Diagnosing and treating lung disease at the cellular level

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As we recognize World Lung Day on September 25, 2020, our goal as a research community remains to pursue cutting-edge research while maintaining close attention to the immediate needs of patients (33). Even though a particular lung disease may present with a distinctive constellation of signs and symptoms, all lung diseases arise from the same components—the cells, structures, and tissues that make up the respiratory system. Current knowledge of these components, how they work together, how they malfunction, and how they repair themselves lays an important foundation for advances in understanding disease and developing new therapies. Fundamental knowledge of the lung and its components, ranging from physiology over the life span to cellular and molecular function, provides a “toolbox” that can be used to study all diseases of the pulmonary system, even newly emerging diseases such as COVID-19. This editorial highlights the current state of the toolbox for lung research, which is rapidly expanding with new technologies for personal monitoring, single-cell analyses, and data science. This toolbox will allow pulmonary researchers unprecedented capabilities for understanding the heterogeneity of disease, both at a population level and at the cellular and molecular levels, promoting discovery of biomarkers that characterize clinical heterogeneity, aid in prognosis, and guide optimal treatment (35). This toolbox will also promote discovery of cellular or molecular targets for precision medicine interventions.

Observational studies have historically taught us about disease risk factors and natural history and have yielded data that can lead to hypotheses regarding mechanisms of pathogenesis. Observational cohort studies also provide a powerful tool to identify correlations, if not causal factors, that may have significant real-world relevance to health and disease. When harmonized for data collection, pooling cohort data can permit researchers to answer questions requiring increased statistical power, such as how lung function decline correlates with smoking cessation and low-intensity smoking (24).

Modern observational studies are increasingly able to incorporate a rich collection of data, including clinical, environmental, behavioral, imaging, genetic, and molecular data types, allowing an unprecedented ability to investigate genetic factors that predispose to pulmonary disease (16), identify subclinical phenotypes that predict lung function decline or mortality (2, 14, 34), and use deep phenotyping to identify patient subtypes that might later be used for prognosis or personalized therapies (22, 36). Imaging and molecular data can be used to develop biomarkers or physiological and molecular hypotheses regarding pathogenesis and disease progression. The literature of lung disease research is replete with examples of insights gained through observational studies. Ideally, hypotheses generated from observational studies will be further supported through experimental approaches, although this is not always possible.

To realize personalized care for lung disease, patients need to be considered not only as distinct from one another but also as characterized by a unique and dynamic spectrum of pathobiologic processes that converge to ultimately define individual responses to treatments and disease evolution. This concept is illustrated in the focus of asthma mechanistic and clinical research over the past several decades. Key findings illustrate that, although asthma patients may present with similar clinical symptoms, there is significant heterogeneity in disease etiology, pathobiology, disease manifestations, and therapeutic responsiveness. Deep phenotyping approaches have yielded multiscale information that revealed the heterogeneous nature of asthma and exacerbations and distinct disease subtypes.

Intrinsic heterogeneity in the pathobiologic processes that underlie asthma, variation in response to therapeutics, and therapeutics that manage or improve the control of symptoms without modifying the underlying disease state or natural history of disease continue to result in significant challenges to optimized clinical care, particularly in those patients with severe disease. Early precision clinical trials, using pathobiologic characteristics, were negative, underscoring the need to integrate pathobiology and clinical symptoms to identify and target specific subpopulations (26).

The National Heart, Lung, and Blood Institute’s (NHLBI) Severe Asthma Research Program has significantly contributed to the concept that severe asthma disease heterogeneity and subpopulations result from interactions among multiple, diverse pathobiologic mechanisms over time (8, 10, 25). This evidence base has provided the opportunity to develop more precise, biologically based approaches to optimize asthma clinical management via the NHLBI Precision Interventions for Severe and/or Exacerbation-Prone Asthma (PrecISE) Network. The study is using predictive biomarkers and defined patient subgroups in a multistage, adaptive design with novel interventions to optimize management and, potentially, provide the ability to modify disease progression and severity.

Another heterogeneous lung disease similar to severe asthma that carries a high mortality rate is acute respiratory distress syndrome (ARDS) (19, 20). The absence of beneficial treatments beyond lung-protective ventilation and regulated fluid management underscores the need to understand disease heterogeneity in order to develop effective therapies. Disease heterogeneity in ARDS stems from a variety of precipitants, including bacterial or viral infections, trauma, or aspiration, as well as heterogeneity of host responses to differing various

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Also be applied to the extremely large and complex data sets collected from many thousands of participants, but “big data” can data sets containing information of many different types collected. During influenza infection KGF increases type II alveolar cell proliferation to augment infectivity and exacerbate influenza-induced lung injury (23).

As for many other lung diseases, age is a risk factor in ARDS and underscores disease heterogeneity. Incidence and mortality increase with age, and ARDS survivors over 70 yr have a protracted recovery period. This evidence supports the importance of understanding determinants of host resilience as paramount to reducing susceptibility and enhancing repair (15, 27). Progress toward understanding disease heterogeneity has advanced through identification and validation of at least two distinct subphenotypes derived from multiple ARDS clinical trial cohorts defined by distinct biomarkers and associated with therapeutic responses and ARDS outcomes (5, 6, 11). Elucidation of more precise mechanisms that define targetable pathobiology and predictable outcomes of a subphenotype of ARDS patients may be necessary for precise and effective therapies.

Rapid technology developments in single-cell analyses, high-resolution imaging, and associated computational tools now allow us to gain novel insights into cellular heterogeneity, including cell identities, cell states, spatial organization, and dynamic trajectory of the cells in the tissue and organ context during normal development and disease. Although single-cell RNA-Seq (scRNA-Seq) is the most frequently used method to profile RNA from freshly dissociated cells and yields most gene numbers per cell (29), single-nucleus RNA-Seq (snRNA-Seq) can profile RNA from previously banked frozen samples (18). snRNA-Seq from serial cryosections of lung tissues can provide transcriptomic information with spatial context. Complementing transcriptomic analysis, single-cell ATAC-seq (scATAC-seq) (7) detects chromatin accessibility to reveal gene regulatory mechanisms. Methods combining imaging and spatial transcriptomics are recent additions to the lung analysis toolbox, including multiplexed error-robust single-molecule fluorescence in situ hybridization (MERFISH) (32) and combining mass cytometry by time of flight (CyTOF).

Joint efforts through the NHLBI-supported LungMAP program (https://lungmap.net) (3), the Lung Cell Atlas (28) under the Human Cell Atlas program (https://www.humancellatlas.org), and others have utilized these single cell-based technologies to identify remarkable cell heterogeneity throughout lung development (9) and have discovered new cell types (21), specific progenitor subgroups (37), cell differentiation trajectories (17), and unique abnormal cells during disease pathogenesis (4, 30). Recent analyses of human single-cell RNA sequencing (scRNA-seq) data sets have revealed that cell subsets that coexpress ACE2/TMPRSS2 include alveolar type II pneumocytes, absorptive enterocytes, and nasal goblet secretory cells and therefore may serve as cellular targets of SARS-CoV-2 (38).

Finally, rapid advancements in biomedical science are increasingly driven by what is commonly referred to as “big data.” The label is often used in reference to complex clinical data sets containing information of many different types collected from many thousands of participants, but “big data” can also be applied to the extremely large and complex data sets generated from modern molecular and cellular techniques. Data sets like those generated by LungMAP (https://lungmap.net) or recently published single-cell RNA-Seq efforts (1, 13) can have thousands of features and tens of millions of data points. Such data sets present robust analytical and visualization challenges but bring with them the opportunity for systems-level discoveries that were not possible even a decade ago. Along with such discoveries comes the conceptual challenge of how to filter results to identify “high-value targets” for in-depth validation and translation. Bringing together large clinical and large molecular/cellular data sets, though significantly more challenging than analyzing either alone, represents an enormous opportunity for accelerating the discovery process. NHLBI’s TOPMed program (https://www.nhlbiwgs.org/) is an excellent representation of what this can look like in practice. The COVID-19 pandemic has highlighted the need for tools and approaches to enable rapid translation of discoveries into practice. The pulmonary science community is well positioned to respond and into the future, with programs such as TOPMed, COPDGene, PVDOMICS, PrecISE, and others having been designed from the beginning to collect and integrate high-density clinical and molecular data.

In summary, we have illustrated several examples of how the ever-growing toolbox of new basic science technologies can be applied to the study of lung diseases to better understand molecular, cellular, and tissue heterogeneity in the context of normal, aberrant, and resilience mechanisms. Combining this knowledge and integrating it with clinical outcomes will hopefully allow scientists to target the primary dysfunction and correct that abnormality at its origin. This tailored systems approach will facilitate more efficient and effective diagnosis and treatment of disorders of the lung, resulting in improved population health.

DISCLAIMERS
The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute, the National Institutes of Health, or the US Department of Health and Human Services.

DISCLOSURES
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AUTHOR CONTRIBUTIONS
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