



## Future Directions in Idiopathic Pulmonary Fibrosis Research An NHLBI Workshop Report

Timothy S. Blackwell<sup>1</sup>, Andrew M. Tager<sup>2</sup>, Zea Borok<sup>3</sup>, Bethany B. Moore<sup>4</sup>, David A. Schwartz<sup>5</sup>, Kevin J. Anstrom<sup>6</sup>, Ziv Bar-Joseph<sup>7</sup>, Peter Bitterman<sup>8</sup>, Michael R. Blackburn<sup>9</sup>, William Bradford<sup>10</sup>, Kevin K. Brown<sup>5</sup>, Harold A. Chapman<sup>11</sup>, Harold R. Collard<sup>11</sup>, Gregory P. Cosgrove<sup>5</sup>, Robin Deterding<sup>5</sup>, Ramona Doyle<sup>12</sup>, Kevin R. Flaherty<sup>4</sup>, Christine Kim Garcia<sup>13</sup>, James S. Hagood<sup>14</sup>, Craig A. Henke<sup>8</sup>, Erica Herzog<sup>15</sup>, Cory M. Hogaboam<sup>4</sup>, Jeffrey C. Horowitz<sup>4</sup>, Talmadge E. King, Jr.<sup>11</sup>, James E. Loyd<sup>1</sup>, William E. Lawson<sup>1</sup>, Clay B. Marsh<sup>16</sup>, Paul W. Noble<sup>17</sup>, Imre Noth<sup>18</sup>, Dean Sheppard<sup>11</sup>, Julie Olsson<sup>12</sup>, Luis A. Ortiz<sup>19</sup>, Thomas G. O’Riordan<sup>20</sup>, Tim D. Oury<sup>19</sup>, Ganesh Raghu<sup>21</sup>, Jesse Roman<sup>22</sup>, Patricia J. Sime<sup>23</sup>, Thomas H. Sisson<sup>4</sup>, Daniel Tschumperlin<sup>24</sup>, Shelia M. Violette<sup>25</sup>, Timothy E. Weaver<sup>26</sup>, Rebecca G. Wells<sup>27</sup>, Eric S. White<sup>4</sup>, Naftali Kaminski<sup>7</sup>, Fernando J. Martinez<sup>4</sup>, Thomas A. Wynn<sup>28</sup>, Victor J. Thannickal<sup>29</sup>, and Jerry P. Eu<sup>30</sup>

<sup>1</sup>Vanderbilt University Medical Center, Nashville, Tennessee; <sup>2</sup>Massachusetts General Hospital, Boston, Massachusetts; <sup>3</sup>University of Southern California, Los Angeles, California; <sup>4</sup>University of Michigan, Ann Arbor, Michigan; <sup>5</sup>National Jewish Health and University of Colorado, Denver, Colorado; <sup>6</sup>Duke Clinical Research Institute, Durham, North Carolina; <sup>7</sup>Carnegie Mellon University, Pittsburgh, Pennsylvania; <sup>8</sup>University of Minnesota Medical School, Minneapolis, Minnesota; <sup>9</sup>The University of Texas Medical School at Houston, Houston, Texas; <sup>10</sup>InterMune, Inc., Brisbane, California; <sup>11</sup>University of California, San Francisco, San Francisco, California; <sup>12</sup>Genentech/Roche, San Francisco, California; <sup>13</sup>University of Texas Southwestern Medical Center, Dallas, Texas; <sup>14</sup>University of California, San Diego, San Diego, California; <sup>15</sup>Yale University School of Medicine, New Haven, Connecticut; <sup>16</sup>The Ohio State University, Columbus, Ohio; <sup>17</sup>Cedars-Sinai Hospital, Los Angeles, California; <sup>18</sup>University of Chicago, Chicago, Illinois; <sup>19</sup>University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; <sup>20</sup>Gilead Sciences, Inc., Foster City, California; <sup>21</sup>University of Washington Medical Center, Seattle, Washington; <sup>22</sup>University of Louisville, Louisville, Kentucky; <sup>23</sup>University of Rochester School of Medicine, Rochester, New York; <sup>24</sup>Harvard School of Public Health, Boston, Massachusetts; <sup>25</sup>Biogen Idec, Cambridge, Massachusetts; <sup>26</sup>Cincinnati Children’s Research Foundation, Cincinnati, Ohio; <sup>27</sup>The University of Pennsylvania, Philadelphia, Pennsylvania; <sup>28</sup>National Institute of Allergy and Infectious Diseases, Bethesda, Maryland; <sup>29</sup>University of Alabama, Birmingham, Alabama; and <sup>30</sup>National Heart, Lung, and Blood Institute, Bethesda, Maryland

### Abstract

The median survival of patients with idiopathic pulmonary fibrosis (IPF) continues to be approximately 3 years from the time of diagnosis, underscoring the lack of effective medical therapies for this disease. In the United States alone, approximately 40,000 patients die of this disease annually. In November 2012, the NHLBI held a workshop aimed at coordinating research efforts and accelerating the development of IPF therapies. Basic, translational, and clinical researchers gathered with representatives from the NHLBI, patient advocacy groups, pharmaceutical companies, and the U.S. Food and Drug Administration to review the current state of IPF research and identify priority areas, opportunities for collaborations, and directions for future research. The workshop was organized into

groups that were tasked with assessing and making recommendations to promote progress in one of the following six critical areas of research: (1) biology of alveolar epithelial injury and aberrant repair; (2) role of extracellular matrix; (3) preclinical modeling; (4) role of inflammation and immunity; (5) genetic, epigenetic, and environmental determinants; (6) translation of discoveries into diagnostics and therapeutics. The workshop recommendations provide a basis for directing future research and strategic planning by scientific, professional, and patient communities and the NHLBI.

**Keywords:** idiopathic pulmonary fibrosis; alveolar epithelial cells; extracellular matrix; interstitial lung disease; inflammation

(Received in original form July 8, 2013; accepted in final form September 19, 2013)

Supported by NHLBI, National Institutes of Health.

Author Contributions: Conception and design: T.S.B., A.M.T., and J.P.E.; writing and contributing important intellectual content: all.

Correspondence and requests for reprints should be addressed to Jerry Eu, M.D., Division of Lung Diseases, National Heart, Lung, and Blood Institute, National Institutes of Health, 6701 Rockledge Drive, Rockledge Centre II, Room 10042, Bethesda, MD 20892. E-mail: jerry.eu@nih.gov

Am J Respir Crit Care Med Vol 189, Iss 2, pp 214–222, Jan 15, 2014

Published 2014 by the American Thoracic Society

Originally Published in Press as DOI: 10.1164/rccm.201306-1141WS on October 25, 2013

Internet address: www.atsjournals.org

## At a Glance Commentary

### Scientific Knowledge on the Subject:

This report summarizes the latest research as well as knowledge gaps in idiopathic pulmonary fibrosis (IPF).

### What This Study Adds to the Field:

The workshop recommendations provide a basis for future strategic planning in IPF research for the scientific, professional, and patient communities and for the NHLBI of the National Institutes of Health.

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive disease of unknown cause that occurs primarily in older adults and is defined by clinicopathologic criteria, including the histologic and/or radiologic pattern of usual interstitial pneumonia (1). Since the NHLBI last convened a workshop on IPF in 2001 (2), researchers have generated important new insights into the pathobiology of this disease; however, to date this information has not been translated into effective therapies.

To focus and coordinate ongoing basic and translational research efforts in IPF, the NHLBI convened a workshop on November 27 and 28, 2012. The workshop was organized into six working groups focused on important areas of perceived need and/or opportunity in the field of IPF research. By necessity, a number of areas of investigation relevant to pulmonary fibrosis, including some aspects of clinical science, were not fully addressed in this forum. This report summarizes discussions by each of the working groups, highlights knowledge gaps, and provides recommendations for strategic planning and future research to better define the pathogenesis of IPF and develop effective therapies. In addition to the group summaries presented here, expanded summaries from two of the working groups, summarizing the role of extracellular matrix (ECM) in IPF and the use of preclinical modeling (3), will be published separately.

## Biology of Alveolar Injury and Aberrant Repair

### Epithelial Injury/Susceptibility in IPF

Recurrent and/or nonresolving injury to the lung epithelium appears to be a key driver of pulmonary fibrosis. Although the cause of

this injury in IPF remains enigmatic, the footprints of lung epithelial injury are manifest as increased epithelial cell death and phenotypic alterations of surviving epithelial cells (4). Emerging data support the concept that altered (or “reprogrammed”) alveolar epithelial cell (AEC) phenotypes, including those produced by increased endoplasmic reticulum stress, predispose to further AEC injury and/or abnormal repair, facilitating the development of pulmonary fibrosis (5, 6). The timing and nature of inciting and subsequent injuries to AECs, as well as how other genetic, epigenetic, and environmental factors affect disease presentation, are poorly understood. This working group suggested that the epithelial abnormalities/dysfunction underlying fibrosis be referred to as “reprogramming,” regardless of whether the initial injury eventually leads to cell death or only elicits profibrotic responses. Better definition of the molecular characteristics of AEC “reprogramming” is needed to improve understanding of IPF pathogenesis. In addition, studies to further investigate epithelial interactions with mesenchymal cells and matrix in animal models and patient samples would be beneficial. It is possible that the “lung-on-a-chip” approach, which has been used to model interactions between epithelial cells and endothelial cells (7), could be modified to incorporate mesenchymal cells and used to study epithelial–mesenchymal cell interactions, as could three-dimensional cell culture systems or decellularized matrix/bioreactor systems.

### Cell Origin(s) of the Myofibroblast

Myofibroblasts, and related mesenchymal cells, are generally accepted as the cells predominantly responsible for fibrotic destruction/distortion of the lung in IPF (8). Lineage tracing experiments in the lung and other organs have identified diverse cellular sources that may give rise to these mesenchymal cells (9–14), but the contributions of these potential cell(s)-of-origin, as well as the potential contributions of stem and differentiated resident cells, circulating mesenchymal cells (fibrocytes), or transdifferentiation from other lineages, to expansion of the lung fibroblast population remain to be definitively determined. Furthermore, the stage of mesenchymal cell differentiation at which IPF myofibroblasts acquire hallmark pathological properties, the mechanisms underlying their pathological

differentiation, and the roles of signals from epithelial cells, immune cells, and matrix in this process all require better elucidation. Progress in these areas would be greatly facilitated by the identification of mesenchymal cell markers able to distinguish the different stages of differentiation of these cells and standardization of methods for isolation, cultivation, and distribution of mesenchymal cells from patients with IPF.

### Role of Stem/Progenitor Cells in Aberrant Repair

Although epithelial cell proliferation and differentiation is a prominent feature of repair after lung injury (15), the importance of expansion and differentiation of small/rare populations of preexisting epithelial stem/progenitor cells in epithelial regeneration is unclear (16, 17). Dedifferentiation of mature epithelial cells followed by their proliferation and differentiation is another plausible mechanism for epithelial regeneration. Depletion or loss of function of airway/epithelial progenitors could favor fibrosis over regeneration of functional lung tissue, but determining the role of progenitor cell failure in IPF will require better understanding of the identity and biology of such cells.

### Recommendations

1. Investigate the molecular basis of AEC “reprogramming” in IPF.
2. Generate novel animal models of pulmonary fibrosis focusing on epithelial injury.
3. Study bidirectional cross-talk between AEC and other cell types that contribute to IPF.
4. Standardize identification and isolation of human AECs, mesenchymal cells, and decellularized lung matrices.
5. Identify distal airway and alveolar stem/progenitor cells and clarify their expansion or contraction in IPF.
6. Identify biomarkers of epithelial injury/dysfunction/reprogramming in IPF.

## Role of ECM

### ECM Composition in IPF and Its Bioactive Components

The matrisome, composed of almost 300 ECM proteins, is one of the most plastic and rapidly evolving compartments of the

proteome (18). Beyond roles in providing architectural support, many ECM components are bioactive (i.e., deliver important signals to fibroblasts and other cells that they contact) (19–25). In addition, ECM mechanical properties can influence cellular phenotypes and organ fibrosis. Newer applications of noninvasive imaging modalities, such as microfocal X-ray computed tomography (26) and magnetic resonance elastography (27), allow for assessment of tissue biomechanical properties of the lung *in vivo*, but our current understanding of ECM mechanics in normal and IPF lungs is still limited. *In vitro*, several groups have shown that fibroblast function and myofibroblast differentiation are tightly linked to the deformability (i.e., the rigidity or stiffness) of the ECM (28–32). “Outside-in” transmission of mechanical forces to the cell, and “inside-out” transmission of traction forces from the cell to the ECM, may both mediate profibrotic cellular responses (33).

#### ECM Dynamics and Fibrosis Resolution

Evidence suggests fibrosis of diverse organ systems is at least partially reversible in experimental animal models and in humans (34, 35). For resolution of fibrosis to occur, recruited/activated myofibroblasts must be eliminated, and deposited ECM must be degraded and cleared. In most cases, it appears these processes occur synchronously (36). Which of these resolution mechanisms are subverted in IPF is currently unknown.

#### ECM Model Systems

The translation of accumulating knowledge of matrix biology to the development of new diagnostic biomarkers and therapeutic agents for IPF could be facilitated by improved ECM model systems. A variety of culture systems are available that mimic the mechanical properties and dimensionality of tissues *in vivo*. One widely used system is mechanically tunable polyacrylamide gels that impart known stiffness to the matrix (28, 29, 31, 37–39). These matrices can be coated with ECM proteins to provide a biologically relevant culture substrate with appropriate (normal) or augmented (fibrotic) stiffness. Alternatively, decellularized human lung ECM (from explanted control and IPF lungs) can be sliced on a vibratome to create individual

culture “discs” that retain both their native stiffness and their ECM composition (40). The decellularized system has the advantages of using fully human matrices and allowing the direct study of matrices from human diseases (or relevant animal models). Further improvements in ECM models are needed to incorporate other mechanical features, including viscosity, flow, stretch, hydrostatic pressure, and heterogeneity.

#### Recommendations

1. Characterize IPF lung ECM and determine how it influences profibrotic cellular phenotypes.
2. Identify ECM components and their receptors that promote fibrotic remodeling.
3. Elucidate mechanisms of matrix stiffening and cell responses to increased stiffness.
4. Determine the lung’s capacity to resolve fibrosis and identify strategies to augment it.
5. Develop model systems that better simulate the lung ECM environment.

#### Preclinical Modeling

##### Usefulness of Animal Models in IPF

Although many investigators have relied on animal models to study the pathogenesis of pulmonary fibrosis, none of the models currently available fully recapitulates the progressive nature of IPF or the histology of usual interstitial pneumonia. In addition, clinical trials based on efficacy signals from animal models (in particular the bleomycin model) have thus far failed to yield an effective medical therapy, leading some to question the usefulness of animal models for studies of IPF pathogenesis and treatment. However, this view discounts valuable insights into mechanisms of fibrosis generated from animal studies (41).

##### Mouse Models of Pulmonary Fibrosis

Although intratracheal installation of bleomycin remains the most commonly used model of lung fibrosis, other exogenous agents, including silica, radiation, fluorescein isothiocyanate, and asbestos, have also been helpful in elucidating pathogenic mechanisms (3). Overexpression of endogenous fibrogenic mediators, such as

transforming growth factor (TGF)- $\beta$ , IL-13, and TGF- $\alpha$  (3, 42), can also be used to model lung fibrosis. TGF- $\beta$  overexpression by adenoviral delivery (43) or by doxycycline-regulated transgenic expression in epithelial cells (44) highlights the ability of this cytokine to promote epithelial cell apoptosis, myofibroblast differentiation, and production of other profibrotic mediators (3, 43–45). Genetic models have been developed based on mutations identified in patients with familial interstitial pneumonia, including surfactant protein C (46, 47), surfactant protein A2 (48), and telomerase components (49, 50). Surprisingly, modeling these mutations has not yet resulted in spontaneous development of fibrosis, although some have produced an increased susceptibility to fibrotic stimuli (51–53), indicating that genetic models have potential to improve understanding of “gene-by-environment” interactions in IPF. As new genetic links to disease are identified, either as rare variants in familial cases or common variants in sporadic IPF, mouse modeling holds promise in dissecting additional pathways pertinent to fibrosis.

#### Other Approaches to Model Human Pulmonary Fibrosis

IPF is age dependent in humans (54), and recent strides have been made in modeling fibrosis in older animals (55–58). In addition, genetic deletion of the receptor for advanced glycation end products (RAGE) (55) or relaxin (57) results in spontaneous age-related lung fibrosis. Such models will be important in helping elucidate biological changes that occur with aging that make the lung more susceptible to fibrosis. Additionally, recent advances have been made to “humanize” mouse fibrosis models using approaches such as infusion of human IPF fibroblasts into immunodeficient NOD/SCID/Beige mice (59, 60).

#### Recommendations

1. New treatments should be studied during the fibrogenic phase, as well as the initiation or inflammatory phase, of preclinical models.
2. Evaluation of fibrosis should incorporate both biochemical measures of ECM deposition and histological assessments of lung architecture.
3. The role of aging should be further investigated in current models.

4. Expansion of humanized models of lung fibrosis is encouraged.

## The Role of Inflammation and Immunity

### Adaptive Immune Cells in IPF

Although the lack of efficacy of immunosuppressive drugs in IPF has led some to suggest inflammation may not play a role in this disease (61), some inflammation/immune activation is consistently found in the lungs of patients with IPF (61–63). Studies in animal lung fibrosis models have also identified important roles for some adaptive immune cells, including CD4<sup>+</sup> T cell subsets (64–66).

### Fibrocytes in IPF

Fibrocytes have been implicated in the immunopathogenesis of many diseases characterized by fibrosis and remodeling (67). Identified by the coexpression of leukocyte markers such as CD45, ECM proteins such as collagen I $\alpha$ , and pluripotency markers such as CD34 (68), fibrocytes are detected with increased frequency in the lungs (69) and circulation of patients with IPF. Elevations in circulating fibrocytes predicted reduced event-free survival in a prospectively recruited cohort of subjects with IPF (70). Animal modeling has also suggested a potential role for fibrocytes in the development and/or maintenance of pulmonary fibrosis (71–74). These cells combine the proinflammatory properties of monocytes and the tissue remodeling properties of fibroblasts and consequently could participate in fibrosis through a number of mechanisms (67); however, studies to date have not definitively determined their role in IPF.

### Macrophages in IPF

Macrophages possess many functions that would be expected to promote fibrosis, including regulation of fibroblast proliferation, recruitment, and activation (75); direct regulation of ECM components (76); and secretion of profibrotic cytokines and growth factors. Macrophages may also possess antifibrotic properties, however (75, 77, 78). Several recent studies have attributed these opposing functions to different macrophage

populations distinguished along the lines of classical (M1) and alternative (M2) macrophage activation (72, 79, 80), with M1 macrophages being antifibrotic and M2 macrophages being either profibrotic or regulatory. However, this classification may be overly simplistic, given recent human studies that demonstrate that IPF mortality is associated with increased concentrations of mediators associated with both M1 and M2 macrophages (81, 82). Although macrophages may be functionally important in IPF, many questions about their contributions to this disease remain unanswered.

### Targeting Inflammatory Cells and Mediators in IPF

Endogenous mediators and mechanisms that naturally slow the progression of fibrosis may be able to be exploited therapeutically, and their identification is consequently of great interest (77). These mediators and mechanisms may include immunosuppressive cytokines like IL-10, decoy receptors like IL-13R $\alpha$ 2 and IL-1RA, regulatory T cells, and various monocyte/macrophage subpopulations that have been shown to suppress inflammation and fibrosis (83).

### Recommendations

1. Better define the roles and importance of specific immune/inflammatory cell populations at all stages of IPF.
2. Investigate whether specific immune cells or the cytokines they secrete can be manipulated to ameliorate lung fibrosis.
3. Characterize and exploit endogenous pathways that inhibit inflammation and fibrosis.

## Genetic, Epigenetic, and Environmental Determinants

### Progress in IPF Genomics

In the last decade, significant progress has been made in identifying genetic causes and genomic characteristics of pulmonary fibrosis. The association of IPF with a common single-nucleotide polymorphism in the promoter of the *MUC5B* gene (84) was recently confirmed by two genome-wide association studies, which also identified other IPF-associated chromosomal loci (85, 86). These additional loci include Toll interacting protein (*TOLLIP*), which negatively regulates Toll-like receptor signaling, and

genes associated with cell–cell adhesion and DNA repair. The *MUC5B* promoter and *TOLLIP* variants associated with IPF have both been linked to survival (86, 87), and the *MUC5B* promoter variant is also associated with interstitial lung abnormalities and fibrosis in the general population (88). Rare variants in genes for surfactant proteins (A and C) (46, 89) and telomerase components (49, 90) have similarly been identified in familial and sporadic IPF. Although these findings transform our current understanding of the genetic predisposition to IPF, additional studies that use whole genome, whole exome, and targeted region sequencing are required to identify all rare risk alleles for IPF in both coding and noncoding regions (91).

### Profiling of Pulmonary Fibrosis Using Novel Techniques

Going beyond genetics, the application and integration of other “omics” approaches, including analyses of coding and noncoding RNA, the epigenome, and the microbiome, to pulmonary fibrosis should enhance our ability to understand, diagnose, and ultimately treat IPF. Classical gene expression profiling studies have progressed in IPF from characterizations of small numbers of human or mouse lung samples to analyses of large cohorts that have led to identification of multiple relevant genes (92–95). Progress has been made in novel profiling of IPF as well: mechanistically relevant changes in microRNA expression profiles in IPF lung have been described (96–98), and studies aimed at determining the lung methylome are ongoing (99, 100). Investigators can now access the Lung Genomics Research Consortium website (<http://www.lung-genomics.org/lgrc>) and download mRNA, microRNA, methylation, and SNP profiles of carefully phenotyped IPF lungs. Although the increased availability of omics data is encouraging, the availability of omics information has not yet translated to personalized medicine approaches that are more precise, predictive, and participatory.

### Gene-to-Function Analyses

Current success in identifying individual genes and pathways in IPF has not yet been accompanied by mechanistic understanding of how these genetic, epigenetic, and microRNA changes result in human disease. Pathways identified by genomic approaches should be studied in preclinical models to

understand disease-relevant mechanisms. Additionally, profiling methods could help identify preclinical animal model(s) that most closely mimic human IPF. An unbiased profiling approach could lead to libraries of relevant animal models and allow detailed phenotyping not possible from human studies.

### Gene–Environment Interactions

A better understanding of the role of environmental factors in IPF requires further epidemiological efforts, including studies in “at-risk” populations, such as carriers of known disease-associated gene variants. Tools are being developed in environmental sciences to precisely measure real-time exposures and the body burden of previous exposures, which should be applied to study IPF whenever possible. Probing the human transcriptome or epigenome for evidence of “profibrosis” exposures and “fibrosis-cumulative” injuries could also have significant impact.

### Going Beyond IPF to Understand IPF

One of the key challenges in interpreting omics information in IPF is that it is difficult to determine which findings of these types of studies are specific to IPF. To better understand IPF, we need to break down individual disease-specific “silos” and consider using genomic, transcriptomic, and epigenetic approaches to compare (1) IPF with other lung diseases such as autoimmune and exposure-related interstitial lung diseases and interstitial lung diseases in children; (2) IPF with fibrotic diseases of liver, kidney, heart, and skin, and (3) IPF with fibrotic lung diseases in other species. These comparative studies may facilitate drug development, particularly of agents that may be effective for multiple fibrotic diseases.

### Recommendations

1. Use genomic approaches to identify novel pathways that influence the development of human IPF.
2. Identify exposure signatures to better understand gene–environment interactions in IPF.
3. Compare omics of IPF to other lung diseases, fibrosis in other organs, and pulmonary fibrosis in other species.
4. Support integrated studies using omics data to develop personalized approaches to IPF.

## Translation of Discoveries into Diagnostics and Therapeutics

### Progress in Clinical and Translational Studies in IPF

Numerous recent accomplishments in this area include: standardization of IPF diagnosis (1), improved understanding that IPF progression is highly variable (1), development of novel clinical staging systems, prediction models (101–103) and potential biomarkers of disease activity (104, 105), and recent publications of practice-altering data from clinical trials conducted by the IPFnet, a network of IPF clinical research centers, and industry.

### Development of Patient Cohorts with Standardized Phenotyping and Longitudinal Clinical Data

In IPF, disease heterogeneity continues to pose challenges for studies of the human disease, management decisions, and clinical trials. The benefits of establishing well-defined cohorts of patients with IPF with standardized, comprehensive, and longitudinal clinical and biological data to guide future clinical investigations, as well as bench research, cannot be overemphasized. For example, the contributions of gastroesophageal reflux (106) and autoantibodies (107) to the pathogenesis of IPF, and their role exacerbations, could be further characterized from such cohorts. The establishment of these cohorts could also facilitate validation studies of genetic variants (86, 87), biomarkers (81, 108), and clinical characteristics (63) that have been linked to IPF progression. The major obstacle to the creation of these cohorts is lack of coordinated efforts among the key stakeholders (clinicians, investigators, patient advocacy groups/foundations, and clinical trial sponsors including government agencies and pharmaceutical companies). The working group supports coordinating national/international efforts to create desired IPF cohorts by standardizing clinical phenotyping of patients with IPF at baseline and over time and linking these data to longitudinal biologic samples, and using these cohorts for genetic, biomarker, clinical, and other studies to develop novel IPF diagnostics and therapeutics.

### Biological Sample Repositories

Translational research linking molecular pathways to disease phenotypes requires comprehensive biorepositories of tissue and biologic samples from carefully phenotyped clinical cohorts. Currently available biorepositories have been collected through the NHLBI-supported Lung Tissue Research Consortium (LTRC), a multicenter observational study (Correlating Outcomes with biochemical Markers to Estimate Time-progression in IPF [COMET]), IPFnet, the National Disease Research Interchange, and a few individual academic centers, including the Pittsburgh genomic and proteomic analysis of disease progression in IPF (GAP) program. Going forward, biorepository efforts to support translational research need to be expanded in a systematic and coordinated fashion, in conjunction with efforts to establish well-defined IPF cohorts advocated above. Standardization of sample collections, agreements to make these biorepositories open-access resources, and regulatory aspects of clinical research still remain to be worked out by all stakeholders.

### Qualification and Validation of Biomarkers

Biologically relevant biomarkers will improve the diagnostic approach to, the clinical management of, and the performance of therapeutic trials for IPF. Obstacles to the development of robust biomarkers, including the investment required for their validation and disease heterogeneity, remain, but a pathway for biomarker development, qualification, and validation has been defined (109). Numerous potential biomarkers have been examined in the IPF population, including measures of pulmonary physiology, chest imaging, patient-reported measures, and tissue and molecular markers. Physiologic measures including FVC, diffusing capacity of the lung for carbon monoxide, TLC, exercise tolerance, and measures of oxygenation have all been used as measures of disease severity, progression, and responsiveness to therapeutic interventions (1). Lung imaging studies, particularly high-resolution computed tomography, play an integral role in IPF diagnosis and may be useful in assessment of disease severity. Structural patterns and semiquantitative measures of individual

radiographic features have been used to define disease progression, and evolving quantitative measures of imaging may improve our ability to monitor disease progression and therapeutic responsiveness. Several instruments of patient-reported outcomes, adapted from other lung disorders and including various measures of dyspnea and health-related quality of life, have been used to assess longitudinal IPF progression and therapeutic response (110). Future collaborative research to develop useful IPF biomarkers by combined efforts of academic consortia, pharmaceutical companies, patient advocacy groups, and the National Institutes of Health is endorsed by the working group.

**The Performance and Impact of Phase I/II/III Clinical Trials**

Although a number of high-quality phase III studies have been performed, the majority of completed clinical trials in IPF have been phase II. Standardization of these studies has improved considerably and proven the feasibility of high-quality, large, global, randomized controlled trials in this disease. Recognized obstacles for trial performance include confusion over strategies to enrich cohorts for measurable disease progression and current debate regarding the optimal primary endpoint (111–114). Although physiological variables have been correlated with survival, whether we have identified a valid surrogate for survival in pivotal clinical trials remains controversial and a key area for future discussion and investigation. The most common study approach has included the use of American Thoracic Society, European Respiratory Society, Japanese Respiratory Society, and Latin American Thoracic Association criteria for IPF diagnosis and the physiologic primary endpoint of FVC, which some advocate as the most practical clinical endpoint for IPF trials (112, 114). Some experts have recently proposed that, in the absence of validated surrogate endpoints, all-cause mortality and nonselective hospitalizations would be more appropriate clinical endpoints than FVC in future phase III clinical trials (111). Although these two endpoints may be ideal in IPF, other experts have countered that IPF clinical trials based on them would be prohibitively large and/or expensive (114), as only a minority of patients with physiologically mild to moderate disease

experience disease progression over the course of clinical trials. A possible solution to this controversy is to use a cohort enrichment strategy that would allow for a more homogenous study population that experiences an increased number of events, and development of such a strategy is critically needed. Further study of the larger, clinically annotated, longitudinal collections of human IPF patient samples advocated by this working group above could also increase the efficiency of IPF clinical studies, by enabling clinical and molecular-based strategies to determine patient numbers, maximize statistical power, and promote future surrogate biomarker evaluation. At present, however, the lack of integrated clinical infrastructure, the cost of clinical drug development, concerns over intellectual property, and the high failure rate of drugs investigated to date all pose challenges for IPF clinical trial efforts.

**Recommendations**

1. Establish national/international cohorts of phenotypically and geographically diverse patients with IPF.
2. Develop/expand centralized open-access biorepositories of tissues from patients with IPF and appropriate control subjects.
3. Develop an IPF-specific patient-reported outcome tool.

4. Develop, select, and validate IPF biomarkers and imaging techniques.
5. Facilitate clinical trials by establishing an infrastructure with regional IPF centers of excellence.
6. Clinical trials should incorporate cohort enrichment and systematic biomarker strategies, should use clinically meaningful endpoints when possible, and should complement patient cohort establishment and biological sample collection efforts.

**Summary**

The goal of this workshop was to summarize important aspects of basic and translational research in IPF, define areas of need in this field, and provide recommendations for future research. In addition to the specific recommendations made by each working group, discussions at the workshop highlighted areas of exceptional need and/or potential to advance the field that are summarized in Table 1. Given the progress that has been made in understanding the biology of pulmonary fibrosis over the last decade, we are optimistic that effective therapies for IPF can be identified in coming years. This outcome will be enhanced and accelerated by transparent and coordinated efforts among all stakeholders. ■

**Table 1:** Areas of Exceptional Needs or Opportunities in Idiopathic Pulmonary Fibrosis Research

---

<p>Development of systems and new approaches to define the molecular characteristics of “reprogrammed” alveolar epithelial cells and interactions of these cells with key components of their environment, including (myo)fibroblasts.</p> <p>Standardization of methods for collection and distribution of human cell types important in IPF (including AECs and fibroblasts).</p> <p>Refinement of methodology to investigate functional components of the ECM.</p> <p>Collaboration among researchers with interests in different organs/systems to expedite identification of common disease mechanisms and conserved therapeutic targets.</p> <p>Development of better preclinical models that incorporate the effects of aging and relevant genetic information.</p> <p>Integration of “omics” data and incorporation of gene–environment studies.</p> <p>Establishment of large, rigorously phenotyped patient cohorts using standardized definitions for long-term clinical and biological data.</p> <p>Enhancement of biorepositories using samples and data from carefully phenotyped patients with longitudinal data</p> <p>Further development and validation of patient-reported outcomes and biomarkers and establishment of an iterative process to incorporate results of ongoing studies into improved design of future therapeutic trials</p>
---

---

*Definition of abbreviations:* AEC = alveolar epithelial cell; ECM = extracellular matrix; IPF = idiopathic pulmonary fibrosis.

**Author disclosures** are available with the text of this article at [www.atsjournals.org](http://www.atsjournals.org).

**Acknowledgement:** Other participants of the workshop are: Tom Croxton, Antonio Punturieri,

Jim Kiley, Michelle Freemer, Lisa Postow, Andrea Harabin, Gail Weinmann, Carol Blaisdell, Weiniu Gan, Sara Lin, Dorothy Gail, Tim Moore, Hannah Peavy (all from NHLBI, Division of Lung Diseases), Ghenima Dirami (Center for Scientific Review,

National Institute of Health), Lydia Gilbert McClain, Banu Karimi-Shah (The U.S. Food and Drug Administration), Dan Rose, Dolly Kervitsky (Pulmonary Fibrosis Foundation), and Teresa Barnes (Coalition for Pulmonary Fibrosis).

## References

- Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA, *et al.*; ATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 2011;183:788–824.
- Crystal RG, Bitterman PB, Mossman B, Schwarz MI, Sheppard D, Almsy L, Chapman HA, Friedman SL, King TE Jr, Leinwand LA, *et al.* Future research directions in idiopathic pulmonary fibrosis: summary of a National Heart, Lung, and Blood Institute working group. *Am J Respir Crit Care Med* 2002;166:236–246.
- Moore B, Lawson W, Oury T, Sisson T, Raghavendran K, Hogaboam C. Animal models of fibrotic lung disease. *Am J Respir Cell Mol Biol* 2013;49:167–179.
- Sakai N, Tager AM. Fibrosis of two: epithelial cell-fibroblast interactions in pulmonary fibrosis. *Biochim Biophys Acta*.
- Selman M, Pardo A. Role of epithelial cells in idiopathic pulmonary fibrosis: from innocent targets to serial killers. *Proc Am Thorac Soc* 2006;3:364–372.
- Tanjore H, Lawson WE, Blackwell TS. Endoplasmic reticulum stress as a pro-fibrotic stimulus. *Biochim Biophys Acta* 2013;1832:911–921.
- Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE. Reconstituting organ-level lung functions on a chip. *Science* 2010;328:1662–1668.
- Scotton CJ, Chambers RC. Molecular targets in pulmonary fibrosis: the myofibroblast in focus. *Chest* 2007;132:1311–1321.
- Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G. The myofibroblast: one function, multiple origins. *Am J Pathol* 2007;170:1807–1816.
- Zeisberg EM, Potenta SE, Sugimoto H, Zeisberg M, Kalluri R. Fibroblasts in kidney fibrosis emerge via endothelial-to-mesenchymal transition. *J Am Soc Nephrol* 2008;19:2282–2287.
- Zeisberg M, Kalluri R. Fibroblasts emerge via epithelial-mesenchymal transition in chronic kidney fibrosis. *Front Biosci* 2008;13:6991–6998.
- Kim KK, Kugler MC, Wolters PJ, Robillard L, Galvez MG, Brumwell AN, Sheppard D, Chapman HA. Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. *Proc Natl Acad Sci USA* 2006;103:13180–13185.
- Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, Valerius MT, McMahon AP, Duffield JS. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *Am J Pathol* 2010;176:85–97.
- Rock JR, Barkauskas CE, Cronce MJ, Xue Y, Harris JR, Liang J, Noble PW, Hogan BL. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. *Proc Natl Acad Sci USA* 2011;108:E1475–E1483.
- Beers MF, Morrissey EE. The three R's of lung health and disease: repair, remodeling, and regeneration. *J Clin Invest* 2011;121:2065–2073.
- Rock JR, Hogan BL. Epithelial progenitor cells in lung development, maintenance, repair, and disease. *Annu Rev Cell Dev Biol* 2011;27:493–512.
- Kotton DN. Next-generation regeneration: the hope and hype of lung stem cell research. *Am J Respir Crit Care Med* 2012;185:1255–1260.
- Hynes RO, Naba A. Overview of the matrisome—an inventory of extracellular matrix constituents and functions. *Cold Spring Harb Perspect Biol* 2012;4:a004903.
- Kliment CR, Englert JM, Gochuico BR, Yu G, Kaminski N, Rosas I, Oury TD. Oxidative stress alters syndecan-1 distribution in lungs with pulmonary fibrosis. *J Biol Chem* 2009;284:3537–3545.
- Naik PK, Bozyk PD, Bentley JK, Popova AP, Birch CM, Wilke CA, Fry CD, White ES, Sisson TH, Tayob N, *et al.*; COMET Investigators. Periostin promotes fibrosis and predicts progression in patients with idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2012;303:L1046–L1056.
- Teder P, Vandivier RW, Jiang D, Liang J, Cohn L, Puré E, Henson PM, Noble PW. Resolution of lung inflammation by CD44. *Science* 2002;296:155–158.
- Okamoto M, Hoshino T, Kitasato Y, Sakazaki Y, Kawayama T, Fujimoto K, Ohshima K, Shiraishi H, Uchida M, Ono J, *et al.* Periostin, a matrix protein, is a novel biomarker for idiopathic interstitial pneumonias. *Eur Respir J* 2011;37:1119–1127.
- Olsen AL, Sackey BK, Marcinkiewicz C, Boettiger D, Wells RG. Fibronectin extra domain-A promotes hepatic stellate cell motility but not differentiation into myofibroblasts. *Gastroenterology* 2012;142:928–937.e3.
- Serini G, Bochaton-Piallat ML, Ropraz P, Geinoz A, Borsi L, Zardi L, Gabbiani G. The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor-beta1. *J Cell Biol* 1998;142:873–881.
- Gao F, Koenitzer JR, Tobolewski JM, Jiang D, Liang J, Noble PW, Oury TD. Extracellular superoxide dismutase inhibits inflammation by preventing oxidative fragmentation of hyaluronan. *J Biol Chem* 2008;283:6058–6066.
- Sera T, Fujioka H, Yokota H, Makinouchi A, Himeno R, Schroter RC, Tanishita K. Localized compliance of small airways in excised rat lungs using microfocal X-ray computed tomography. *J Appl Physiol* (1985) 2004;96:1665–1673.
- McGee KP, Hubmayr RD, Ehman RL. MR elastography of the lung with hyperpolarized <sup>3</sup>He. *Magn Reson Med* 2008;59:14–18.
- Li Z, Dranoff JA, Chan EP, Uemura M, Sévigny J, Wells RG. Transforming growth factor-beta and substrate stiffness regulate portal fibroblast activation in culture. *Hepatology* 2007;46:1246–1256.
- Liu F, Mih JD, Shea BS, Kho AT, Sharif AS, Tager AM, Tschumperlin DJ. Feedback amplification of fibrosis through matrix stiffening and COX-2 suppression. *J Cell Biol* 2010;190:693–706.
- Balestrini JL, Chaudhry S, Sarrazy V, Koehler A, Hinz B. The mechanical memory of lung myofibroblasts. *Integr Biol (Camb)* 2012;4:410–421.
- Huang X, Yang N, Fiore VF, Barker TH, Sun Y, Morris SW, Ding Q, Thannickal VJ, Zhou Y. Matrix stiffness-induced myofibroblast differentiation is mediated by intrinsic mechanotransduction. *Am J Respir Cell Mol Biol* 2012;47:340–348.
- Wipff PJ, Rifkin DB, Meister JJ, Hinz B. Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. *J Cell Biol* 2007;179:1311–1323.
- Goldmann WH. Mechanotransduction and focal adhesions. *Cell Biol Int* 2012;36:649–652.
- Duffield JS, Lupher M, Thannickal VJ, Wynn TA. Host responses in tissue repair and fibrosis. *Annu Rev Pathol* 2013;24:241–276.
- Henderson NC, Iredale JP. Liver fibrosis: cellular mechanisms of progression and resolution. *Clin Sci (Lond)* 2007;112:265–280.
- Iredale JP, Benyon RC, Pickering J, McCullen M, Northrop M, Pawley S, Hovell C, Arthur MJ. Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. *J Clin Invest* 1998;102:538–549.
- Georges PC, Hui JJ, Gombos Z, McCormick ME, Wang AY, Uemura M, Mick R, Janmey PA, Furth EE, Wells RG. Increased stiffness of the rat liver precedes matrix deposition: implications for fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2007;293:G1147–G1154.

38. Wells RG. The role of matrix stiffness in hepatic stellate cell activation and liver fibrosis. *J Clin Gastroenterol* 2005;39:S158–S161.
39. Tschumperlin DJ, Dai G, Maly IV, Kikuchi T, Laiho LH, McVittie AK, Haley KJ, Lilly CM, So PT, Lauffenburger DA, et al. Mechanotransduction through growth-factor shedding into the extracellular space. *Nature* 2004;429:83–86.
40. Booth AJ, Hadley R, Cornett AM, Dreffs AA, Matthes SA, Tsui JL, Weiss K, Horowitz JC, Fiore VF, Barker TH, et al. Acellular normal and fibrotic human lung matrices as a culture system for in vitro investigation. *Am J Respir Crit Care Med* 2012;186:866–876.
41. Peng R, Sridhar S, Tyagi G, Phillips JE, Garrido R, Harris P, Burns L, Renteria L, Woods J, Chen L, et al. Bleomycin induces molecular changes directly relevant to idiopathic pulmonary fibrosis: a model for “active” disease. *PLoS ONE* 2013;8:e59348.
42. Baughman RP, Lower EE, Miller MA, Bejarano PA, Heffelfinger SC. Overexpression of transforming growth factor- $\alpha$  and epidermal growth factor-receptor in idiopathic pulmonary fibrosis. *Sarcoidosis Vasc Diffuse Lung Dis* 1999;16:57–61.
43. Sime PJ, Xing Z, Graham FL, Csaky KG, Gauldie J. Adenovector-mediated gene transfer of active transforming growth factor- $\beta$ 1 induces prolonged severe fibrosis in rat lung. *J Clin Invest* 1997;100:768–776.
44. Lee CG, Cho SJ, Kang MJ, Chapoval SP, Lee PJ, Noble PW, Yehualaeshet T, Lu B, Flavell RA, Milbrandt J, et al. Early growth response gene 1-mediated apoptosis is essential for transforming growth factor  $\beta$ 1-induced pulmonary fibrosis. *J Exp Med* 2004;200:377–389.
45. Fernandez IE, Eickelberg O. The impact of TGF- $\beta$  on lung fibrosis: from targeting to biomarkers. *Proc Am Thorac Soc* 2012;9:111–116.
46. Thomas AQ, Lane K, Phillips J III, Prince M, Markin C, Speer M, Schwartz DA, Gaddipati R, Marney A, Johnson J, et al. Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. *Am J Respir Crit Care Med* 2002;165:1322–1328.
47. Lawson WE, Grant SW, Ambrosini V, Womble KE, Dawson EP, Lane KB, Markin C, Renzoni E, Lympny P, Thomas AQ, et al. Genetic mutations in surfactant protein C are a rare cause of sporadic cases of IPF. *Thorax* 2004;59:977–980.
48. Wang Y, Kuan PJ, Xing C, Cronkhite JT, Torres F, Rosenblatt RL, DiMaio JM, Kinch LN, Grishin NV, Garcia CK. Genetic defects in surfactant protein A2 are associated with pulmonary fibrosis and lung cancer. *Am J Hum Genet* 2009;84:52–59.
49. Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C, Lawson WE, Xie M, Vulto I, Phillips JA III, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med* 2007;356:1317–1326.
50. Tsakiri KD, Cronkhite JT, Kuan PJ, Xing C, Raghu G, Weissler JC, Rosenblatt RL, Shay JW, Garcia CK. Adult-onset pulmonary fibrosis caused by mutations in telomerase. *Proc Natl Acad Sci U S A* 2007;104:7552–7557.
51. Lawson WE, Polosukhin VV, Stathopoulos GT, Zoia O, Han W, Lane KB, Li B, Donnelly EF, Holburn GE, Lewis KG, et al. Increased and prolonged pulmonary fibrosis in surfactant protein C-deficient mice following intratracheal bleomycin. *Am J Pathol* 2005;167:1267–1277.
52. Lawson WE, Cheng DS, Degryse AL, Tanjore H, Polosukhin VV, Xu XC, Newcomb DC, Jones BR, Roldan J, Lane KB, et al. Endoplasmic reticulum stress enhances fibrotic remodeling in the lungs. *Proc Natl Acad Sci USA* 2011;108:10562–10567.
53. Degryse AL, Xu XC, Newman JL, Mitchell DB, Tanjore H, Polosukhin VV, Jones BR, McMahon FB, Gleaves LA, Phillips JA, 3rd, et al. Telomerase deficiency does not alter bleomycin-induced fibrosis in mice. *Exp Lung Res* 2012;38:124–134.
54. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). *Am J Respir Crit Care Med* 2000;161:646–664.
55. Englert JM, Hanford LE, Kaminski N, Tobolewski JM, Tan RJ, Fattman CL, Ramsgaard L, Richards TJ, Loutaev I, Nawroth PP, et al. A role for the receptor for advanced glycation end products in idiopathic pulmonary fibrosis. *Am J Pathol* 2008;172:583–591.
56. Naik P, Horowitz JC, Moore TA, Wilke CA, Toews GB, Moore BB. Pulmonary fibrosis induced by  $\gamma$ -herpesvirus in aged mice is associated with increased fibroblast responsiveness to transforming growth factor- $\beta$ . *J Gerontol A Biol Sci Med Sci* 2012;67:714–725.
57. Samuel CS, Zhao C, Bathgate RA, Bond CP, Burton MD, Parry LJ, Summers RJ, Tang ML, Amento EP, Tregear GW. Relaxin deficiency in mice is associated with an age-related progression of pulmonary fibrosis. *FASEB J* 2003;17:121–123.
58. Torres-González E, Bueno M, Tanaka A, Krug LT, Cheng DS, Polosukhin VV, Sorescu D, Lawson WE, Blackwell TS, Rojas M, et al. Role of endoplasmic reticulum stress in age-related susceptibility to lung fibrosis. *Am J Respir Cell Mol Biol* 2012;46:748–756.
59. Pierce EM, Carpenter K, Jakubzick C, Kunkel SL, Flaherty KR, Martinez FJ, Hogaboam CM. Therapeutic targeting of CC ligand 21 or CC chemokine receptor 7 abrogates pulmonary fibrosis induced by the adoptive transfer of human pulmonary fibroblasts to immunodeficient mice. *Am J Pathol* 2007;170:1152–1164.
60. Trujillo G, Meneghin A, Flaherty KR, Sholl LM, Myers JL, Kazerooni EA, Gross BH, Oak SR, Coelho AL, Evanoff H, et al. TLR9 differentiates rapidly from slowly progressing forms of idiopathic pulmonary fibrosis. *Sci Transl Med* 2010;2:57ra82.
61. Selman M, King TE, Pardo A; American Thoracic Society; European Respiratory Society; American College of Chest Physicians. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. *Ann Intern Med* 2001;134:136–151.
62. Gilani SR, Vuga LJ, Lindell KO, Gibson KF, Xue J, Kaminski N, Valentine VG, Lindsay EK, George MP, Steele C, et al. CD28 down-regulation on circulating CD4 T-cells is associated with poor prognoses of patients with idiopathic pulmonary fibrosis. *PLoS ONE* 2010;5:e8959.
63. Ley B, Collard HR, King TE Jr. Clinical course and prediction of survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2011;183:431–440.
64. Wilson MS, Elnekave E, Mentink-Kane MM, Hodges MG, Pesce JT, Ramalingam TR, Thompson RW, Kamanaka M, Flavell RA, Keane-Myers A, et al. IL-13 $\alpha$ 2 and IL-10 coordinately suppress airway inflammation, airway-hyperreactivity, and fibrosis in mice. *J Clin Invest* 2007;117:2941–2951.
65. Wilson MS, Madala SK, Ramalingam TR, Gochuico BR, Rosas IO, Cheever AW, Wynn TA. Bleomycin and IL-1 $\beta$ -mediated pulmonary fibrosis is IL-17A dependent. *J Exp Med* 2010;207:535–552.
66. Wynn TA. Fibrotic disease and the T(h)1/T(h)2 paradigm. *Nat Rev Immunol* 2004;4:583–594.
67. Reilkoff RA, Bucala R, Herzog EL. Fibrocytes: emerging effector cells in chronic inflammation. *Nat Rev Immunol* 2011;11:427–435.
68. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. *Mol Med* 1994;1:71–81.
69. Peng X, Mathai SK, Murray LA, Russell T, Reilkoff R, Chen Q, Gulati M, Elias JA, Bucala R, Gan Y, et al. Local apoptosis promotes collagen production by monocyte-derived cells in transforming growth factor  $\beta$ 1-induced lung fibrosis. *Fibrogenesis Tissue Repair* 2011;4:12.
70. Moeller A, Gilpin SE, Ask K, Cox G, Cook D, Gauldie J, Margetts PJ, Farkas L, Dobranowski J, Boylan C, et al. Circulating fibrocytes are an indicator of poor prognosis in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2009;179:588–594.
71. Moore BB, Murray L, Das A, Wilke CA, Herrygers AB, Toews GB. The role of CCL12 in the recruitment of fibrocytes and lung fibrosis. *Am J Respir Cell Mol Biol* 2006;35:175–181.
72. Murray LA, Chen Q, Kramer MS, Hesson DP, Argentieri RL, Peng X, Gulati M, Homer RJ, Russell T, van Rooijen N, et al. TGF- $\beta$  driven lung fibrosis is macrophage dependent and blocked by Serum amyloid P. *Int J Biochem Cell Biol* 2011;43:154–162.
73. Phillips RJ, Burdick MD, Hong K, Lutz MA, Murray LA, Xue YY, Belperio JA, Keane MP, Strieter RM. Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. *J Clin Invest* 2004;114:438–446.
74. Mehrad B, Burdick MD, Strieter RM. Fibrocyte CXCR4 regulation as a therapeutic target in pulmonary fibrosis. *Int J Biochem Cell Biol* 2009;41:1708–1718.



75. Song E, Ouyang N, Hörbelt M, Antus B, Wang M, Exton MS. Influence of alternatively and classically activated macrophages on fibrogenic activities of human fibroblasts. *Cell Immunol* 2000;204:19–28.
76. Atabei K, Jame S, Azhar N, Kuo A, Lam M, McKleroy W, Dehart G, Rahman S, Xia DD, Melton AC, et al. Mfge8 diminishes the severity of tissue fibrosis in mice by binding and targeting collagen for uptake by macrophages. *J Clin Invest* 2009;119:3713–3722.
77. Wynn TA, Barron L. Macrophages: master regulators of inflammation and fibrosis. *Semin Liver Dis* 2010;30:245–257.
78. Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. *Nat Rev Immunol* 2011;11:723–737.
79. Murray LA, Rosada R, Moreira AP, Joshi A, Kramer MS, Hesson DP, Argentieri RL, Mathai S, Gulati M, Herzog EL, et al. Serum amyloid P therapeutically attenuates murine bleomycin-induced pulmonary fibrosis via its effects on macrophages. *PLoS ONE* 2010;5:e9683.
80. Gibbons MA, MacKinnon AC, Ramachandran P, Dhaliwal K, Duffin R, Phythian-Adams AT, van Rooijen N, Haslett C, Howie SE, Simpson AJ, et al. Ly6Chi monocytes direct alternatively activated profibrotic macrophage regulation of lung fibrosis. *Am J Respir Crit Care Med* 2011;184:569–581.
81. Richards TJ, Kaminski N, Baribaud F, Flavin S, Brodmerkel C, Horowitz D, Li K, Choi J, Vuga LJ, Lindell KO, et al. Peripheral blood proteins predict mortality in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2012;185:67–76.
82. Prasse A, Probst C, Bargagli E, Zissel G, Toews GB, Flaherty KR, Olschewski M, Rottoli P, Müller-Quernheim J. Serum CC-chemokine ligand 18 concentration predicts outcome in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2009;179:717–723.
83. Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat Med* 2012;18:1028–1040.
84. Seibold MA, Wise AL, Speer MC, Steele MP, Brown KK, Loyd JE, Fingerlin TE, Zhang W, Gudmundsson G, Groshong SD, et al. A common MUC5B promoter polymorphism and pulmonary fibrosis. *N Engl J Med* 2011;364:1503–1512.
85. Fingerlin TE, Murphy E, Zhang W, Peljto AL, Brown KK, Steele MP, Loyd JE, Cosgrove GP, Lynch D, Groshong S, et al. Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis. *Nat Genet* 2013;45:613–620.
86. Noth I, Zhang Y, Ma S-F, Flores C, Barber M, Huang Y, Broderick SM, Wade MS, Hysi P, Scuirba J, et al. Genetic variants associated with idiopathic pulmonary fibrosis susceptibility and mortality: a genome-wide association study. *Lancet Respir Med* 2013;1:309–317.
87. Peljto AL, Zhang Y, Fingerlin TE, Ma SF, Garcia JG, Richards TJ, Silveira LJ, Lindell KO, Steele MP, Loyd JE, et al. Association between the MUC5B promoter polymorphism and survival in patients with idiopathic pulmonary fibrosis. *JAMA* 2013;309:2232–2239.
88. Hunninghake GM, Hatabu H, Okajima Y, Gao W, Dupuis J, Latourelle JC, Nishino M, Araki T, Zazueta OE, Kurugol S, et al. MUC5B promoter polymorphism and interstitial lung abnormalities. *N Engl J Med* 2013;368:2192–2200.
89. Maitra M, Cano CA, Garcia CK. Mutant surfactant A2 proteins associated with familial pulmonary fibrosis and lung cancer induce TGF- $\beta$ 1 secretion. *Proc Natl Acad Sci USA* 2012;109:21064–21069.
90. Tsakiri KD, Cronkhite JT, Kuan PJ, Xing C, Raghu G, Weissler JC, Rosenblatt RL, Shay JW, Garcia CK. Adult-onset pulmonary fibrosis caused by mutations in telomerase. *Proc Natl Acad Sci USA* 2007;104:7552–7557.
91. Yang IV, Schwartz DA. The next generation of complex lung genetic studies. *Am J Respir Crit Care Med* 2012;186:1087–1094.
92. Konishi K, Gibson KF, Lindell KO, Richards TJ, Zhang Y, Dhir R, Bisceglia M, Gilbert S, Yousem SA, Song JW, et al. Gene expression profiles of acute exacerbations of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2009;180:167–175.
93. Yang IV, Burch LH, Steele MP, Savov JD, Hollingsworth JW, McElvania-Tekippe E, Berman KG, Speer MC, Sporn TA, Brown KK, et al. Gene expression profiling of familial and sporadic interstitial pneumonia. *Am J Respir Crit Care Med* 2007;175:45–54.
94. Yang IV, Luna LG, Cotter J, Talbert J, Leach SM, Kidd R, Turner J, Kummer N, Kervitsky D, Brown KK, et al. The peripheral blood transcriptome identifies the presence and extent of disease in idiopathic pulmonary fibrosis. *PLoS ONE* 2012;7:e37708.
95. Zuo F, Kaminski N, Eugui E, Allard J, Yakhini Z, Ben-Dor A, Lollini L, Morris D, Kim Y, DeLustro B, et al. Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. *Proc Natl Acad Sci USA* 2002;99:6292–6297.
96. Oak SR, Murray L, Herath A, Sleeman M, Anderson I, Joshi AD, Coelho AL, Flaherty KR, Toews GB, Knight D, et al. A micro RNA processing defect in rapidly progressing idiopathic pulmonary fibrosis. *PLoS ONE* 2011;6:e21253.
97. Pandit KV, Milosevic J, Kaminski N. MicroRNAs in idiopathic pulmonary fibrosis. *Transl Res* 2011;157:191–199.
98. Milosevic J, Pandit K, Magister M, Rabinovich E, Ellwanger DC, Yu G, Vuga LJ, Weksler B, Benos PV, Gibson KF, et al. Profibrotic role of miR-154 in pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2012;47:879–887.
99. Sanders YY, Tollefsbol TO, Varisco BM, Hagood JS. Epigenetic regulation of thy-1 by histone deacetylase inhibitor in rat lung fibroblasts. *Am J Respir Cell Mol Biol* 2011;45:16–23.
100. Rabinovich EI, Kapetanaki MG, Steinfeld I, Gibson KF, Pandit KV, Yu G, Yakhini Z, Kaminski N. Global methylation patterns in idiopathic pulmonary fibrosis. *PLoS ONE* 2012;7:e33770.
101. du Bois RM, Weycker D, Albera C, Bradford WZ, Costabel U, Kartashov A, Lancaster L, Noble PW, Raghu G, Sahn SA, et al. Ascertainment of individual risk of mortality for patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2011;184:459–466.
102. Ley B, Ryerson CJ, Vittinghoff E, Ryu JH, Tomassetti S, Lee JS, Poletti V, Bucciolini M, Elicker BM, Jones KD, et al. A multidimensional index and staging system for idiopathic pulmonary fibrosis. *Ann Intern Med* 2012;156:684–691.
103. Wynn TA. Integrating mechanisms of pulmonary fibrosis. *J Exp Med* 2011;208:1339–1350.
104. Vij R, Noth I. Peripheral blood biomarkers in idiopathic pulmonary fibrosis. *Transl Res* 2012;159:218–227.
105. Zhang Y, Kaminski N. Biomarkers in idiopathic pulmonary fibrosis. *Curr Opin Pulm Med* 2012;18:441–446.
106. Lee JS, Ryu JH, Elicker BM, Lydell CP, Jones KD, Wolters PJ, King TE Jr, Collard HR. Gastroesophageal reflux therapy is associated with longer survival in patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2011;184:1390–1394.
107. Kahloon RA, Xue J, Bhargava A, Cszmadia E, Otterbein L, Kass DJ, Bon J, Soejima M, Levesque MC, Lindell KO, et al. Patients with idiopathic pulmonary fibrosis with antibodies to heat shock protein 70 have poor prognoses. *Am J Respir Crit Care Med* 2013;187:768–775.
108. Selman M, Carrillo G, Estrada A, Mejia M, Becerril C, Cisneros J, Gaxiola M, Pérez-Padilla R, Navarro C, Richards T, et al. Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern. *PLoS ONE* 2007;2:e482.
109. Micheel C, Ball J, editors. Evaluation of biomarkers and surrogate endpoints in chronic disease. Washington, DC: The National Academic Press; 2010.
110. Swigris JJ, Fairclough D. Patient-reported outcomes in idiopathic pulmonary fibrosis research. *Chest* 2012;142:291–297.
111. Raghu G, Collard HR, Anstrom KJ, Flaherty KR, Fleming TR, King TE Jr, Martinez FJ, Brown KK. Idiopathic pulmonary fibrosis: clinically meaningful primary endpoints in phase 3 clinical trials. *Am J Respir Crit Care Med* 2012;185:1044–1048.
112. du Bois RM, Nathan SD, Richeldi L, Schwarz MI, Noble PW. Idiopathic pulmonary fibrosis: lung function is a clinically meaningful endpoint for phase III trials. *Am J Respir Crit Care Med* 2012;186:712–715.
113. Olson AL, Swigris JJ, Brown KK. Clinical trials and tribulations—lessons from pulmonary fibrosis. *QJM* 2012;105:1043–1047.
114. Wells AU. Forced vital capacity as a primary end point in idiopathic pulmonary fibrosis treatment trials: making a silk purse from a sow's ear. *Thorax* 2013;68:309–310.