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Precision-Cut Lung Slices: Emerging Tools for Preclinical and Translational Lung Research

An Official American Thoracic Society Workshop Report

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Abstract

The urgent need for effective treatments for acute and chronic lung diseases underscores the significance of developing innovative preclinical human research tools. The 2023 American Thoracic Society Workshop on Precision-Cut Lung Slices (PCLSs) brought together 35 experts to discuss and address the role of human tissue–derived PCLSs as a unique tool for target and drug discovery and validation in pulmonary medicine. With increasing interest and usage, together with advancements in methods and technology, there is a growing need for consensus on PCLS methodology and readouts. The current document recommends standard reporting criteria and emphasizes the requirement for careful collection and integration of clinical metadata. We further discuss current clinically relevant readouts that can be applied to PCLSs and highlight recent developments and future steps for implementing novel technologies for PCLS modeling and analysis. The collection and correlation of clinical metadata and multiomic analysis will further advance the integration of this preclinical platform into patient endotyping and the development of tailored therapies for patients with lung disease.

Keywords: human *ex vivo* model; precision-cut lung slice; translational research; preclinical research; biomarkers

Overview

The purpose of this report is to provide an overview and guidance to the application of precision-cut lung slices (PCLSs), which have emerged as a human lung tissue–based translational model in pulmonary science and medicine. The overall goal is to provide a current framework and consensus criteria for the generation, applications, and experimental readouts for the use of PCLSs, which can serve as a standard for the field.

The key findings of this workshop are as follows:

- The interest in and use of PCLSs has grown exponentially in recent years, and, together with advancements in

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methods and technologies, there is an emerging need for consensus on PCLS methodology and readouts.

- Human PCLS generation is complex, and the success and suitability of PCLSs is highly dependent on tissue procurement and processing, followed by PCLS generation and culture. We propose a set of standard reporting criteria that are required to foster PCLS protocol alignment and enhance evaluation across different laboratories.
- Several molecular and biochemical readouts can be assessed in PCLSs, and novel technologies, such as omic approaches, have been applied. Further studies are needed to improve functional and clinically relevant readouts that can also be assessed in other biospecimens.
- We emphasize the importance of collection and integration of clinical metadata with human-derived PCLSs to allow for improved phenotyping and foster clinical translation.

Introduction

Acute and chronic lung diseases are among the leading causes of death worldwide and represent a major health and financial burden on both patients and society. The pathology of lung diseases is complex and heterogeneous, complicating the discovery and development of new therapeutics, leaving most lung diseases without a cure. Thus, there is an urgent unmet need to identify more effective and targeted treatments. To achieve this, novel approaches and clinically relevant human model systems are needed to explore disease mechanisms and, more importantly, to validate potential new targets and drugs.

The present document summarizes the 2023 American Thoracic Society (ATS) workshop, "Precision-Cut Lung Slices: Emerging Tools for Preclinical and Translational Lung Research," hosted by the *Journal* together with Respiratory Structure and Function; Allergy, Immunology, and Inflammation; Clinical Problems; and Environmental, Occupational, and Population Health assemblies. This workshop focused on PCLSs as a rising human lung tissue–based translational model. As such, improved methodology and accessibility to human tissue has led to increased interest and usage of PCLSs by a rapidly increasing number of investigators within and outside the ATS community. PCLSs from healthy and diseased human tissue are a promising tool, which can *1*) recapitulate the complexity of the lung's native environment; *2*) enable the study of the complex interactions among different cell types and the extracellular matrix (ECM) in the lungs' native three-dimensional (3D) architecture; *3*) allow for high-resolution (live) imaging of cellular functions in several dimensions; *4*) mimic the onset and progression of lung diseases, complementing studies in end-stage diseased tissue; and *5*) enable testing of potential therapeutics in a disease-relevant human model.

In the workshop and follow-up work of the project, common constraints to the use of PCLSs and the emergence of novel technologies that are being developed to overcome them were discussed. These constraints include the following: First, the lifespan of PCLSs is limited in culture, with culture time ranging from several days to a few weeks, restricting their use for long-term studies of chronic disease development or therapeutic resolution. Second, the slices lack mechanical ventilation, resulting in both limited contact with air as well as no physiological stretch. Third, PCLSs are neither innervated nor perfused, limiting systemic signals, including hormone and immune cell recruitment upon injury, exposures that are critical for replicating certain pathophysiological responses. Novel technologies addressing some of these constraints are discussed in this document.

Notably, although in general PCLS models are relatively low cost and feasible for many labs, some assessments and readouts require specialized equipment and expertise, and sufficient human tissue access is often a limiting factor. To overcome this challenge, several protocols are under development that allow and/or processed tissues to be preserved and shared.

Altogether, PCLSs are a remarkable tool to bridge the gap between target identification and translation into more advanced and expensive testing platforms and clinical studies. Their broad applications extend from basic to clinical research in pulmonary medicine.

The main objective of the 2-day workshop was to discuss and summarize recent advances in the PCLS field; define a series of consensus criteria for the generation, applications, and experimental assessments in the use of PCLSs; and

assemble a set of recommendations to guide the larger scientific community toward more interpretable and comparative data. We largely focused our discussion on human tissue–derived PCLSs, given their relevance for mechanism and target identification, drug discovery, validation, efficacy, and safety studies.

Methods

ATS Workshop Participants

The 35 investigators participating in the workshop consisted of a diverse group of international participants comprising early-career and established professionals from academia, government, and industry (*see* Figure E1 in the data supplement). We began with a survey of participants' demographics, expertise, and current challenges and opportunities in PCLS research. Most participants regularly use PCLSs in their labs, and many plan to expand their usage, primarily with human tissue (Figure E2). Based on survey feedback, we set the workshop agenda. This resulting workshop report discusses and summarizes experience in the generation and culture of PCLSs, the application of diseased PCLSs and PCLS models, as well as current quality assessments, readouts, and endpoints. We further highlight recent developments and future steps for implementing novel technologies for PCLS analysis, advancing PCLS modeling, and the value of PCLSs for translational and personalized medicine.

PCLS Generation, Culture, and Preservation

PCLSs have emerged as a highly relevant *ex vivo* model for studying injury and repair responses, and for the development of novel therapies. The use of tissue slices was initiated in the 1920s, when liver slices were usually cut manually, resulting in high thickness, high variability, and limited viability. The development of mechanical microtomes in the 1980s increased homogeneity and reproducibility (1, 2). For the soft tissue of the lung, a breakthrough was achieved with the infusion of low-melting-point agarose, preventing collapse of the alveoli during slicing while preserving the 3D structure (3, 4). The overall generation and culture of PCLSs requires several variable steps that affect quality and potential for downstream application. We outline key steps in the process and discuss challenges and open questions. Based on these, we discuss and provide minimum criteria (Figure 1 and Table 1) that are strongly recommended in implementing and reporting future PCLS studies.

Human Lung Tissue Procurement

Human lung tissue for PCLSs can be obtained from various sources, including excess surgical pathology tissue from lobectomy procedures, lung biopsy samples, research organ donations, or explanted transplant lungs. Coordination with caregivers and obtaining appropriate ethical approval and consent are crucial steps in this process of human subject (live donor) research. In addition, donor lungs not suitable for transplant, procured through the U.S. United Network for Organ Sharing and the associated organ procurement organizations, either local to research centers or via the broader distribution by nonprofit organizations like The National Disease Research Interchange and the International Institute for Advancement of Medicine, serve as another important source. These lung donors typically experienced a medical emergency and were on mechanical ventilation, with lungs deemed unsuitable for

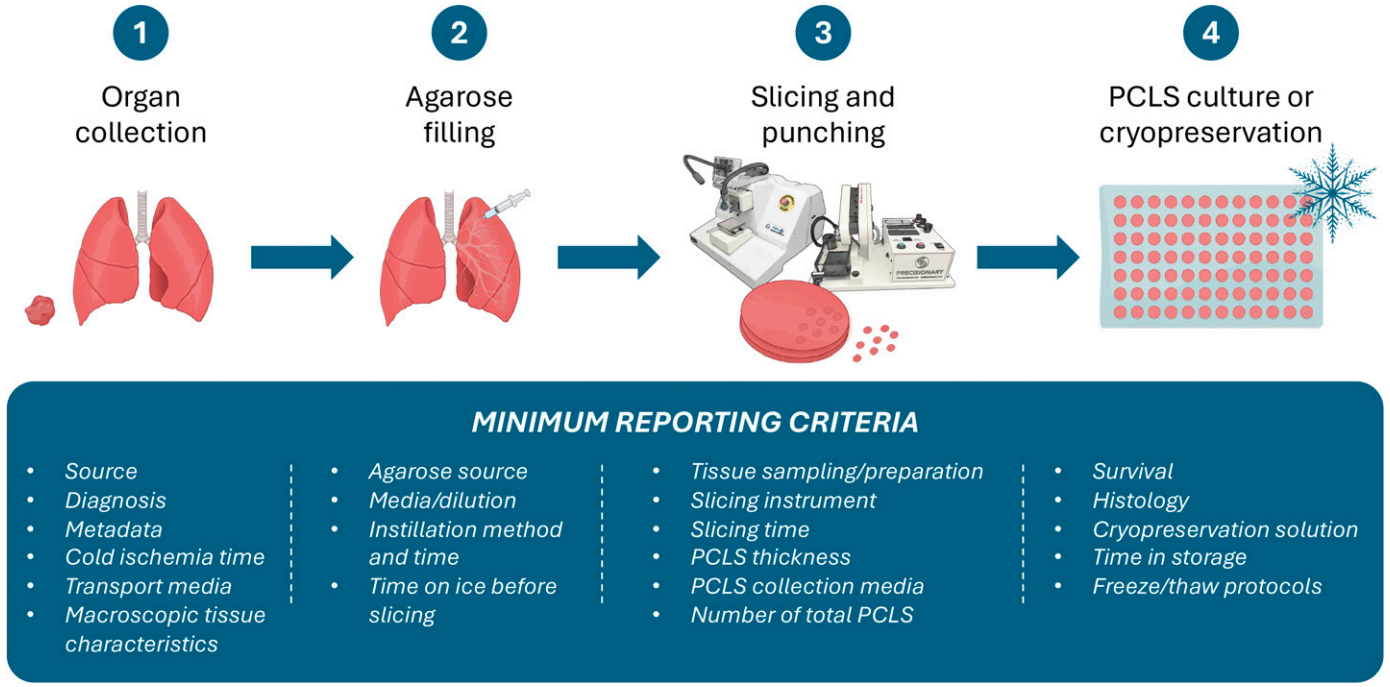

Figure 1. Recommended minimum reporting criteria for precision-cut lung slices (PCLSs). PCLS generation comprises multiple variable steps. Standardization of the protocols is needed, and the implementation of minimum reporting criteria is recommended for tissue procurement and PCLS generation, culture, and storage.

Table 1. Minimum Reporting Criteria Recommended by the Panel

Human PCLS Minimum Reporting Criteria	
Category	**Notes**
Source	Living donor, cadaveric donor, explant, or lung resection. Source repository if applicable
Diagnosis	Patient diagnosis for explanted lungs or resections: what was the patient diagnosis? For donor lungs, what was the cause of death?
Metadata	As comprehensive as possible, including patient history, exposome, genetics, and medication, to the extent available and in agreement with HIPAA and GDPR regulations
Transport media	Preservation media used for transport, supplements, and concentrations (antibiotics, antimycotics, antioxidants, trophic factors, etc.)
Warm ischemia time	What was the total time between cessation of blood circulation and active cooling and buffer perfusion and/or immersion?
Cold ischemia time	What was the total time of cold ischemia?
Tissue characteristics	Full lobe (which one) or resection, macroscopic anatomy (include pictures). Was a vascular flush of the tissue done and with what fluid?
Agarose filling	Agarose source, grade, catalog number, concentration (wt/vol), temperature, and media or buffer used; inflation parameters (infusion speed and pressure if available, total volume; if a peristaltic pump was used, include infusion parameters); gelation time and temperature
Sampling	Was the tissue cut in blocks or cores? Include approximate cubic size or diameter and core length. Total number of blocks and/or cores
Total time before slicing	Lapsing time between blocking or coring and slicing into PCLS. Storage temperature and media used
Slicing instrument	Include brand and slicing parameters (blade angle if available, media temperature, speed, and frequency; slicing media composition)
PCLS thickness	In micrometers
PCLS collection and storage until use	Were the PCLSs collected in cold or RT media preservation solution? Were they rinsed before use? If yes, in which solution and how many times?
Cryopreservation or cold storage	Were they cryopreserved or cold-stored? In which solution? As cores or single slices? For how long? At which temperature? How were they thawed? Results of functional and/or cellular integrity tested after thawing?
Adaptation period	Were the PCLSs cultured before treatment initiation? For how long and at what temperature? Media composition, culture conditions
Culture and treatment conditions	How were the PCLSs cultured (culture media used, frequency of media changes, volume)? Total duration. Which samples were taken? Technical replicates and number of PCLSs pooled
PCLS viability	Viability: WST1/MTT/LDH/live/dead staining and imaging/Alamar Blue (Resazurin) including use of controls for loss of viability (i.e., heat-killed control).
Histology	H&E, Trichome stain. Immunofluorescence (indicate antibody, source, and protocol)

Definition of abbreviations: HIPAA = Health Insurance Portability and Accountability Act; GDPR = general data protection regulation; H&E = hematoxylin and eosin; LDH = lactatedehydrogenase; MTT = (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide); PCLS = precision-cut lung slice; RT = room temperature; WST1 = water-soluble tetrazolium dye 1.

transplant for various reasons due to size, past or current health history, or lack of tissue-matched eligible recipient. Regardless of the source of the tissue, collecting key metadata, such as demographic information and donor history, including factors like smoking, occupational exposures, and concurrent and past medical conditions and therapies, as well as the condition of the lung at the time of recovery (i.e., pneumonia, contusion, acute respiratory distress syndrome), is essential for ensuring the quality and relevance of the recovered tissue. Gross and histopathological assessments of the lung tissue to be further processed into PCLS models are also critical, as not all abnormalities are detected by clinical history and medical testing.

Human Lung Tissue Processing

After resection, fresh lung tissue should be immediately kept in a cold organ transplant solution (Celsior, University of Wisconsin solution, histidine-tryptophan-ketoglutarate solution, Custodiol) or Dulbecco's modified Eagle medium/F12 culture media. Because these samples are derived from the nonsterile respiratory tract, addition of antibiotics and/or antifungals may be helpful. Transport and processing should be done immediately, so cold ischemia time does not exceed 18 hours. Prolonged cold ischemia time impacts tissue viability, which needs to be considered, especially when overnight shipping is needed for remote donation. The size of the lung lobe or explant is important when determining agarose volume and filling pressure. The infused medium comprises sufficient volume of liquified low-melting-point agarose solution in physiological buffer, prepared typically as a 1.5–3% wt/vol solution, depending on desired gel hardness. To minimize the risk of stretch-induced damage, particular care should be taken not to overinflate the tissue. Because the agarose needs to be dissolved in liquid at increased temperature, it is important to consider the temperature of the agarose solution at the time of instillation: too warm (>37°C) may damage the tissue, too cool and the agarose will begin to thicken and not flow well through the airways. Because of the size and rapid cooling of the agarose that can occur in a human lung lobe, it may be helpful to suspend the lung lobe in a 37°C transplant

buffer or Dulbecco's modified Eagle medium bath while instilling the agarose. If the entire lobe is not needed for PCLSs, it is possible to clamp off a portion to restrict agarose inflation of neighboring tissue that can be processed for alternative purposes. Furthermore, during inflation, when the agarose is injected with a syringe attached to a cannula, consideration should be given to cannula diameter, injection pressure, and solution viscosity. Although agarose filling through the bronchus is the most common method for complete lobes, human tissue is often obtained from surgical resection samples. In these cases, agarose inflation is quite difficult because of agarose leaking through open airways and exposed lung tissue. In such an event, if small airways are available, agarose can be instilled with a fine cannula (0.3–0.5 mm in diameter), using clamps to limit leaks or using artificial pleura (5). Otherwise, multiple small injections directly into the lung parenchyma with a fine-gauge needle can be used.

PCLS Generation

We refer to excellent peer-reviewed publications and review articles for detailed procedures (6–10). The agarose-filled lungs are cooled to below 25°C to allow rapid solidification, cut in blocks or cored out, and PCLSs are then prepared by either slicing top-down (vibratome), bottom-up (Alabama R&D Tissue Slicer/Krumdieck), or sideways (Leica and Precisionary Instruments' Compresstome) (Figure 1). Each slicing method has its own advantages and disadvantages. For example, slicing top-down allows for visual selection of slices containing regions of interest, whereas slicing from the bottom up facilitates slicing of more fibrous tissue, fixing the tissue between the core and the slicing base. Attention to uniformity of slice thickness, overall area, and volume, as well as presence or absence of major lung structures included or excluded from the PCLSs, is important to reduce variability in downstream experimental results. Depending on the purpose of the PCLSs, one might focus on peripheral alveolar tissue or areas with somewhat larger airways or vessels. The presence of vessels or bronchi can complicate the PCLS sectioning. Using a tissue coring tool is helpful in starting with uniform cores from which to slice the PCLSs. It is important to note that during tissue resection, blocking, coring, and slicing, there is diffuse tissue and cell damage, which facilitates immune cell activation and proinflammatory signal release (11, 12). Tissues should be washed and incubated in medium for up to 24 hours before commencing experiments or cryopreserving, with consideration for adding antibiotics and antifungals to the media (13). Open questions include the variable success of inflation and slicing of individual lungs with different disease extents, such as extremes of fibrosis and emphysema, and whether clinical metrics can serve as criteria for tissue inclusion or exclusion. The committee strongly recommends collecting and reporting regional staining for histological measures like hematoxylin and eosin and Masson's trichrome, in sequential slices. Combined with comprehensive metadata, including patient history, exposures, and medications, these data can help establish disease severity, confirm diagnosis, or assess compartmentalization in cases of infectious complications. Table 1 lists complete minimum reporting criteria.

PCLS Culture

PCLSs can be successfully kept in culture for several days, in some cases even weeks (8, 10, 14–17). However, based on available data reporting tissue integrity and viability, most research teams will only culture human PCLSs for 5–7 days. There are several excellent peer-reviewed publications and reviews with detailed procedures to optimize PCLS culture using different culture conditions (8, 14–16). However, currently, there are no standardized recommendations for PCLS culture. One of the open questions in the field is related to the extent to which agarose remains in cultured PCLSs. This seems to vary depending on specific protocols and requires consideration, because it may impact downstream applications and outcomes (5). Investigators have attempted to determine the agarose status using a dye (such as Evan's blue or fluorescence dyes) to visualize agarose as well as assessing agarose remnants in histological specimens.

To open the path to a more standardized protocol for PCLSs, the workshop participants discussed and recommended the inclusion of minimum reporting criteria (Table 1). Some of those criteria are based on assays that do not require specialized equipment, including overall viability and histological assessment over time in culture. Although further quality-control experiments are highly dependent on the research question and project, functional tests are highly recommended. These include, but are not limited to, airway contraction, cytokine release, immune responsiveness, mitochondrial activity, or stiffness. Additional questions depending on the study focus include: *1*) What are the suitable cutoffs for quality control or functional tests, and why? *2*) Should functional versus cellular changes be prioritized? and *3*) Given the evidence of variable survival of different cell types over time, should reporting be cell specific? Importantly, there are several new bioengineering approaches to improve and extend survival of PCLS cultures. Poly(ethylene glycol)-based hydrogel, alginate, or solubilized ECM encapsulation have been shown to enhance viability outcomes (17, 18). Specifically, PCLSs have been embedded in hydrogel, which prolongs viability of alveolar epithelial type 2 (AT2) cells (18). Moreover, superimposition of (patho)physiological stretch mimicking breathing (19) and/or mechanical ventilation and/or perfusion using microfluidic chambers of circulating blood-borne cells and other factors are promising new routes (*see below*). Some of these approaches require specialized expertise and equipment, limiting their wide use across laboratories worldwide. The committee recognizes this as a major limitation and encourages collaborations with specialized experts for specific scientific questions. We further support the establishment of a worldwide PCLS network that combines training and exchange of specialized procedures.

PCLS Cold Storage and Cryopreservation

An effective way to overcome limited PCLS viability is cryopreservation or cold storage (15, 20–26). Using this approach, high numbers (up to hundreds) of PCLSs can be collected per human lung, cold-stored or frozen, and then rewarmed or thawed on demand. Thus, the timing of experiments becomes uncoupled from that of preparation, and the use of precious samples is maximized. Moreover, the establishment of PCLS biobanks (22, 23), including diseased tissues, has facilitated expanded PCLS use in drug discovery and (patho)physiological studies. This allows not only sharing of tissue with other institutions but also analysis of several individual lungs at once. Specific cryopreservation protocols have improved viability and immune responsiveness for up to 4 weeks after thawing (21, 22, 27).

However, these methods remain technically demanding and challenging because of ice crystal formation resulting in loss of intracellular water and destruction of cellular architecture (28). Although PCLSs seem to withstand the freeze–thaw cycle with only little loss in viability and airway constriction, a decrease in metabolic activity has been observed (21). Optimum freezing media for PCLSs continue to be explored. Addition of serum and 5–10% DMSO, similar to media used in peripheral blood mononuclear cell cryopreservation, may be beneficial. The rate of freezing and of thawing will also affect PCLS cell viability. As an alternative, long-term hypothermic storage of PCLSs in specifically designed preservation solutions for up to 28 days at 4°C has been optimized (15). Several open questions remain: *1*) Could the use of lung tissue cores simplify cryopreservation and long-term cold storage? *2*) How does agarose impact slice quality after preservation? Moreover, studies on long-term cold storage or cryopreservation need generalization to verify functional responses, tissue integrity and viability, and cell-specific outcomes measured across labs.

In conclusion, the success and suitability of PCLSs is highly dependent on tissue procurement and processing, as well as PCLS generation and culture. We recommend the implementation of minimum reporting criteria (Table 1) in current and future studies to allow for standardized and comparable protocols and results. These criteria will be helpful to establish cutoff for acceptance or rejection of human lung tissue–derived PCLSs for inclusion into preclinical studies. In addition, we acknowledge that the extent of experiments that can be performed will vary based on research focus, tissue availability, and equipment and resources. Nevertheless, the workshop participants agreed that functional readouts should be included, and clear rationale and data should be provided to support the research targets.

PCLSs to Investigate and Model Lung Disease

Using PCLSs to investigate specific diseases is of utmost interest to many researchers. Conceptually, two approaches are possible. Either diseased PCLSs are generated to study mechanisms and therapies in the diseased tissue, or PCLSs are generated from tissue recovery from healthy donors or tumor resections and used for *ex vivo* induction and modeling of disease (Figure 2). In general, a major advantage of PCLSs is that they allow for matched analyses of multiple treatments on tissue from the same patient or donor. In addition, PCLSs can be generated from different locations within the same lung to represent lung and disease heterogeneity and progression. Here, we describe advantages and disadvantages of using primary diseased tissue as well as nondiseased tissue from lung donors that are subjected to specific disease inducers *ex vivo*.

PCLSs Generated from Diseased Lung Tissue

Although primary diseased human tissue represents the most physiologically relevant model, accessibility to diseased lung tissue is a limiting factor. Tissues suitable for PCLS generation are largely limited to explanted tissue from patients undergoing lung

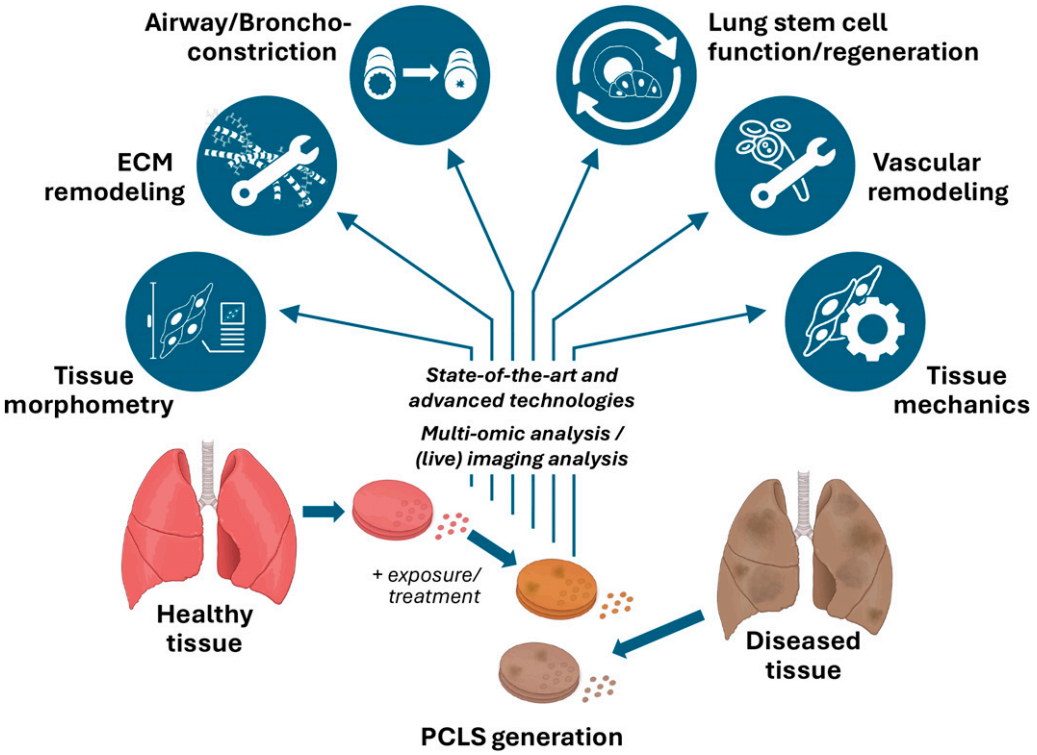

Figure 2. Assessment of disease relevant readouts in PCLSs. PCLSs can be generated from diseased lung tissue, mainly from explants, and from nondiseased donor tissue, which can be subjected to disease-modeling approaches. In both cases, a variety of disease-relevant readouts have been established in PCLSs. The implementation of novel advanced technologies has improved several of these readouts. ECM = extracellular matrix.

transplantation or lung surgery or resection, which mainly include patients with interstitial lung disease, including idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), cystic fibrosis, or pulmonary arterial hypertension (29). For diseases such as asthma, in which transplantation is rare, access to diseased tissue is extremely limited and primarily through endobronchial or transbronchial biopsies. Donor lungs not suitable for transplant are another potential source; in this case, asthma is one cause of lungs being rejected for transplant. Working criteria and definitions when using lungs from donors with asthma and any other chronic lung disease should be made as transparent as practically possible. Although biopsies are potentially routinely available for many disease entities, the current protocols for PCLS generation require larger tissue amounts, and efforts to adapt protocols to the use of smaller tissues are underway. Overall, access to explant tissue is very limited and usually restricted to a few labs that are associated with a lung transplant center. To overcome some of these challenges, investigators have put major efforts into cryopreservation of tissue, which will allow not only sharing of tissue with other institutions but also the analysis of several individual lungs at once (*see above*).

Another important factor to consider is that—especially with explant tissue—investigations are mostly restricted to end-stage lung disease. Although reversal of end-stage disease would be the holy grail, this will most likely remain a challenge. In addition, molecular pathogenic pathways are likely to change with disease progression, and pathways and biomarkers of early disease are not necessarily preserved in end-stage diseased tissue. Furthermore, only a few of the subjects from whom these tissues are obtained are naive for clinical treatments, and they are more likely to have received multiple and varied medications, which further hinders standardized analysis. These considerations are significant to keep in mind, further highlighting the need for extensive collection of metadata, including prior medications. Moreover, it is critical to observe and report intradonor and interdonor disease PCLS heterogeneity, which requires careful inspection and selection of PCLSs, as well as appropriate number of donors and slices per donor to yield robust and rigorous results (30, 31). Although the number of replicates depends on the expected effect size and specific scientific question, typically at least five or six donors are used in PCLS experiments, with higher variability predicted when using diseased tissues. Importantly, there are several studies using diseased lung tissue for validation of novel compounds and drugs, which have demonstrated important proof of concept and paved the way for future PCLS studies to identify and test therapeutics (32–38). Many of these studies have been done in explanted tissue from patients with either IPF or COPD who had undergone transplantation and have subsequently led to further clinical development of novel therapeutics.

Modeling Disease with PCLSs Generated from Donor Lung Tissue

Given the restrictions and potential limitations regarding diseased lung tissue as a source for PCLS studies mentioned above, another attractive option is to induce features of disease in otherwise healthy lung tissue from donors. This option has gained widespread attention over the last few years. Tissue access is still an important consideration but is more widely possible with different organ donor agencies that preserve and supply tissues in most states and/or nationwide in the United States. Access to donor tissue varies globally and is typically more restricted in Europe and Australia. Alternatives include the use of nondiseased margins from tumor resections. Modeling diseases in human PCLSs with specific inducers and exposures has been performed for both chronic and acute lung diseases, and specific examples are outlined below. In general, induction of disease-defining elements can be achieved by exposure to mitogens, cytokines, growth factors, or environmental triggers such as cigarette smoke, bacteria, and viruses; physical and chemical stimulation including high or low oxygen treatments, chemical exposure (e.g., lipopolysaccharide and bleomycin), or γ or UV irradiation (reviewed in References 10 and 39) can also be used. Notably, the route of exposure to cells such as epithelium versus endothelium is not, or is minimally, controlled in PCLSs in culture. Although this has been successfully applied to induce disease-defining elements, for example COPD, IPF, asthma, or acute lung injury, the currently limited length of viable PCLS cultures presents a challenge for long-term exposure and manifestation of chronic injury and remodeling processes. It does, however, enable the identification of putative early mechanisms of disease and associated biomarkers. Another advantage of using donor tissue for disease modeling is that intraindividual treatment controls from the same individual tissue are available and can give important information toward disease-specificity of treatments. An important consideration for induced disease models in PCLSs is the absence of recruitable immune cells. In some instances, PCLSs cocultured with peripheral blood mononuclear cells might be desired to mimic immune cell recruitment in response to lung tissue damage.

PCLSs used for disease modeling are mostly generated from "healthy" donor tissue, which is largely restricted to tissue rejected for transplant, nondiseased margins from tumor resections, and excess tissue during transplantation, often with limited information and metadata available. It is important to reinforce to those who recover these tissues for research the importance of collecting and providing not only the precious tissue but also the clinical metadata and history that are critical to interpretation of the experiments for which they are used. As outlined above, the detailed description and collection of available demographic information, tissue quality, and histology are required criteria to ensure robustness of all PCLS data (Table 1).

Both in the case of primary diseased tissue and in the case of disease induction, the relatively short time of culture (currently 5–7 d to maximum of a few weeks) limits outcome measurements to specific (early) endpoints. Although initial molecular endpoints established in mouse models or in a clinical setting can be tested in PCLSs, these endpoints need to be adapted for the PCLS system for reverse translation into patient cohorts to ascertain translational potential (*see below*). Nevertheless, although the relatively short culture time restricts the use of endpoints that rely on long-term restructuring of the tissue, the system allows for some longitudinal assessment of pathogenic features and disease-relevant endpoints in human PCLSs (Figure 2).

Disease-Relevant Endpoints in Human PCLSs

ECM Remodeling

Changes in ECM secretion, deposition, and crosslinking are key features of different

chronic lung diseases, and tissue remodeling and fibrosis can be assessed in PCLSs. Measurements include gene expression of ECM markers, including Type I Collagen, Fibronectin, and Tenascin C, all of which show increased levels in slices from patients with IPF, as well as in models (30, 40). Similarly, the total collagen content as well as the secreted collagens can be measured via Western blotting, ELISA, high-performance liquid chromatography, or hydroxyproline assays (41–43), the last of which is one of the gold standard assessments for fibrosis in animal models. The use of measuring secreted mediators has the advantage of enabling longitudinal sampling using the same PCLSs and enables measuring of clinically relevant biomarkers, including collagens, fibronectin, tissue inhibitor of metalloproteinase 1, and matrix metalloproteinase 7. In addition, histological staining methods including Sirius Red or Masson's Trichrome are used for visual assessment of collagen fiber alignment and density in red or blue, respectively (30, 40, 44–48). Immunofluorescence or immunohistochemistry targeting specific ECM components, such as Fibronectin and Tenascin C, can highlight spatial localization to convey spatial information into the distribution and localization of these biomarkers within the tissue (30, 40). Moreover, the formation and degradation of ECM proteins has been investigated using neoepitope biomarkers, including PRO-C1, PRO-C3, and PRO-C6, for types I, III, and VI collagen, as well as the interstitial collagen degradation marker C3M for type III collagen (49, 50). Notably, some of these neoepitopes are used as exploratory biomarkers in pulmonary fibrosis and thus can serve as *ex vivo* correlation tools to inform clinical trials. Incorporation of radioisotopes allows for quantification of newly synthesized versus shed or degraded ECM proteins and metabolites (51). Regarding COPD, further studies of elastin or collagen turnover are needed and may be accomplished in PCLS culture. Although staining for elastin and morphological changes have been reported in human COPD-derived PCLSs (52), the short time frame of most studies thus far has precluded measurements of active remodeling.

Tissue Morphometry

Morphometrical analysis is one of the gold standard readouts for COPD models that mimic emphysema (53–56). Increased airspace dimensions analyzed by morphometry have been reported in PCLSs (57–62); however, challenges remain for proper inflation, fixation, and inclusion criteria for use. Severely emphysematous lungs yield slices with little tissue mass. Given the heterogeneity of emphysema in COPD lungs, a robust approach for sampling is needed, and minimum reporting criteria (Table 1) will be helpful to develop a more standardized approach.

Mechanical Properties

Changes in the ECM lead to significant alteration in mechanical properties, especially tissue stiffness. Tissue stiffness not only governs lung expansion, ventilation, gas exchange (52), and constituent cell function (53), but it is also altered by diseases. For example, although lung stiffness increases during pulmonary fibrosis leading to decreased compliance, the tissue softens during emphysema, a major subtype of COPD, resulting in increased compliance. Thus, restoring normal lung compliance is a major therapeutic goal (63). To assess lung stiffness both at the tissue and the alveolar levels, the conventional approach is to use the atomic force microscope (64). An atomic force microscope can be used to measure the stiffness of lung tissue microscopically by indenting the tissue surface with a nanoscale tip and recording the force response. This technique is applied to PCLSs attached to a stretchable membrane, provides precise measurements of tissue stiffness, and can highlight heterogeneity within the lung tissue (58, 65–67). More recently, the introduction of a biomechanical device to impose uniform stretch to the PCLSs, and for simultaneously quantifying strain, allowed calculation of PCLS stiffness in a simple and fast way (64). This method showed that tissue softening and associated ECM degradation leads to a loss of function in COPD lungs.

Airway and Bronchoconstriction

Investigation of airway or bronchoconstriction and dilation is probably one of the longest-used and best-characterized readouts in PCLSs, especially in the context of asthma and COPD studies (57, 68–71). Spontaneous constriction, as well as responses to a variety of environmental exposures, allergens, inflammatory cytokines, or therapeutic interventions, have been used to generate numerous examples of clinically relevant readouts (60, 72–74). More recently, studies have focused on the effects on bronchoconstriction in the context of muscle contractile forces (19) and superimposed mechanical stretch mimicking tidal and deep breathing (75). In addition, disease settings change bronchoconstriction, as in the case of pulmonary fibrosis characterized by augmented basal and carbachol-induced bronchoconstriction upon profibrotic stimuli (31, 49, 76–78). Ciliary beating can be observed in PCLSs in real time and is impaired upon exposure to toxicants, including e-cigarette condensate (79). The introduction or maintenance of a mucus layer in the airway compartment remains a challenge, given the requirement of a media milieu that compromises reliable assessments of mucociliary action in PCLSs.

Vascular Remodeling

Vascular dysfunction and remodeling are crucial mechanisms in many lung diseases, contributing either to the initial disease pathogenesis or to the progression of secondary pulmonary hypertension. PCLS have been effectively used to investigate pulmonary vasoconstriction and dilation in response to various vasoactive substances or conditions over time (47, 80–85). Specifically, calcium flux has been monitored in living PCLSs in real time (76) as a mechanistic readout. However, these studies have largely been performed in rodent-derived PCLSs. More recently, human PCLSs derived from explant tissue of patients with pulmonary hypertension have been used in the studies of pulmonary arterial hypertension (77). By combining a novel drug delivery method via inhalation using microparticles and tissular distribution-function analysis with PCLSs, spatially resolved drug localization and reduced pulmonary vascular remodeling with a significant reduction of drug toxicity have been demonstrated (80). This study supports PCLSs as a platform for the development of drug delivery systems.

Lung Stem Cell Function and Regeneration

Proper lung epithelial stem cell function is critical for lung repair and regeneration. The major lung stem cells include airway basal cells, club cells, and AT2 cells. PCLSs have been used to determine AT2 cell function by assessing the expression and secretion of Surfactant Protein C (33, 52, 61, 86). More recently, live imaging approaches (*see* also below) have been applied to PCLSs to observe AT2 cell migration and potential transdifferentiation into AT1 cells (81, 87, 88).

Although the use of human-derived PCLSs does not allow for genetic labeling including lineage tracing, an interesting approach has been to isolate epithelial cells from treated or modified PCLSs, which can then be further characterized in an organoid assay to assess progenitor cell potential (89). Notably, the use of human PCLSs allows the study of structures that are absent in rodents, including epithelial cells of the terminal bronchioles and their regenerative potential (90). Ultimately, a highly important regenerative endpoint would be changes in tissue morphometry indicating an improvement in structure including formation of alveoli (*see* also above). Supporting the use of PCLSs as a platform for tissue regeneration, studies of patient-derived COPD PCLS samples show evidence of reparative alveolar epithelial proportions with pharmacologic enhancement of Wnt signaling (52).

The Future: Emerging Advances in PCLS Assessment Using Novel Technologies

The advent of single-cell multiomics in combination with recent imaging advances that allow multiday live cell and structural imaging provide new opportunities to understand mechanisms of lung regeneration and development using PCLSs.

Imaging

Combining PCLSs with live imaging techniques enables observation of cellular and extracellular processes over time, extending beyond static endpoints measured largely in two-dimensional fixed tissue. Currently, live imaging studies are largely done using rodent-derived PCLSs, which allows the application of transgenic fluorescent reporter mice. For example, PCLSs taken from a neonatal mouse demonstrated changes in tissue architecture that closely mimic alveologenesis *in vivo* (87, 91). After injury with bleomycin or other profibrotic agents, PCLSs demonstrated septal wall thickening and increased matrix expression (92). Live imaging using wide-field microscopy and epithelial cell adhesion molecule labeling observed epithelial cell movements in murine PCLSs taken from lungs during the saccular-to-alveolar transition (87).

Recent advances in light sheet and two-photon microscopy allow 3D rapid volumetric sectioning through increased tissue depth across a multiday period (72–96 h). Investigation of the alveolar–saccular stage transition by scanned oblique plane imaging generated surprising observations that alveoli appear to form through a process of epithelial aggregation and budding through alveolar myofibroblast-rich mesenchymal rings (88, 93). In time-lapse imaging of PCLSs, lineage-labeled AT2 cells spread across multiple alveolar spaces, with similar flattening movements as observed in neonatal mice, thus shedding light into unappreciated dynamic AT2 cell mobility (81).

Live imaging of human PCLSs remains challenging. Recent methodological developments have established first approaches for genetic modifications in PCLSs (94–96), and future work is needed to achieve cell-type specificity. Another elegant approach is antibody labeling of the cell surface; however, this limits cell labeling at significant tissue depth. Alternative strategies include using membrane labeling, using viral vectors to express fluorescent proteins, and adding membrane-permeable dyes for live labeling of cell-type–specific organelles (e.g., lysotracker for AT2 cells). Promising strategies for future unlabeled imaging include second harmonic generation (97) for fibrillar collagen or multimodal and nonlinear thermal imaging system (98). Together, metrics for cellular movements and changes in cellular structure under normal developmental and homeostatic conditions can establish benchmarks for quantifying perturbations in the setting of pathway modulators or in response to injury.

Omics

PCLSs have been used for a wide range of molecular readouts, as illustrated above. Single-cell omic and multiomic technologies studying disaggregated cells can be readily applied to PCLSs. Sections from PCLSs can be used for evolving multiplexed platforms preserving spatial patterning of both mRNA and proteins. PCLSs may allow assays to be performed after direct and targeted modification of lung cells and tissues. These approaches have the potential to elucidate the contributions of different cell populations to normal and pathologic changes during lung development, injury, and repair. With the advent of these technologies, new questions are emerging, including how to integrate omics findings in PCLSs with existing single-cell atlases and spatial transcriptomics from the whole lung. Preliminary work directly comparing single-cell transcriptomics from human PCLSs after 5 days in culture to "fresh" noncultured native lung shows reasonable transcriptional fidelity in epithelial and mesenchymal cells (99). Furthermore, single-nucleus sequencing applied to a human fibrosis model of PCLSs demonstrated the appearance of cell subpopulations, initially identified in patients with IPF *in vivo* and in human tissue *ex vivo* (31). Importantly, inclusion of novel spatial omics technologies will increase our understanding of regional relationships between changing cell states and types in the lung micro- and macroenvironment and heterogeneity across a PCLS. Lingering questions specific to PCLS technology (e.g., edge damage) can also be addressed using spatial transcriptomics.

In summary, live imaging, omics, and next-generation spatial technologies, when combined with PCLSs, have the potential to generate robust datasets from human lungs *ex vivo*. Integration of these approaches with concomitant morphological, mechanical, and airway/vascular constriction measurements will broaden our understanding of dynamic lung processes, such as development, injury, and repair, including the earliest stages of abnormal repair that lead to chronic lung disease. Standardization of PCLS readouts and aggregation and comparisons between experiments across broad experimental approaches, as outlined above, are critical and will support the translational goal of harnessing live imaging and omics of PCLSs for therapeutic discovery.

The Future: Emerging Concepts in PCLS Modeling

Ventilation and Perfusion

More recently, models such as a "breathing lung slice model" are being developed to incorporate stretching using PCLSs. For instance, the Flexcell Tension System stretches PCLSs to model ventilation-induced lung injury. Another method embeds elastic substrates prepared in multiwell formats (19) suitable for high-throughput screening. Despite these advancements, optimizing these models remains a challenge, especially in separating stretch effects on different lung compartments. Computational and structural models are emerging to address

this issue, potentially integrating physiological and biochemical data for a more comprehensive understanding. One possible direction is mapping stiffness values across tissues to model local stretch differences based on tissue structure, which may allow studying local mechanotransduction. Subsequently, these approaches can be applied to different disease models such as fibrotic lungs, underdeveloped lungs from preterm neonates, and lungs used for aging research. Similarly, approaches incorporating perfusion using microfluidic chambers with circulating blood-borne cells and other factors are promising new avenues (100–108).

Aging

Aging is a main risk factor for chronic lung disease. PCLSs are generated from human tissue from across the lifespan and offer the opportunity to investigate age-related effects. Moreover, hallmarks of aging, such as mitochondrial dysfunction or cellular senescence, can be modeled in PCLSs, thus allowing us to dissect cell-specific effects of different aging inducers (89). The epigenetic clock (DNA methylation) can be also measured by genomic DNA isolated from PCLSs to estimate biological age acceleration (109, 110).

The Future: Biomarker, Drug Discovery Platform, and Personalized Medicine Approaches

Biomarkers are of specific importance for early diagnosis and assessment for therapeutic response in clinical settings. In the context of preclinical models, where clinical readouts such as lung function cannot be assessed, other biomarkers with strong clinical correlation are needed. To date, endpoints for PCLS studies have been driven by the state of knowledge in specific disease processes. Moving ahead, measurement of secreted mediators in PCLSs emerges as a promising tool for biomarker discovery and validation. These analyses can help to identify biomarkers that predict a mechanism of action for a specific target and/or drug, therefore presenting valuable candidates for companion diagnostic approaches. Many secreted mediators, including growth factors, cytokines, chemokines, enzymes involved in ECM reshaping, as well as other factors such as surfactant protein C, can be detected in the supernatant of PCLSs (35, 49, 97, 111, 112). Analysis of these media provides a relatively high-throughput approach for integration with other systems, including clinical trials

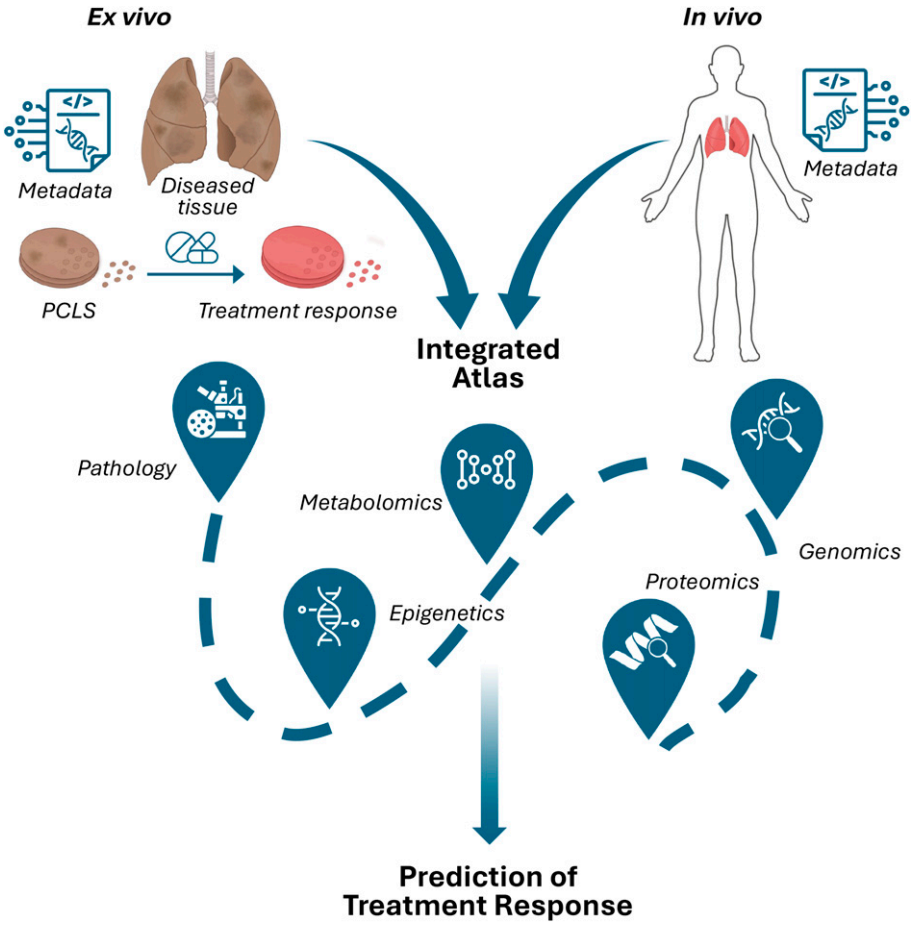

Figure 3. Integrated atlas of *ex vivo* and *in vivo* omics data for personalized medicine. PCLSs can be a unique tool to build a personalized medicine approach for lung disease. This relies on careful collection of metadata as well as integration of multiple *ex vivo* and *in vivo* datasets to enable identification of molecular phenotypes and will close the "cellular gap" of endpoints that are currently used. Moreover, an integrated atlas based on *ex vivo* perturbations and treatment can help in the prediction of therapy response.

and *in vivo* experiments, supporting the use of PCLSs to close the gap to identifying therapeutic biomarkers.

PCLSs are already being used to help evaluate novel therapeutic strategies. Clinical trial candidates, such as PLN-74809 or saracatinib, currently under evaluation for IPF, showed antifibrotic effects in PCLSs (113, 114). Future clinical results will shed more light on the utility of PCLSs as translational drug discovery and validation tools. To facilitate transfer to clinical scenarios, standardized protocols for PCLSs need to be implemented (*see* Table 1 for minimum reporting criteria), ideally applying principles of Good Laboratory Practice to the *ex vivo* setup (Good *In Vitro* Method Practice), including Good Cell Culture Practice (115–117).

Although this report mainly focuses on the use of human PCLSs, we want to mention that the use of PCLSs from experimental models, such as mouse models, do have a specific value in relating *in vivo* to *ex vivo* responses in the PCLSs in the context of biomarker and endpoint discovery. Translating findings from human PCLSs to rodent PCLSs and related *in vivo* models ensures translational significance, especially for evaluating failed clinical trial compounds in preclinical settings.

The careful collection of metadata will be crucial in identifying patient subpopulations and specific responses to treatment and is necessary if we want to make a significant contribution to personalized medicine approaches in pulmonary medicine (Figure 3). This includes anonymized patient information, such as genetics, medications, epigenetics, history of exposure, and, importantly, clinical presentation. Additional PCLS metadata could include morphology and imaging, including radiomics, and physiological and functional readouts in response to exogenous stimuli. This combined information will enable correlations with molecular phenotypes determined by spatial sequencing, single cell or single nuclei RNA sequencing, bulk RNA sequencing, and proteomics, as well as epigenetic profiling. It will further allow us to close the "cellular gap" of endpoints that are currently used and will help to recalibrate endpoints from mere observational levels to mechanistic endpoints that tell us about disease pathomechanisms. PCLSs are a specifically suitable model for this, because they allow for the longitudinal characterization and, as such, the dynamic change of biomarkers and do not rely on one "endpoint measurement." It will be of crucial importance that we take the information gained in PCLSs back to population studies of diseases. The preparation of an atlas integrating PCLS data as well as patient data will allow for computational correlation of patient profiles to *ex vivo* perturbations and potentially to prediction of therapy response (Figure 3). Furthermore, we hope that the use of PCLSs will contribute to the discovery of molecular endpoints and biomarkers that can inform clinical trials and represent the complex molecular changes in chronic lung diseases, including IPF and COPD.

Summary and Conclusion

Over recent years, PCLSs have gained immense attention as a novel tool to advance our understanding of human lung disease pathobiology, target and drug identification, and validation and, thus, have emerged as an important pillar to support the development of potential new therapeutics for lung disease.

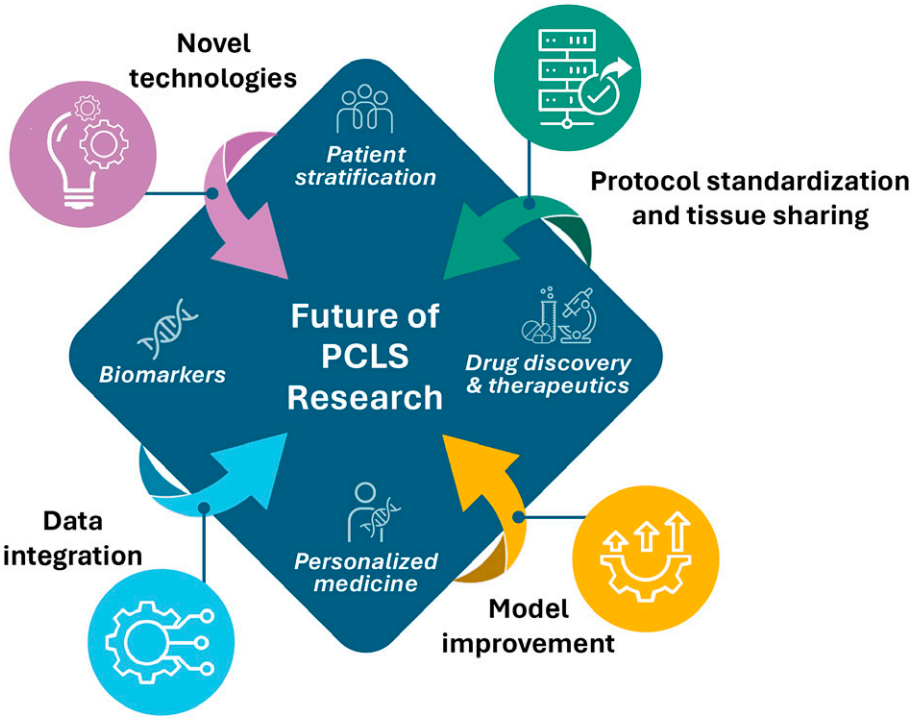

Figure 4. The future of PCLS research. PCLSs have emerged as an important pillar to support the development of potential new therapeutics for lung disease. Main development areas that will help to further expand and advance PCLS applications include: *1*) protocol standardization and tissue-sharing opportunities due to novel storage solutions; *2*) model improvement with bioengineering approaches; *3*) implementation of novel technologies, such as live imaging and spatial omics; and *4*) integration of metadata as well as matched omics data from *ex vivo* and *in vivo* datasets.

The implementation of standardized protocols, minimum reporting criteria as outlined in Table 1, and novel technologies to capture clinically relevant readouts will be instrumental for future studies using PCLSs. Several new and exciting technologies can be applied to PCLSs and will undoubtedly expand our understanding of human lung pathology and advance drug discovery and validation (Figure 4). The collection and correlation of clinical metadata and multiomic analysis will further advance the integration of this preclinical platform into patient endotyping and precision medicine approaches. PCLSs are a unique tool to bridge basic and translational findings and clinical trials and personalized medicine. ■

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