

mechanical ventilation again if deemed necessary, and they speculated that the “remembering-self” was probably the leading reason for this affirmation.

This is in keeping with another study that showed that patients would choose to receive aggressive treatment, but only if survival was not associated with severe functional or cognitive impairment (15).

The major factor in a patient–clinician interview is how it is conducted, and this detail is not specified in the article. It has been suggested that “little white lies” in this bidirectional communication are quite common; sometimes in an attempt to “please my doctor who saves my life,” a patient may minimize his or her symptoms or bad experiences (16).

In conclusion, this study, besides being well conducted and providing important clinical information, clarifies the issue that perception in medicine may be very misleading. Therefore, the patient and clinician, allied together, should never give up on the dream to liberate the patient from prolonged ventilation and recover a satisfactory quality of life. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Defining the Cell Types That Drive Idiopathic Pulmonary Fibrosis Using Single-Cell RNA Sequencing

Few novel technologies have been welcomed with more excitement by the scientific community than single-cell RNA sequencing (scRNA-seq). The report by Reyfman and colleagues (pp. 1517–1536) in this issue of the *Journal* provides important insight into

pathogenic cell types in lung fibrosis on an unprecedented scale (1), making idiopathic pulmonary fibrosis (IPF) the first chronic lung disease to be analyzed using scRNA-seq (#CureIPF).

A single cell is the fundamental unit of life. Dissecting the heterogeneity, dynamics, and interactions of cells will truly unravel how we, as well as diseases we are trying to cure, develop and grow. Until recently, the characterization of specific cell types (of the lung) relied on *ex vivo* labeling or generating large numbers of cells based, at times, on poorly understood isolation techniques, followed by analysis of pooled RNA or proteins for hybridization or sequencing. Although these approaches have revealed robust cell

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Originally Published in Press as DOI: 10.1164/rccm.201901-0197ED on February 4, 2019

type-specific markers (e.g., surfactant proteins) and detailed mRNA expression profiles of cell populations, they were generated using pooled cells, leaving any information about cellular heterogeneity hidden.

This knowledge gap is now filled by scRNA-seq analysis, which allows the simultaneous characterization of thousands of cells, providing an elaborate expression signature of mRNAs, microRNAs, and possibly soon proteins in each single cell (2). Importantly, scRNA-seq approaches do not require prior knowledge of cell-specific markers to define heterogeneity within cell populations. New or rare cell types have been discovered already using scRNA-seq, such as the recently described ionocyte in the epithelial lining of the trachea (3, 4). Technological advances in capturing and isolating RNA from individual cells, as well as the rapidly evolving field of systems biology and artificial intelligence, are now enabling investigators to perform single-cell studies with unprecedented scale and ease (5), and to decipher the ontogeny of cell lineages in the absence of lineage tracing. Global efforts are underway to sequence the transcriptomes of all cells in the human body, led by the Human Cell Atlas consortium (<https://www.humancellatlas.org>) (6). Our community thus will have access to the most comprehensive and accurate reference map of human cells. Ideally, this will form the basis for mechanistic studies, with the goal of establishing causative links between cellular composition, heterogeneity, and disease.

The current publication by Reyfman and colleagues provides the first detailed map of single-cell transcriptomes in human lung fibrosis (1). IPF is a progressive and ultimately fatal disease characterized by airway and interstitial inflammation, destruction of functional alveoli, and the formation of fibrotic tissue. Although previous studies using conventional approaches have discovered and confirmed altered cell types that are enriched in IPF (e.g., activated myofibroblasts and hyperplastic epithelial cells), an unbiased approach to detect and quantify these populations has not been available thus far. Smaller-scale, single-cell profiles of epithelial cells from six patients with IPF (540 epithelial cells in total) were previously reported (7, 8); however, Reyfman and colleagues' data set (76,070 cells in total) significantly extends those observations, representing the largest data set in lung fibrosis reported to date. Their study included eight human subjects with pulmonary fibrosis and eight donor lung samples. The authors analyzed and visualized their data as one integrated set and also as individual data sets for each patient, revealing patient-specific cell types. The authors analyzed differential gene expression between donor and fibrotic lungs in macrophages, alveolar type II cells, and fibroblasts, and confirmed the results by sequencing "bulk" RNA of pooled, flow cytometry-sorted cells. Importantly, an in-depth analysis of macrophages and epithelial cells revealed an unexpected heterogeneity within these two populations, and distinct profibrotic macrophage populations in IPF. Because the transcriptome of these macrophages is now available in detail, future studies will test whether modification of any of these novel genes would inhibit lung fibrosis in model systems. The authors obtained equally exciting results by subclustering epithelial cells and mapping the expression of Wnt pathway components, as this signaling pathway is important in IPF (9–11). This analysis revealed that Wnt ligands are expressed in nonoverlapping fashion, at times only in select single cells, whereas neighboring cells

express different Wnt ligands, underscoring the importance of (mesenchymal–epithelial) cell–cell communication for transmitting Wnt signals.

Macrophages have been at the center of several recent publications in the field. Aran and colleagues identified a profibrotic macrophage subtype that appears after bleomycin injury, resembling a transitional cell type between alveolar and monocyte-derived macrophages (12). Cohen and colleagues used scRNA-seq to reconstruct dynamic changes in cellular composition during mouse lung development, and identified cell–cell interactions based on receptor–ligand pair screening (13). This identified lung-resident basophils as regulators of maturation and immunomodulatory functions of lung macrophages, indicating that basophils may dictate macrophage function in fibrosis.

As with any new technology, there are several aspects of scRNA-seq data that need to be interpreted with caution. One surprising caveat is the "ectopic" expression of genes that supposedly are restricted to only a certain cell type. One example is immunoglobulins, which appear to be expressed by macrophages, alveolar type II cells, and ciliated cells. Another example is surfactant genes, which appear to be among the most sharply downregulated mRNAs in fibroblasts. The authors explain this phenomenon by the presence of cell-free ambient RNA in the cell suspension, which is released by dying cells during the preparations. Certainly, bioinformatic algorithms to address this issue in scRNA-seq data are being refined.

Another important issue regarding scRNA-seq data is the interpretation of the relative frequencies of distinct cell types. The relative abundance of each cell type is highly dependent on the cell isolation protocol used, as different cell types are recovered from fibrotic and healthy lung tissue with different efficiencies. One possible way to assess cell frequencies more accurately would be to perform, in parallel to scRNA-seq, a bulk RNA-seq analysis of undigested samples followed by digital deconvolution to assess the relative frequencies of the cell types identified by scRNA-seq. As opposed to traditional algorithms, which use *a priori*-defined signatures derived, e.g., from *in vitro* studies, such coupled deconvolution would use cell signatures derived from the scRNA-seq analysis itself, as recently described (14).

In summary, this publication by Reyfman and colleagues demonstrates the unique power of scRNA-seq for discovering cell heterogeneity in human lung disease (here, IPF). The authors should be applauded for undertaking this elaborate study and, most importantly, for making the data available to the community. This will most certainly lead to a number of follow-up studies and ultimately refine our understanding of IPF pathogenesis. We are convinced that this will be a landmark publication in the field. The entire data set is available on an interactive website for anyone to explore differential cell abundance and gene expression in individual cells in control and IPF lungs. Kudos to the authors. Let's dig in and collaborate! ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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⊗ Toward Personalized Medicine in Bronchiolitis

Nothing essential has changed in the definition of bronchiolitis since its description nearly 80 years ago (1, 2). The syndrome affects infants and young children at different ages, presents with a few overlapping symptoms of varying intensity and other manifestations often associated with specific pathogens (e.g., fever and pharyngitis in influenza or wheezing with respiratory syncytial virus), and is caused by infection with one or a combination of half a dozen viruses with different inflammatory, antiviral, and/or atopic phenotypes (3). Conceived as a single entity, a number of candidate drugs failed as therapeutics against it in randomized trials (4). To date, management recommendations for hospitalized patients are based on supportive therapy (2). Etiologic testing is discouraged (2).

Times are changing. Paradigm shifts usually announce themselves through periods characterized by coexisting contradictory practices, concepts, and models. We are living in the dawn of the omics era and precision medicine, and steadily moving away from the illusion of the panacea (one medicine that cures all diseases) to re(de)fine our diagnoses and provide tailored, personalized treatments. We are only now coming to

terms with the idea that bronchiolitis is, in fact, a constellation of clinical manifestations arbitrarily considered a single disease, instead conformed by several distinct pathophysiological entities. In this issue of the *Journal*, Jones and colleagues (pp. 1537–1549) perform transcriptomics profiling of peripheral blood mononuclear cells and nasal mucosal scrapings from 26 infants and 27 young children who suffered acute bronchiolitis, and provide evidence supporting the need to discriminate apples from oranges. Samples were obtained during acute infection and at convalescence to characterize the immune-inflammatory response networks associated with the entities within the syndrome (5). Adding nasal mucosal scrapings expanded the analysis to two biological sources of data, whereas previous reports limited their sampling to either blood or nasal scrapings (6–8).

The first message emerging from these observations is that, during acute bronchiolitis, IFN seems to matter, particularly for infants (most of them infected with respiratory syncytial virus [RSV]). Infants with bronchiolitis exhibit hyperactivation of type I IFN transcription pathways, validated at the protein level (5). During RSV infection, IFN is seldom found in respiratory secretions, and consequently has been speculated to play a minor role in disease pathogenesis (9). However, type I IFN levels peak early after infection, and RSV bronchiolitis has a long incubation period followed by a prodromal phase. Therefore, samples may have been collected too late in earlier studies to detect its antiviral effects (9). For the same reason, and given the nature of study design, the observed up-regulation of IFN during acute illness does not imply a positive association between levels and severity. Earlier works in asthma nicely show that timing is everything when

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Funded by a grant from the Bill and Melinda Gates Foundation and the Director's Challenge Award from the National Institute of Environmental Health Sciences from the National Institutes of Health (F.P.P.).

Originally Published in Press as DOI: 10.1164/rccm.201812-2371ED on January 18, 2019