery efforts and help inform clinical trials by allowing for more 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 and cell–cell interactions between tumor cells, stromal fibroblasts, endothelial cells, immune subtypes and their individual responses (cytokine release, mechanotransduction) to immunotherapy and other systemic treatments [90].

## Funding

This research (to CLC) was partially funded by NIH/NCI, grant number 1R21CA264353-01 and U54 CA217297-04. This research (to J. A.T) was supported by NIH/NCI grants 1R01CA269766-01A1, the Max and Minnie Tomerlin Voelcker Fund Young Investigator Award, NIH/NCATS grants KL2 TR001118 and KL2 TR002646.

## CRediT authorship contribution statement

**Josephine A. Taverna:** Conceptualization, Formal analysis, Supervision, Writing – original draft. **Chia-Nung Hung:** Writing – original draft. **Madison Williams:** Investigation. **Ryan Williams:** Investigation. **Meizhen Chen:** Investigation, Data curation. **Samaneh Kamali:** Writing – original draft. **Vaishnavi Sambandam:** Writing – original draft. **Cheryl Hsiang-Ling Chiu:** Visualization. **Pawel A. Osmulski:** Writing – review & editing. **Maria E. Gaczynska:** Writing – review & editing. **Daniel T. DeArmond:** Writing – review & editing. **Christine Gaspard:** Writing – original draft, Methodology. **Maria Mancini:** Writing – original draft. **Meena Kusi:** Writing – original draft, Data curation. **Abhishek N. Pandya:** Writing – review & editing. **Lina Song:** Writing – review & editing. **Lingtao Jin:** Writing – review & editing, Writing – original draft. **Paolo Schiavini:** Writing – review & editing. **Chun-Liang Chen:** Writing – original draft, Supervision, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

In this section, you can acknowledge any support given which is not covered by the author contribution or funding sections. This may include administrative and technical support, or donations in kind (e.g., materials used for experiments).

## References

[1] H. Sung, J. Ferlay, R.L. Siegel, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. 71 (2021) 209–249.
[2] L.A. Pikor, V.R. Ramnarine, S. Lam, et al., Genetic alterations defining NSCLC subtypes and their therapeutic implications, Lung Cancer 82 (2013) 179–189.
[3] C.M. Rudin, E. Brambilla, C. Faivre-Finn, et al., Small-cell lung cancer, Nat. Rev. Dis. Primers 7 (2021) 3.
[4] L.A. Byers, C.M. Rudin, Small cell lung cancer: where do we go from here? Cancer 121 (2015) 664–672.
[5] F.R. Hirsch, G.V. Scagliotti, J.L. Mulshine, et al., Lung cancer: current therapies and new targeted treatments, Lancet 389 (2017) 299–311.
[6] M. Reck, D. Rodriguez-Abreu, A.G. Robinson, et al., Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer, N. Engl. J. Med. 375 (2016) 1823–1833.
[7] Z.F. Lim, P.C. Ma, Emerging insights of tumor heterogeneity and drug resistance mechanisms in lung cancer targeted therapy, J. Hematol. Oncol. 12 (2019) 134.
[8] N.K. Altorki, G.J. Markowitz, D. Gao, et al., The lung microenvironment: an important regulator of tumour growth and metastasis, Nat. Rev. Cancer 19 (2019) 9–31.
[9] J.W. Tyner, F. Haderk, A. Kumaraswamy, et al., Understanding drug sensitivity and tackling resistance in cancer, Cancer Res. 82 (2022) 1448–1460.
[10] M. Yuan, Y. Zhao, H.T. Arkenau, et al., Signal pathways and precision therapy of small-cell lung cancer, Signal Transduct. Target. Ther. 7 (2022) 187.
[11] Z. Niu, R. Jin, Y. Zhang, et al., Signaling pathways and targeted therapies in lung squamous cell carcinoma: mechanisms and clinical trials, Signal Transduct. Target. Ther. 7 (2022) 353.
[12] J. Rotow, T.G. Bivona, Understanding and targeting resistance mechanisms in NSCLC, Nat. Rev. Cancer 17 (2017) 637–658.
[13] K.J. Hiam-Galvez, B.M. Allen, M.H. Spitzer, Systemic immunity in cancer, Nat. Rev. Cancer 21 (2021) 345–359.
[14] W.H. Douglas, J.A. McAteer, J.R. Smith, et al., Type II alveolar pneumonocytes in vitro, Int. Rev. Cytol. Suppl. (1979) 45–65.
[15] J. Choi, E. Iich, J.H. Lee, Organogenesis of adult lung in a dish: differentiation, disease and therapy, Dev. Biol. 420 (2016) 278–286.
[16] M. Bleijs, M. van de Wetering, H. Clevers, et al., Xenograft and organoid model systems in cancer research, EMBO J. 38 (2019) e101654.
[17] M. Kim, H. Mun, C.O. Sung, et al., Patient-derived lung cancer organoids as in vitro cancer models for therapeutic screening, Nat. Commun. 10 (2019) 3991.
[18] K.M. Morgan, G.M. Riedlinger, J. Rosenfeld, et al., Patient-derived xenograft models of non-small cell lung cancer and their potential utility in personalized medicine, Front. Oncol. 7 (2017) 2.
[19] A.L. Bredenoord, H. Clevers, J.A. Knoblich, Human tissues in a dish: the research and ethical implications of organoid technology, Science 355 (2017) 20.
[20] H.C. Clevers, Organoids: Avatars for personalized medicine, Keio J. Med. 68 (2019) 95.
[21] N. Iakobachvili, P.J. Peters, Humans in a dish: the potential of organoids in modeling immunity and infectious diseases, Front. Microbiol. 8 (2017) 2402.
[22] D. Gao, I. Vela, A. Sboner, et al., Organoid cultures derived from patients with advanced prostate cancer, Cell 159 (2014) 176–187.
[23] H. Clevers, Modeling development and disease with organoids, Cell 165 (2016) 1586–1597.
[24] J. Drost, H. Clevers, Organoids in cancer research, Nat. Rev. Cancer 18 (2018) 407–418.
[25] N. Sachs, J. de Ligt, O. Kopper, et al., A living biobank of breast cancer organoids captures disease heterogeneity, Cell 172 (373–386) (2018) e310.
[26] E. Driehuis, K. Kretzschmar, H. Clevers, Establishment of patient-derived cancer organoids for drug-screening applications, Nat. Protoc. 15 (2020) 3380–3409.
[27] J.H. Yu, S. Ma, Organoids as research models for hepatocellular carcinoma, Exp. Cell Res. 411 (2022) 112987.
[28] A. Baiao, S. Dias, A.F. Soares, et al., Advances in the use of 3D colorectal cancer models for novel drug discovery, Expert Opin. Drug Discov. (2022) 1–12.
[29] J. Doffo, S.A. Bamopoulos, H. Kose, et al., NOXA expression drives synthetic lethality to RUNX1 inhibition in pancreatic cancer, PNAS 119 (2022).
[30] J.R. Rock, M.W. Onaitis, E.L. Rawlins, et al., Basal cells as stem cells of the mouse trachea and human airway epithelium, PNAS 106 (2009) 12771–12775.
[31] Hild M, Jaffe AB. Production of 3-D Airway Organoids From Primary Human Airway Basal Cells and Their Use in High-Throughput Screening. *Curr Protoc Stem Cell Biol* 2016;37:IE 9 1-IE 9 15.
[32] R.R. Nadkarni, S. Abed, J.S. Draper, Organoids as a model system for studying human lung development and disease, Biochem. Biophys. Res. Commun. 473 (2016) 675–682.
[33] Y.W. Chen, S.X. Huang, A. de Carvalho, et al., A three-dimensional model of human lung development and disease from pluripotent stem cells, Nat. Cell Biol. 19 (2017) 542–549.
[34] Y. Han, X. Duan, L. Yang, et al., Identification of SARS-CoV-2 inhibitors using lung and colonic organoids, Nature 589 (2021) 270–275.
[35] P. Kadur Lakshminarasimha Murthy, V. Sontake, A. Tata, et al., Human distal lung maps and lineage hierarchies reveal a bipotent progenitor, Nature 604 (2022) 111–119.
[36] T. Sato, R.G. Vries, H.J. Snippert, et al., Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche, Nature 459 (2009) 262–265.
[37] J. Abbasi, Patient-derived organoids predict cancer treatment response, J. Am. Med. Assoc. 319 (2018) 1427.
[38] C.L. Chen, X. Yu, I.O. James, et al., Heparin-binding EGF-like growth factor protects intestinal stem cells from injury in a rat model of necrotizing enterocolitis, Lab. Invest. 92 (2012) 331–344.
[39] H. Clevers, E. Bender, Q&A: hans clevers, Banking on Organoids. *Nature* 521 (2015) S15.
[40] F. Weeber, M. van de Wetering, M. Hoogstraat, et al., Preserved genetic diversity in organoids cultured from biopsies of human colorectal cancer metastases, PNAS 112 (2015) 13308–13311.
[41] N. Sachs, A. Papaspyropoulos, D.D. Zomer-van Ommen, et al., Long-term expanding human airway organoids for disease modeling, EMBO J. 38 (2019) 15.

[42] Verissimo CS, Overmeer RM, Ponsioen B, et al. Targeting mutant RAS in patient-derived colorectal cancer organoids by combinatorial drug screening. *Elife* 2016;5.
[43] C. Pauli, B.D. Hopkins, D. Prandi, et al., Personalized in vitro and in vivo cancer models to guide Precision medicine, Cancer Discov. 7 (2017) 462–477.
[44] Y. Wang, T. Jiang, Z. Qin, et al., HER2 exon 20 insertions in non-small-cell lung cancer are sensitive to the irreversible pan-HER receptor tyrosine kinase inhibitor pyrotinib, Ann. Oncol. 30 (2019) 447–455.
[45] J.H. Chen, X.P. Chu, J.T. Zhang, et al., Genomic characteristics and drug screening among organoids derived from non-small cell lung cancer patients, Thorac Cancer 11 (2020) 2279–2290.
[46] Z. Zhang, W.R. Karthaus, Y.S. Lee, et al., Tumor microenvironment-derived NRG1 promotes antiandrogen resistance in prostate cancer, Cancer Cell 38 (279–296) (2020) e279.
[47] Y.F. Li, Y. Gao, B.W. Liang, et al., Patient-derived organoids of non-small cells lung cancer and their application for drug screening, Neoplasma 67 (2020) 430–437.
[48] Li Z, Qian Y, Li W, et al. Human Lung Adenocarcinoma-Derived Organoid Models for Drug Screening. *iScience* 2020;23:101411.
[49] H. Tamura, A. Higa, H. Hoshi, et al., Evaluation of anticancer agents using patient-derived tumor organoids characteristically similar to source tissues, Oncol. Rep. 40 (2018) 635–646.
[50] N. Takahashi, H. Hoshi, A. Higa, et al., An in vitro system for evaluating Molecular Targeted drugs using lung patient-derived tumor organoids, Cells 8 (2019).
[51] S.-Y. Kim, S.-M. Kim, S. Lim, et al., Modeling clinical responses to Targeted therapies by patient-derived organoids of advanced lung Adenocarcinoma, Clinical Cancer Res. 27 (2021) 4397–4409.
[52] A. Pomerenke, Organotypic models of lung cancer, Curr. Top. Microbiol. Immunol. 09 (2018) 09.
[53] D.J. Jung, T.H. Shin, M. Kim, et al., A one-stop microfluidic-based lung cancer organoid culture platform for testing drug sensitivity, Lab Chip 19 (2019) 2854–2865.
[54] Gmeiner WH, Miller LD, Chou JW, et al. Dysregulated Pyrimidine Biosynthesis Contributes to 5-FU Resistance in SCLC Patient-Derived Organoids but Response to a Novel Polymeric Fluoropyrimidine, CF10. *Cancers (Basel)* 2020;12.
[55] T. Du, S. Lin, M. Ji, et al., A novel orally active microtubule destabilizing agent S-40 targets the colchicine-binding site and shows potent antitumor activity, Cancer Lett. 495 (2020) 22–32.
[56] M. Verduin, A. Hoeben, D. De Ruysscher, et al., Patient-derived cancer organoids as predictors of treatment response, Front. Oncol. 11 (2021) 641980.
[57] P. Saraon, J. Snider, W. Schormann, et al., Chemical genetics screen identifies COPB2 tool compounds that alters ER stress response and induces RTK dysregulation in lung cancer cells, J. Mol. Biol. 433 (2021) 167294.
[58] Y. Hu, X. Sui, F. Song, et al., Lung cancer organoids analyzed on microwell arrays predict drug responses of patients within a week, Nat. Commun. 12 (2021) 2581.
[59] X. Le, R. Cornelissen, M. Garassino, et al., Poziotinib in non-small-cell lung cancer Harboring HER2 exon 20 insertion mutations after prior therapies: ZENITH20-2 trial, J. Clin. Oncol. 40 (2022) 710–718.
[60] J.F. Gainor, G. Curigliano, D.W. Kim, et al., Pralsetinib for RET fusion-positive non-small-cell lung cancer (ARROW): a multi-cohort, open-label, phase 1/2 study, Lancet Oncol. 22 (2021) 959–969.
[61] H. Shigematsu, T. Takahashi, M. Nomura, et al., Somatic mutations of the HER2 kinase domain in lung adenocarcinomas, Cancer Res. 65 (2005) 1642–1646.
[62] J. De Greve, E. Teugels, C. Geers, et al., Clinical activity of afatinib (BIBW 2992) in patients with lung adenocarcinoma with mutations in the kinase domain of HER2/neu, Lung Cancer 76 (2012) 123–127.
[63] M.G. Kris, D.R. Camidge, G. Giaccone, et al., Targeting HER2 aberrations as actionable drivers in lung cancers: phase II trial of the pan-HER tyrosine kinase inhibitor dacomitinib in patients with HER2-mutant or amplified tumors, Ann. Oncol. 26 (2015) 1421–1427.
[64] J. Mazieres, F. Barlesi, T. Filleron, et al., Lung cancer patients with HER2 mutations treated with chemotherapy and HER2-targeted drugs: results from the european EUHER2 cohort, Ann. Oncol. 27 (2016) 281–286.
[65] D.M. Hyman, S.A. Piha-Paul, H. Won, et al., HER kinase inhibition in patients with HER2- and HER3-mutant cancers, Nature 554 (2018) 189–194.
[66] Z. Song, D. Lv, S.Q. Chen, et al., Pyrotinib in patients with HER2-amplified advanced non-small cell lung cancer: a prospective, multicenter, Single-Arm Trial. *Clin Cancer Res* 28 (2022) 461–467.
[67] L. Pacini, A.D. Jenks, N.C. Lima, et al., Targeting the fibroblast growth factor receptor (FGFR) family in lung cancer, Cells (2021) 10.
[68] M.A. Krook, J.W. Reeser, G. Ernst, et al., Fibroblast growth factor receptors in cancer: genetic alterations, diagnostics, therapeutic targets and mechanisms of resistance, Br. J. Cancer 124 (2021) 880–892.
[69] R. Shi, N. Radulovich, C. Ng, et al., Organoid cultures as preclinical models of non-small cell lung cancer, Clin. Cancer Res. 26 (2020) 1162–1174.
[70] F. Facchinetti, A. Hollebecque, R. Bahleda, et al., Facts and new hopes on selective FGFR inhibitors in solid tumors, Clin. Cancer Res. 26 (2020) 764–774.
[71] S. Vyse, P.H. Huang, Targeting EGFR exon 20 insertion mutations in non-small cell lung cancer, Signal Transduct. Target. Ther. 4 (2019) 5.
[72] J. Yun, S.H. Lee, S.Y. Kim, et al., Antitumor activity of amivantamab (JNJ-61186372), an EGFR-cMet bispecific antibody, in diverse models of EGFR exon 20 insertion-driven NSCLC, Cancer Discov. 15 (2020) 15.
[73] K. Park, E.B. Haura, N.B. Leighl, et al., Amivantamab in EGFR exon 20 insertion-mutated non-small-cell lung cancer progressing on platinum chemotherapy: initial results from the CHRYSALIS phase I study, J. Clin. Oncol. 39 (2021) 3391–3402.
[74] M. Banda, K.L. McKim, M.B. Myers, et al., Outgrowth of erlotinib-resistant subpopulations recapitulated in patient-derived lung tumor spheroids and organoids, PLoS One 15 (2020) e0238862.
[75] S. Li, L. Li, Y. Zhu, et al., Coexistence of EGFR with KRAS, or BRAF, or PIK3CA somatic mutations in lung cancer: a comprehensive mutation profiling from 5125 chinese cohorts, Br. J. Cancer 110 (2014) 2812–2820.
[76] J.A. Taverna, C.N. Hung, D.T. DeArmond, et al., Single-cell proteomic profiling identifies combined AXL and JAK1 inhibition as a novel therapeutic strategy for lung cancer, Cancer Res. 80 (2020) 1551–1563.
[77] A.F.M. Dost, A.L. Moye, M. Vedaie, et al., Organoids model transcriptional Hallmarks of oncogenic KRAS activation in lung epithelial progenitor cells, Cell Stem Cell 27 (2020) 663–678.e668.
[78] G. Vlachogiannis, S. Hedayat, A. Vatsiou, et al., Patient-derived organoids model treatment response of metastatic gastrointestinal cancers, Science 359 (2018) 920–926.
[79] L. Liu, L. Yu, Z. Li, et al., Patient-derived organoid (PDO) platforms to facilitate clinical decision making, J. Transl. Med. 19 (2021) 40.
[80] M. Putker, R. Millen, R. Overmeer, et al., Medium-throughput drug- and radiotherapy screening assay using patient-derived organoids, J. Vis. Exp. (JoVE 2021.).
[81] K. Dzobo, Taking a full snapshot of cancer biology: deciphering the tumor microenvironment for effective cancer therapy in the oncology clinic, OMICS 24 (2020) 175–179.
[82] A. Mazzocchi, M. Devarasetty, S. Herberg, et al., Pleural effusion aspirate for use in 3D lung cancer modeling and chemotherapy screening, ACS Biomater Sci. Eng. 5 (2019) 1937–1943.
[83] A. Mazzocchi, A. Dominijanni, S. Soker, Pleural effusion aspirate for use in 3D lung cancer modeling and chemotherapy screening, Methods Mol. Biol. 2394 (2022) 471–483.
[84] N. Palechor-Ceron, E. Krawczyk, A. Dakic, et al., Conditional reprogramming for patient-derived cancer models and next-generation living biobanks, Cells 8 (2019) 27.
[85] K.K. Dijkstra, K. Monkhorst, L.J. Schipper, et al., Challenges in establishing pure lung cancer organoids limit their utility for personalized medicine, Cell Rep. 31 (2020) 107588.
[86] X. Zeng, Q. Ma, X.K. Li, et al., Patient-derived organoids of lung cancer based on organoids-on-a-chip: enhancing clinical and translational applications, Front. Bioeng. Biotechnol. 11 (2023) 1205157.
[87] H. Xu, Y. Jiao, S. Qin, et al., Organoid technology in disease modelling, drug development, personalized treatment and regeneration medicine, Exp. Hematol. Oncol. 7 (2018) 30.
[88] S.N. Bhatia, D.E. Ingber, Microfluidic organs-on-chips, Nat. Biotechnol. 32 (2014) 760–772.
[89] X. Zhang, M. Karim, M.M. Hasan, et al., Cancer-on-a-Chip: models for studying metastasis, Cancers (basel) (2022) 14.
[90] D.E. Ingber, From tensegrity to human organs-on-chips: implications for mechanobiology and mechanotherapeutics, Biochem. J 480 (2023) 243–257.
[91] N. Sachs, A. Papaspyropoulos, D.D. Zomer-van Ommen, et al., Long-term expanding human airway organoids for disease modeling, EMBO J. 38 (2019).
[92] D. Barbosa Rabago, C.M. Blakely, F. Haderk, et al., Profiling sensitivity to Targeted therapies in EGFR-mutant NSCLC patient-derived organoids, J. Vis. Exp. JoVE (2021).