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New Insights into the Pathogenesis and Treatment of IPF: An Update

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Abstract

Idiopathic pulmonary fibrosis (IPF) is the most common and lethal of the idiopathic interstitial pneumonias. There are currently no effective pharmacological therapies approved for the treatment of IPF. Despite the focus on targeting fibrogenic pathways, recent clinical trials have been largely disappointing. Progress is being made in elucidating key cellular processes and molecular pathways critical to IPF pathogenesis, and this should facilitate the development of more effective therapeutics for this recalcitrant disease. Emerging pathobiological concepts include the role of aging and cellular senescence, oxidative stress, endoplasmic reticulum stress, cellular plasticity, microRNAs, and mechanotransduction. Therapeutic approaches that target molecular pathways to modulate aberrant cellular phenotypes and promote tissue homeostasis in the lung must be developed. Heterogeneity in biological and clinical phenotypes of IPF warrants a personalized medicine approach to diagnosis and treatment of this lung disorder.

I. INTRODUCTION

IPF is the most common form of idiopathic interstitial pneumonia affecting over 100,000 persons in the United States alone. It is a fatal lung disease with a median survival after diagnosis of only 3 years¹. IPF is a disease that affects older individuals and is slightly more prevalent among men². It is characterized histologically by the presence of usual interstitial pneumonia on surgical lung biopsy and clinically by the progressive loss of lung function with resultant dyspnea, non-productive cough and exercise limitation. There are currently no effective pharmacological therapies approved by the U.S. Food and Drug Administration (FDA) for IPF. Despite more recent efforts in developing anti-fibrotic therapeutic agents, the few drugs tested to-date have not proven to be clinically beneficial. Lung transplantation is often the only option available for patients with advanced IPF, and even this has its own limitations – it is expensive, available to only a selected minority, and median survival post-lung transplant is only about five years. There is an urgent need to identify effective anti-fibrotic agents for IPF. In this review, evolving concepts in IPF pathogenesis and emerging therapeutic targets for this lethal lung disease will be discussed.

II. CURRENT APPROACH TO TREATMENT OF IPF

Until effective drug treatments become available, the management of IPF patients is, for the most part, focused on measures to improve quality of life, preserve mobility and independence. Patients with IPF have diminished exercise capacity, increased

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breathlessness, depression, severe fatigue, diminished cognitive function, and diminished quality of life compared to the general population. Pulmonary rehabilitation has been shown to favorably impact many of these problems in the chronic obstructive pulmonary disease (COPD) population and is recommended for patients with IPF¹. However, large and well-designed prospective studies exploring the role of pulmonary rehabilitation in IPF are lacking. Hypoxemia is often associated with disease progression³; however, no conclusive studies have been performed on the effects of long-term oxygen therapy on survival. A number of small studies do suggest favorable impact of oxygen therapy on exercise capacity and quality of life⁴⁻⁵; patients with IPF and hypoxemia are prescribed supplemental oxygen. Cough can be quite disabling in IPF and it is important to recognize that many patients will have associated problems such as upper airway cough syndrome and gastroesophageal reflux (GER) that ought to be treated aggressively⁶. GER is prevalent in IPF and may be potentially related to disease progression⁷⁻⁹. Most physicians now recommend aggressive treatment of GER in IPF patients with an acid-reducing drug; however, well-designed studies are needed to elucidate whether acid suppression improves IPF outcomes⁹.

Sleep-disordered breathing is also common in IPF; it is often under-diagnosed and negatively impacts quality of life in IPF¹⁰. Sleep-disordered breathing can often be treated with non-invasive positive pressure ventilation, although no studies have defined the impact of this treatment in IPF. Patients with IPF have increased risk of acute coronary syndrome and deep vein thrombosis when compared to the general population¹¹. Furthermore, the concomitant presence of severe coronary artery disease and IPF may be related to worse outcomes¹². IPF patients should be managed aggressively with risk factor modifying strategies; coronary artery disease ought to be suspected in any IPF patient presenting with worsening dyspnea and/or deteriorating exercise capacity. Pulmonary hypertension is also common in IPF and may be associated with poor survival¹³⁻¹⁴; however, further studies are needed to define the best diagnostic approaches and treatment modalities in this population.

In summary, until effective drug therapies become available, IPF patients should be referred to tertiary care centers to be considered for clinical trials and lung transplantation. In addition to symptom control and quality of life management, one also has to consider the co-morbidities that may be associated with IPF. Most patients are appropriate candidates for pulmonary rehabilitation, and oxygen therapy should be offered for those with hypoxemia. GERD and risk factors for coronary artery disease should be treated aggressively. If sleep-disordered breathing is diagnosed, a trial of non-invasive positive pressure ventilation can be considered if no contraindications are found. Further studies are needed to define the best strategies to diagnose and treat pulmonary hypertension secondary to IPF.

III. WHAT WE HAVE LEARNED FROM IPF CLINICAL TRIALS

Since the late 1990s, when a more uniform definition of IPF was proposed¹⁵, more than 3,000 patients have been enrolled in clinical trials exploring novel therapies (Table 1). Most of these studies allowed the use of corticosteroids in the “placebo” arm. A small minority of these studies have met their primary endpoints, but none demonstrated survival advantage or meaningful clinical benefit in IPF. These studies have shed light, however, on the natural history of IPF. A detailed analysis of the placebo arm of a large multicenter, randomized placebo controlled study of interferon γ -1b in patients with moderate IPF demonstrated that lung physiology had minimal decline during the first 12 months following randomization¹⁶. In fact, only later studies that followed patients beyond 72 weeks observed mean declines in FVC around or beyond the 10% threshold considered to be associated with increased risk of mortality¹⁷⁻¹⁸. The same group of investigators also found that approximately half the deaths that occurred acutely were preceded by a period of rapid clinical deterioration that did not have an identifiable cause besides “disease progression”. Although there was an

overall trend towards worsening dyspnea and deterioration of physiological parameters just prior to one's death, there was enormous variability among patients and the initial FVC did not seem to predict these events. These findings emphasize the generally unpredictable course of IPF and the need for early referral to a tertiary care center for early lung transplant evaluation.

The IFIGENIA study that compared the effectiveness of one year therapy of the antioxidant, N-Acetylcysteine (NAC), in addition to combination therapy with prednisone and azathioprine is one of only three Phase III clinical trials in IPF that met its primary endpoint¹⁹. At the end of the study, patients on triple therapy had significantly less decline in vital capacity and diffusion capacity compared to patients who took only prednisone and azathioprine. The lack of a placebo-only control group and the significant rate of dropouts during the study have prevented the scientific community from embracing this therapy as standard of care in IPF. The NIH-sponsored IPF Clinical Research Network (IPFnet) is currently enrolling patients in a three-arm randomized and controlled study comparing triple therapy (prednisone, azathioprine, and NAC) to NAC alone and to placebo. This study (PANTHER-IPF) is expected to complete enrollment in the fall of 2011.

Kubo and collaborators conducted a prospective, randomized, non-blinded study of 64 patients with well-characterized IPF admitted to a hospital in Japan to evaluate potential for anticoagulation to improve mortality in IPF²⁰. All patients had demonstrated prior evidence of clinical deterioration, and were on corticosteroids at the time of enrollment. Patients were randomized to receive either anticoagulants ($n = 31$) plus corticosteroids or corticosteroids alone ($n = 33$). The anticoagulant regimen consisted of outpatient oral coumadin titrated to an INR 2–3 and intravenous low molecular weight heparin during hospitalizations for acute exacerbations. All patients also received intravenous methylprednisolone when hospitalized with acute respiratory deterioration related to IPF. However, corticosteroids were stopped whenever patients were hospitalized with either bacterial infection or heart failure. The group overall had relatively mild physiological impairment (forced vital capacity = 70 % of predicted; $D_LCO = 60\%$ of predicted). As 8/31 subjects withdrew in the anticoagulant group, the analyzed cohort constituted a group of 56 patients, 23 of whom received anticoagulants. There was a significant improvement in survival in the anticoagulant-treated group (hazard ratio of 2.9 – 95% CI 1.0 – 8.0, $p = 0.04$). Furthermore, the mortality associated with the episodes of acute exacerbation was also significantly reduced in the anticoagulation-treated group (18% vs. 71%, $p = 0.008$)²⁰. This clinical trial provides us with initial evidence of a possible survival benefit of anticoagulation in human IPF; however, some concerns regarding the methodology have been raised. This study enrolled patients at the time of their hospitalization and most subjects did not have advanced disease, making it more difficult to explain why survival in the control group was much lower than reported in other recent IPF clinical trials. Furthermore, an intent-to-treat analysis of the whole randomized patient cohort might have been more appropriate. The results of this study are certainly exciting and promising, but warrant confirmation with a larger, multicenter, randomized and placebo-controlled clinical trial before the use of anticoagulation can be recommended as standard of care for IPF patients. The IPFnet is currently enrolling patients in a study (ACE-IPF) comparing warfarin to placebo for patients with IPF. The network expects to complete enrollment for ACE-IPF by the end of 2011.

Pirfenidone is a molecule believed to have antifibrotic effect through its anti-inflammatory and anti-fibrotic effects. In 2005, Azuma and collaborators published a clinical trial in which 107 IPF patients were randomized to receive either pirfenidone or placebo²¹. Although this study failed to reach its primary endpoint (change in lowest oxygen saturation by pulse oximetry between baseline and 9 months during a 6-minute exercise test), the number of individuals with improved or stable vital capacity and total lung capacity was greater in the

pirfenidone group than the placebo group. The study was stopped earlier by the DSMB because more patients in the placebo group experienced acute exacerbations of IPF²¹. These promising findings led to three subsequent large clinical trials on pirfenidone. Taniguchi and collaborators randomized 275 Japanese IPF patients to either 1,800 mg or 1,200 mg of pirfenidone, or placebo²². Patients randomized to the higher dose group had significantly lower decline in vital capacity and a similarly significant difference was seen in progression free-survival, which was a secondary endpoint²². A major methodological concern is that the primary endpoint was changed after the trial was started; the handling of missing data may have also magnified the treatment effect of pirfenidone. In the U.S., two large clinical trials of pirfenidone were recently concluded. Both studies recruited patients with mild to moderate IPF and were powered with “change in lung function over time” as the primary endpoint. PIPF 004 (www.clinicaltrials.gov: NCT00287716) randomized 435 patients in a 2:2:1 model to a higher dose group, a placebo group, or a lower dose group. PIPF 006 (www.clinicaltrials.gov: NCT00287729) enrolled 344 patients in a 1:1 model to either a high dose group or a placebo group. Although PIPF 004 did meet its primary endpoint, PIPF 006 did not. The data from the above studies have been presented to the scientific community in the form of an abstract¹⁸, and it is available to the public for review at the FDA website (<http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/Pulmonary-AllergyDrugsAdvisoryCommittee/UCM206399.pdf>). Although promising, the role of pirfenidone for the treatment of IPF remains uncertain.

Many challenges lie ahead, as future clinical trials in IPF are anticipated. The scientific community and regulatory authorities ought to agree on which endpoints are meaningful. Clinical trials powered around a mortality end-point require larger numbers of patients, longer periods for enrollment and follow up, and carry increased cost. Other endpoints such as change in lung function over time are felt by many to be adequate surrogates of mortality, but are yet to be rigorously validated.

IV. RISK FACTORS

A. Inhalational Exposures

IPF is a disease process that appears limited to the lungs, without clear evidence at the current time that other organs systems are involved. Thus, it seems intuitive that the injurious agent or antigen may have been transmitted to the lung *via* the inhaled route. Many studies have looked at the possible role of inhalational exposures and risk of developing IPF. Most of the studies are case-control studies and suggest associations rather than causality. Additionally, most of these studies were reported prior to the 2002 joint American Thoracic Society and European Respiratory Society consensus statement on the classification of idiopathic interstitial pneumonias¹, which raises concerns regarding the reliability of the diagnoses.

Inhalational exposures to metal or wood dust and smoking have been most studied. There are several case-control studies in patients with a diagnosis of IPF that demonstrated increased odds ratios for developing IPF with exposure to metal dust, with odds ratios ranging from 1.34 to 10.97^{23–28}. Case-control studies also suggest a link between exposure to wood dust and risk of IPF^{23–24, 26–27}. Only one of these studies was able to show a statistically significant association²⁴. Another study showed a significant association between wood burning stoves in the home and IPF²³. More recently, a study of Swedish subjects with exposure to birch dust or hardwood dust demonstrated an elevated risk of developing IPF²⁹. In a United States study, subjects who worked in wood buildings had increased risk of developing IPF²⁸. Other occupational exposures that have reported as

possibly associated with IPF include agriculture and farming^{25–26}, livestock^{23, 26}, textile dust²⁴, and exposures to sand, stone and silica^{24, 26, 30–31}.

There is considerable evidence that cigarette smoking is associated with a risk of developing IPF. Six case-control studies have shown a positive association between ever having smoked cigarettes and the development of IPF^{24, 26–27, 32–33}, with a combined odds ratio of 1.58³⁴. Additionally, a recent study from Mexico demonstrated that former smoking history is associated with IPF³⁵. It has also been demonstrated that those IPF patients with a smoking history have decreased survival when compared to IPF patients without a smoking history³⁶. Two different studies suggest that current smokers may have a better outcome compared with former smokers, which may reflect less severe disease at presentation and may represent a healthy smoker effect^{36–37}. While there is substantial epidemiologic evidence that suggests associations between inhalational exposures, there are significant numbers of IPF patients with no occupational exposure history and who have no smoking history.

B. Gastroesophageal Reflux

There is accumulating evidence of an association between gastroesophageal reflux (GER) and IPF. Multiple epidemiologic studies demonstrate a high prevalence of GER in patients with IPF^{7–8, 38–39}. Initially, Tobin et al⁷ demonstrated that almost all patients with IPF in their cohort had GER, whereas only 50% in the control cohort of patients with other interstitial lung diseases had GER. Raghu et al⁸ were able to demonstrate that 87% of IPF patients vs. 68% of asthma patients studied had GER. In both of these studies, a majority of the patients with GER did not have the typical symptoms of GER^{7–8}. More recently, it was demonstrated that systemic sclerosis patients with interstitial lung disease (ILD) had more severe GER and more proximal esophageal reflux when compared to systemic sclerosis patients without ILD⁴⁰. It should be recognized, however, that most end-stage lung disease are associated with a high prevalence of GER^{41–42}, and a cause-effect relationship remains unclear. The most compelling evidence for a role in GER in the pathogenesis in IPF is a published case series in which four patients with IPF and GER who were treated only for their reflux disease had stabilization or improvement in their clinical parameters⁹. Two of these patients had deterioration in pulmonary function tests and symptoms when non-compliant with anti-reflux measures that again stabilized with treatment of their reflux⁹. While this alone does not definitively link IPF and GERD, it does warrant significant consideration for GERD as a contributor to the progression of this disease, at least in a subgroup patients.

C. Diabetes Mellitus

There is epidemiologic evidence that supports an association between IPF and diabetes mellitus. In a case control study in Japan, a higher prevalence of diabetes was observed in IPF patients compared to the control group (odds ratio of 4.06; CI, 1.8–9.15)³². In another case control study, the use of insulin was associated with the diagnosis of IPF³⁹. In a more recent study from Mexico, diabetes was identified as an independent risk factor associated with IPF³⁵. The potential mechanism(s) by which diabetes might contribute to the risk of developing IPF is unknown. It has been shown that there is accumulation of advanced glycation end-product (AGE) modified proteins in alveolar macrophages in IPF patients⁴³, and that blocking AGE formation can prevent bleomycin-induced pulmonary fibrosis in a mouse model⁴⁴. However, other studies have suggested that the receptor for AGE, RAGE, might be protective from pulmonary fibrosis. IPF patients have decreased levels of RAGE in lung tissue and on type II alveolar epithelial cells^{45–46}, and RAGE-null mice have more severe pulmonary fibrosis in response to asbestos injury⁴⁵. Further studies are warranted to elucidate the precise relationship between diabetes and increased the risk of IPF.

D. Viral Infection

There has been growing interest in viral infections as a potential factor in the etiology of pulmonary fibrosis, in acute exacerbations, or in the progression of disease. The herpesviruses, and in particular Epstein Barr Virus (EBV), cytomegalovirus (CMV) and Kaposi's sarcoma-associated herpesvirus (KSHV), have received the most attention. The detection of herpesvirus in the lungs of IPF patients has been reported by multiple groups. In the United Kingdom, it has been shown that EBV can be detected in lung tissue by immunohistochemistry and PCR in 40–60% of IPF patients and in 0–4% of control patients^{47–49}. In the United States, Tang et al⁵⁰, demonstrated that 97% of IPF lung specimens had evidence of EBV, CMV, KSHV or human herpesvirus 7 (HHV-7) while only 36% of control lung specimens had evidence of one of these viruses; in this study, 57% of the IPF cases compared to only 8% of the control cases had evidence of two or more of these viruses. These authors also presented a case report of a patient with familial IPF who had evidence of EBV infection, and whose pulmonary function tests stabilized with anti-viral therapy⁵⁰. More recent studies report that EBV, CMV or KSHV was detected in the alveolar epithelium of 65% of lung specimens from IPF patients and in none of the control specimens⁵¹. Other studies report a failure to find evidence for herpesviral infection in IPF patients. Hayakawa et al⁵², in Japan, and Zamo et al⁵³, in Italy, were unable to detect herpesvirus DNA in any of the IPF and control lung specimens that they studied.

Animal models of fibrosis support the notion that herpesviruses may participate in the fibrotic process. It has been demonstrated that in Th2-biased mice, murine gammaherpesvirus-68 (a strain that is similar to EBV and KSHV in humans) infection induces progressive pulmonary fibrosis⁵⁴. It has also been demonstrated that this same virus can exacerbate established fluoroscein isothiocyanate (FITC)-induced pulmonary fibrosis in mice⁵⁵. Further studies are required to investigate the precise role(s) that these viruses play in lungs of patients with IPF.

V. EMERGING NEW CONCEPTS IN PATHOGENESIS OF IPF

Steady progress has been made in our understanding of IPF pathogenesis over the last several years. Concepts on disease pathogenesis have evolved from chronic inflammation⁵⁶, to aberrant wound healing⁵⁷, to current paradigms of a multi-factorial and heterogeneous disease process in which cellular senescence, oxidative stress, endoplasmic reticulum stress, cellular plasticity, mechanotransduction, and the role of epigenetic mechanisms involving micro-RNAs play critical roles. In the genetically/epigenetically susceptible host, exposure to injurious agents may result in phenotypic alterations of structural cells of the lung that leads to aberrant epithelial-mesenchymal interactions and eventually to fibrosis. IPF can thus be viewed as a fundamental problem of altered tissue homeostasis and aberrant communication between cells of the lung (Figure 1).

A. Aging and Cellular Senescence

The diagnosis of IPF is rarely made in patients less than forty years of age and both the incidence and prevalence of IPF markedly increase with advancing age². Further, the survival rate for IPF patients decreases with age^{37, 58}. Age-related diseases may be associated with telomere dysfunction⁵⁹, and shortening of telomeres leads to reduced capacity for stem cell renewal and cellular senescence⁶⁰. Recent studies of familial and sporadic cases of IPF have been associated with telomere shortening^{61–63}, suggesting that IPF may represent a degenerative disease process associated with advancing age⁶⁴. The mechanisms of stem cell renewal, differentiation, senescence, and other fates of the different cell types involved in lung fibrogenesis require further study. Few animal studies have evaluated the role of aging on fibrosis, although a study published in 1984 suggested that

chronic cigarette smoke exposure in older mice, in contrast to young mice, produced morphologic and functional changes suggestive of pulmonary fibrosis⁶⁵. This suggests that age-dependent changes in pulmonary physiology, which occur even in the absence of disease, may contribute to susceptibility of the lungs to injury and/or a predisposition to fibrosis. Understanding age-dependent changes in lung physiology and the role of aging on lung injury/repair responses will provide novel insights into the pathogenesis of IPF and potential targets for therapeutic intervention.

B. Oxidative Stress

Oxidative stress is defined as an imbalance of the generation of reactive oxygen species (ROS) in excess of the capacity of cells/tissues to detoxify or scavenge them. Such a state of oxidative stress may alter the structure/function of cellular macromolecules that eventually leads to tissue/organ dysfunction. There is a substantial and growing body of evidence indicating that oxidative stress is not only a pathological feature of IPF, but that it likely plays an important role in the development of fibrosis in multiple organ systems^{66–67}.

Lung tissue from IPF patients demonstrate “signatures” of chronic oxidative damage^{66, 68–70}. Although ROS generation may be involved in a number of normal physiological functions, overproduction of ROS may lead to oxidative stress that alter cellular phenotypes and cause tissue damage over time⁷¹. Oxidative stress can influence cellular phenotypes and fates, including senescence and apoptosis, which may promote a tissue microenvironment that favors fibrosis over regeneration. For example, increased oxidative stress leads to premature induction of cellular senescence^{72–73}, and fibroblasts acquire an apoptosis-resistance phenotype as a consequence of senescence^{74–75}. Senescent fibroblasts stop replicating, yet they remain metabolically active and generate higher levels of ROS⁷⁶. Lung myofibroblasts secrete hydrogen peroxide (H₂O₂), which may mediate fibrogenic effects in tissues by inducing epithelial cell apoptosis by a paracrine mechanism⁷⁷, or by inducing matrix crosslinking reactions in the presence of extracellular heme peroxidases⁷⁸. Although there is an established role for oxidative stress in mediating various cellular behaviors that influence tissue homeostasis and the outcome of repair/regeneration, age-dependent oxidative changes may provide a positive-feedback mechanism for perpetuating a pro-fibrotic tissue microenvironment.

C. Endoplasmic Reticulum Stress

Endoplasmic reticulum (ER) stress of alveolar epithelial cells has been implicated in IPF. ER is a membrane-enclosed intracellular organelle that serves as the site for protein folding, processing, and trafficking. Accumulation of improperly folded proteins in ER induces ER stress in the form of the adaptive response, called unfolded protein response (UPR)⁷⁹. Many proteins are involved in the UPR, including the ER chaperone Grp78 (BiP), PKR-like ER kinase (PERK), ATF-4, and ATF-6⁷⁹. UPR tends to limit the *de novo* entry of proteins into the ER and facilitate both ER protein folding and degradation, allowing protection from cell death. However, when protein misfolding is persistent or excessive, ER stress triggers cell death, typically apoptosis through the Bcl-2 signaling pathway⁷⁹. ER stress has been found in lung samples from both familial IPF and sporadic IPF lungs^{51, 80}. Epithelial cells in IPF lungs show significant staining of BiP, EDEM, and XBP-1 when compared to control lung tissues, suggesting ER stress in the epithelial cells in IPF⁵¹. Expression of pro-SP-C mutant protein in A549 cells increases BiP expression, demonstrating the presence of ER stress in these cells due to improper folding of the pro-SP-C mutant protein⁵¹. In another study, expression of p50ATF-6, ATF-4, and CHOP are increased in sporadic IPF lungs when compared to control lung tissues, further supporting that ER stress exists in IPF lungs⁸⁰. Importantly, the apoptotic signaling is increased in IPF in lung tissue when compared to donor control lungs⁸⁰, suggesting a connection between ER stress and lung epithelial cell

death. Although viral infection and oxidative stress have been proposed as potential mechanisms for ER stress in IPF^{51, 80}, further investigation is needed to understand the exact molecular mechanisms leading to ER stress in IPF.

D. Cellular Plasticity

There is growing recognition for expanded plasticity of somatic cells, even those thought to be terminally differentiated. This “plasticity” typically occurs under certain regenerative, reparative, and fibrotic conditions, and involves a number of cell types, including epithelial, endothelial, and mesenchymal cell types.

1. Epithelial-Mesenchymal Transition (EpMT)—Recent *in vivo* studies demonstrate that fibroblasts can be derived from lung epithelium during the development of lung fibrosis in animal models. EpMT is a process by which cell lose epithelial markers/phenotype and acquire mesenchymal characteristics^{81–82}. Animal models support that EpMT contributes to the accumulation of fibroblasts in lung fibrosis^{83–84}. Loss of expression of E-cadherin and surfactant-protein C, and gain of expression of α -smooth muscle actin (α -SMA) and S100A4, have been observed in fibrotic lungs induced by overexpression of transforming growth factor (TGF)- β 1⁸³ or bleomycin administration⁸⁴. In the latter study, about one-third of the S100A4-positive fibroblasts were derived from lung epithelium two-weeks after bleomycin treatment; in contrast, few S100A4-positive cells were found in lungs in untreated mice⁸⁴. *In vitro* studies indicate that transforming growth factor β 1 (TGF- β 1) in combination with epidermal growth factor (EGF) can induce EpMT; E-cadherin expression is lost, while S100A4 expression is gained in type II alveolar epithelial cells treated with both TGF- β 1 and EGF⁸⁴. Interestingly, few epithelium-derived S100A4-positive fibroblasts in the bleomycin-induced lung fibrosis model are α -SMA-positive⁸⁴, while a subset of epithelium-derived fibroblasts in the TGF- β 1 overexpression model of lung fibrosis model are α -SMA-positive⁸³. These observations suggest that different cellular phenotypes of fibroblasts and myofibroblasts co-exist during the development of lung fibrosis and contribute to varying degrees in different animal models of lung fibrosis.

The role and significance of EpMT in the pathogenesis of human IPF remains unclear. Co-expression of the myofibroblast marker (α -SMA) and alveolar epithelial cell marker (pro-surfactant protein B) have been observed in cells lining alveolar spaces in human IPF lung tissues⁸². Immunohistochemical staining of IPF lung tissues also support a role for Wnt/ β -catenin signaling in IPF⁸⁵. Laminin-5- γ 2 chain is associated with increased cell motility and its expression is mainly restricted to basal cell sheets located between luminal bronchiolar cells and myofibroblast clusters of fibroblast foci in human IPF lungs, suggesting that abnormal proliferation and migration of bronchiolar basal cells have a role in the remodeling process characterizing IPF⁸⁶. The molecular mechanisms and cellular phenotypes/fates of epithelial cells with apparent expanded cell plasticity during fibrogenesis warrants further investigation.

2. Endothelial-Mesenchymal Transition (EnMT)—*In vivo* data from the bleomycin-induced lung fibrosis model in mice indicate that fibroblasts in fibrotic lungs can be derived from lung capillary endothelial cells, through EnMT-like process⁸⁷. EnMT accounted for approximately 16% of lung fibroblasts isolated from bleomycin-treated mice, and only about 15% of these endothelial-derived fibroblasts are α -SMA positive⁸⁷.

3. Fibroblast Heterogeneity and Plasticity—Fibroblasts likely play key regulatory functions in normal homeostasis of the adult lung^{88–89}, although their roles in ECM synthesis and remodeling are better appreciated. Fibroblasts are perhaps best defined by their elongated spindle-like morphology, their varied functions and plasticity. To date, no

“fibroblast-specific” marker has been identified. In most cases, the absence of more specific markers of other cell types and a combination of mesenchymal markers are used. Fibroblasts are known to be highly heterogeneous with respect to their cytoskeletal protein expression and architecture; the expression of myogenic or smooth muscle cell markers, such as α -SMA identify a subset of myofibroblasts that are key effectors of tissue contraction and architectural remodeling characteristic of most chronic fibrotic disorders⁹⁰. Fibroblast heterogeneity is also observed with respect to the expression of specific cell surface glycoproteins^{91–92}. Thy-1 is a lipid raft glycoprotein without a cytoplasmic tail⁹³. Published data demonstrate that Thy-1(–) and Thy-1(+) lung fibroblasts have different roles during lung fibrosis^{92, 94}. Thy-1 is involved in the regulation of myofibroblast differentiation and survival, and the latent TGF- β activation^{92, 94}. Importantly, Thy-1 deficiency promotes lung fibrosis in the bleomycin-induced lung fibrosis model in mice, suggesting that Thy-1 expression may limit fibrotic responses to tissue injury⁹¹.

E. MicroRNAs in Matrix Remodeling and Fibrosis

MicroRNAs (miRNAs) are 21–22 nucleotide non-coding small RNAs⁹⁵. The expression of miRNAs is often modulated by transcriptional factors in response to cellular stimuli. The primary transcripts of miRNAs (pri-miRs) are processed by the nuclear RNaseIII Drosha to form 70–80 nt precursor miRNAs (pre-miRs). Pre-miRs are then exported to the cytoplasm, where they are processed by Dicer into mature miRNAs. Mature miRNAs, together with Dicer and Argonaute proteins, form the miRNA induced silencing complex (miRISC). Guided by miRNA through base pairing, the miRNA complex binds to the 3' UTR of target genes and thereby represses translation of target genes and/or induces degradation of target gene mRNA⁹⁵.

A number of recent studies have demonstrated that miRNAs play an important role in maintaining the homeostasis of extracellular matrix. Aberrant expression of miRNAs has been linked to fibrotic diseases in various organs^{96–100}. In the heart, miR-133 expression is reduced during nicotine-induced atrial fibrosis and exogenous miR-133 expression in cultured atrial fibroblasts decreases levels of TGF- β 1 and TGF- β 2 and production of collagen¹⁰¹. Another study showed that both miR-133 and miR-30 regulate the expression of connective tissue growth factor (CTGF)¹⁰². In this study, downregulation of miR-133 and miR-30 increases the CTGF expression in cultured cardiomyocytes and fibroblasts; furthermore, overexpression of miR-133 or miR-30c decreased CTGF levels, which was accompanied by decreased production of collagens¹⁰². miR-133 and miR-30 directly target CTGF through conserved binding sites within 3' UTR of CTGF mRNA¹⁰². miR-29 family members are downregulated in the region of the heart adjacent to myocardial infarct; downregulation of miR-29 induces the expression of collagens, whereas over-expression of miR-29 in fibroblasts reduces collagen expression⁹⁶. Recent studies by Cushing et al¹⁰³ demonstrate that miR-29 is downregulated in lungs of mice during fibrotic remodeling following bleomycin injury. In this study, miR-29 was found to be suppressed by TGF- β 1 in lung fibroblasts and many of the fibrosis-associated genes upregulated by TGF- β 1 were derepressed by miR-29 knockdown. miR-29 also appears to regulate other fibrosis-associated genes, independent of TGF- β 1¹⁰³, suggesting that strategies to induce expression of miR-29 in fibrotic lung fibroblasts may be effective.

miR-21 is one of the most studied miRNAs and has been shown to be involved in carcinogenesis, inflammation and cardiovascular diseases^{104–106}. miR-21 can activate the extracellular signal-regulated kinase (ERK) through targeting the sprouty homologue 1 (Spry1) in cardiac fibroblasts, and thereby contribute to cardiomyocyte hypertrophy and cardiac fibrosis⁹⁷. miR-21 also regulates the expression of metalloprotease-2 in cardiac fibroblasts¹⁰⁷, representing another mechanism by which miR-21 regulates cardiac remodeling. miR-216a and miR-217 regulate Akt activation through downregulation of

phosphatase and tensin homologue, an inhibitor of Akt activation¹⁰⁸. Overexpression of miR-17 decreases cell adhesion, migration and proliferation, and overexpressing miR-17 directly downregulates fibronectin and induces overall growth retardation in transgenic mice¹⁰⁹.

Recent evidence also support that miRNAs can regulate cellular plasticity. miR-145 expression affects fibroblast differentiation into a smooth muscle cell phenotype. Inhibition of miR-145 with antisense oligonucleotide blocks α -SMA expression¹¹⁰, suggesting that miRNAs may regulate (myo)fibroblast plasticity. However, it is currently unknown if miR-145 is expressed in the lungs or whether its expression is altered in lung fibrosis.

Kaminski and colleagues first reported dysregulation of miRNA expression in the lungs of IPF patients⁹⁸. They found a number of miRNAs upregulated, while others were downregulated in IPF lungs⁹⁸. One of the downregulated miRNAs, let-7d, regulates epithelial-mesenchymal transition in alveolar epithelial cells by targeting a transcriptional factor, HMGA2. Let-7d expression is downregulated by TGF- β 1 and participates in TGF- β 1-induced EMT. More importantly, HMGA2 expression is remarkably decreased in IPF lungs and inversely correlates with the levels of mesenchymal cell markers⁹⁸. These data argue strongly for a role of miRNAs in the pathogenesis of IPF. miR-155 expression was found to be significantly increased in the lungs of mice with bleomycin induced lung fibrosis. miR-155 targets the angiotensin II type 1 receptor [AT(I)R] and KGF, suggesting that miR-155 may participate in the pathogenesis of lung fibrosis^{111–112}. miR-21 expression is markedly increased in the lungs of mice with bleomycin induced pulmonary fibrosis and in the lungs of IPF patients⁹⁹. The enhanced expression of miR-21 is primarily localized to lung fibroblasts, and TGF- β 1 was found to upregulate miR-21 expression in human lung fibroblasts⁹⁹. Overexpression of miR-21 promotes, whereas downregulation of miR-21 diminishes TGF- β 1 induced expression of ECM proteins. These data suggests that miR-21 regulates the profibrotic effects of TGF- β 1 signaling, at least in part, by targeting the inhibitory Smad protein, Smad7⁹⁹. This supports the possibility that miR-21 acts in a feed-forward loop to amplify TGF- β 1 effects and promote lung fibrosis.

miR-200 family members inhibit epithelial-mesenchymal transition and migration in mammary epithelial cancer cells by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. The level of miR-200 family is decreased during EMT in mammary epithelial cancer cells in response to TGF- β 1^{113–115}. However, roles for miR-200 family members specifically in pulmonary fibrosis have not been demonstrated.

F. Mechanotransduction

The biophysical properties of the ECM may regulate cellular plasticity and fate *via* integrin signaling¹¹⁶ or growth factor activation¹¹⁷. Excessive TGF- β 1 production/activation is central to the pathogenesis of IPF. TGF- β 1 is initially secreted as an inactive latent complex (termed the small latent TGF- β , SLC) that consists of an N-terminal latency-associated peptide (LAP) and a C-terminal active TGF- β domain. The association of LAP prevents active TGF- β from binding to its receptors for biological functions^{118–120}. Most cells secrete TGF- β as a large latent complex in which the SLC binds to a second protein, latent TGF- β binding protein (LTBP)^{121–122}. The binding of LTBPs (LTBPs-1, 3 and 4) facilitates TGF- β secretion, deposition into the ECM and latent complex activation^{123–124}. Both *in vivo* and *in vitro* studies have demonstrated that latent TGF- β 1 activation, a process that results in the release/exposure of active TGF- β 1 from the latent complex, is a crucial step in lung fibrosis^{125–127}. Active TGF- β 1 promotes epithelial cell apoptosis, stimulates fibroblast proliferation, migration and differentiation into myofibroblasts, induces synthesis of ECM proteins and inhibits their degradation, and prevents myofibroblast apoptosis^{128–131}.

Recent studies demonstrate that mechanical forces induce latent TGF- β 1 activation^{117, 132–133}. Lung myofibroblasts acquire contractile activities by formation of α -SMA-containing stress fibers. Myosin-produced contractile forces transmit to the ECM through transmembrane integrins, primary integrin $\alpha_v\beta_5$ ¹¹⁷. The latent TGF- β 1 complex binds to integrin β_5 subunits with an Arg-Gly-Asp (RGD) motif and matrix fibronectin or fibrillin-1 with LTBP-1^{134–136}. Myofibroblast stress fiber-generated forces transmit to the latent TGF- β 1 complex, causing a conformational change that releases or exposes active TGF- β 1 portion. Since active TGF- β 1 is sufficient for myofibroblast differentiation, it is plausible that myofibroblast contraction-induced latent TGF- β 1 activation may be a driving force for persistence of myofibroblasts in IPF. It has been demonstrated that integrin $\alpha_v\beta_6$ -mediated epithelial TGF- β 1 activation is critical for bleomycin-induced lung fibrogenesis¹²⁵. Mechanisms underlying $\alpha_v\beta_6$ -mediated TGF- β 1 activation are not fully understood. Evidence that $\alpha_v\beta_6$ -mediated latent TGF- β 1 activation is proteinase-independent¹²⁵, is augmented by Rho/Rho-kinase activation¹³⁷, and that truncated β_6 lacking cytoplasmic domains prevents latent TGF- β 1 activation¹³⁸ suggest that cytoskeleton-dependent traction forces might mediate $\alpha_v\beta_6$ -mediated latent TGF- β 1 activation.

The activation of ECM-bound latent TGF- β 1 activation by fibrogenic lung fibroblasts lacking a GPI-linked cell surface glycoprotein Thy-1 requires LTBP-4^{94, 139}. LTBP-4 presents latent TGF- β 1 in a soluble form which facilitates matrix metalloproteinase-mediated latent TGF- β 1 activation⁹². Thus, LTBP-4 may be required for latent TGF- β 1 activation by fibrogenic lung fibroblasts and lung fibroblast-to-myofibroblast differentiation, whereas myofibroblast contraction-induced latent TGF- β 1 activation may be more important in the maintaining myofibroblastic phenotype in persistent lung fibrosis.

VI. EMERGING APPROACHES TO TREATMENT OF IPF

A. Protein Kinase Inhibitors

Focal adhesion kinase (FAK) and Src kinases are non-receptor tyrosine kinases. FAK plays an important role in mediating cell migration, proliferation, and differentiation.¹⁴⁰ FAK activation is necessary for fibroblast-to-myofibroblast differentiation^{141–142}. Inhibition of FAK activation, by expression autophosphorylation-deficient mutant¹⁴¹, a negative FAK regulator (FAK-related non-kinase, FRNK)^{142–143}, or by using pharmacologic inhibitors^{141, 143} abrogates TGF- β 1-induced myofibroblast differentiation in human lung fibroblasts. FAK contributes to the pro-survival signaling and promotes an anoikis-resistant phenotype of myofibroblasts in response to TGF- β 1¹⁴⁴. Recent observations indicate that FAK activity is increased in lung fibroblasts derived from IPF patients, while the expression of the endogenous FAK inhibitor, FRNK, is decreased in the same IPF lung fibroblasts¹⁴³. FAK inhibitors have been studied in tumor cells and in animal tumor models with promising results on inhibition of tumor growth, progression, and metastasis; this has led to ongoing clinical trials of FAK inhibitors in multiple cancers¹⁴⁰. FAK and Src can reciprocally activate each other¹⁴⁵, and Src kinases appear to regulate fibroblast activation in systemic sclerosis¹⁴⁶. Given the findings that Src/FAK play an essential role in myofibroblast differentiation and survival, inhibition of Src and/or FAK signaling through either pharmacologic inhibitors or upregulation of FRNK may prove to be an effective therapeutic strategy for the treatment of IPF¹⁴⁷.

Mitogen activated protein kinases (MAPKs) are involved in TGF- β -induced signaling and responses^{142, 148–149}. TGF- β 1 can activate the MAPK signaling cascade, and activated MAPK signaling is required for TGF- β 1-induced protein expression, including collagen and other extracellular matrix proteins^{142, 148–149}. The three classical MAPKs, extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinases/stress-activated protein

kinase (JNK/SAPK), are involved in the regulation of Smad proteins and TGF- β -induced myofibroblast differentiation^{142, 150–151}. Inhibition of ERK and p38 activation by FRNK overexpression inhibits myofibroblast differentiation induced by TGF- β 1¹⁴². Pharmacologic inhibitors of JNK and p38 MAPKs have been used to inhibit collagen production in cells *in vitro*, and inhibit the development of fibrosis in animal models. p38 MAPK inhibitors are being evaluated in phase II clinical studies in several diseases, including rheumatoid arthritis, psoriasis, and COPD. The potential utility and safety of MAPK inhibitors in fibrotic disorders require further study.

B. MicroRNAs

Due to their small size, miRNAs can be modified for *in vivo* administration. Blocking miRNA activities *in vivo* has been achieved in a number of studies using oligonucleotide probes with different chemical modifications to enhance their stability, such as locked nucleotide acid (LNA) and 2'-OMe sugar modifications^{97, 152}. Oligonucleotide probes have sequences complementary to specific miRNAs and irreversibly bind to target miRNAs. Expressing a mature miRNA *in vivo* has been more difficult due to the instability of single-stranded RNAs; employing viral vectors and direct intravenous administration of miRNA mimetics have been reported^{153–154}.

Given the important roles of miRNAs in the regulation of ECM homeostasis and organ fibrosis, including IPF, they are appealing targets for the design of novel therapeutics. In recent studies of lung fibrosis, LNA-modified anti-miR-21 probes were employed to block miR-21 activity in a murine model of injury-repair. When given intratracheally, the anti-miR-21 probes effectively sequestered mature miR-21, and significantly diminished bleomycin-induced pulmonary fibrosis in mice⁹⁹. This is the first example of targeting miRNAs to experimentally treat pulmonary fibrosis. In another study, Kaminski and colleagues showed that downregulating let-7d by antagomirs induces EpMT in mouse lungs⁹⁸. Given that let-7d is downregulated in the lungs of IPF patients, augmentation of let-7d in the lung may prove effective. In spite of the early successes of miRNA modulation in treating a variety of diseases, including pulmonary fibrosis, cell type- and organ-specific targeting has not been achieved and unexpected side effects may occur.

C. Targeting Myofibroblast Contractility

Myofibroblasts are key effector cells for the connective tissue remodeling characteristic of IPF. Persistent myofibroblast differentiation/survival may play a central role in the pathogenesis of IPF. Targeting the myofibroblast contractile phenotype represents a promising new strategy for developing therapeutic interventions for IPF.

Myofibroblasts are specialized contractile cells that produce contractile forces with α -SMA-containing microfilament bundles or stress fibers. The finding that myofibroblast-produced contractile forces are transmitted to fibrogenic signals by induction of latent TGF- β 1 activation suggests that blocking myofibroblast force generation or targeting the mechanotransduction machinery associated with latent TGF- β 1 activation represents a potential anti-fibrotic strategy. Myofibroblast force generation is mainly regulated by myosin light chain (MLC) phosphorylation, a process that is controlled by the opposing activities of myosin light chain phosphatase (MLCP) and myosin light chain kinase (MLCK). Regulation of MLCP activity has been proposed as the primary mechanism for sustained myofibroblast contraction¹⁵⁵. Rho/Rho kinase (ROCK) pathway plays an important role in regulation of myofibroblast force formation. Activated Rho kinase (ROCK) inactivates myosin phosphatase by phosphorylating the myosin-binding subunit of this enzyme complex which results in increased MLC phosphorylation and myofibroblast contraction. Conversely, inhibition of ROCK promotes myosin phosphatase activity which

decreases MLC phosphorylation and inhibits myofibroblast contraction^{156–157}. ROCK inhibitors are a newly developed class of drugs with potential therapeutic benefits in treating numerous pathologic processes including tissue fibrosis^{158–161}. The ROCK inhibitor, Y-27632, has been shown to inhibit tubulointerstitial kidney fibrosis and liver fibrosis in rodent models^{162–163}. Another ROCK inhibitor, fasudil, attenuates hypertensive glomerulosclerosis in Dahl salt-sensitive rats¹⁶⁴. SLX-2119, a newly developed ROCK inhibitor, reduces fibroblast stress fiber formation in radiation-induced intestinal fibrosis¹⁶⁵. Inhibition of myofibroblast contractile forces by ROCK inhibitors may represent an effective mechanism-based therapeutic intervention for developing drugs for the treatment of IPF. In addition, it has been shown that α -SMA expression and its incorporation into the stress fibers increase myofibroblast contractile forces¹⁶⁶. Delivery of AcEEED, a sequence specific to N-terminal α -SMA (SMA-FP), significantly reduces myofibroblast contractility¹⁶⁷.

Transmembrane integrins are an important mechanotransducer on the cell surface. Integrin $\alpha_v\beta_5$ is the primary integrin responsible for mechano-induced latent TGF- β 1 activation by lung myofibroblasts¹¹⁷. The small latent TGF- β 1 complex binds to β_5 subunit with a RGD motif in its LAP portion. Blocking the interactions between β_5 and LAP with either synthetic RGD peptides or neutralizing antibodies against $\alpha_v\beta_5$ abolishes mechano-induced latent TGF- β 1 activation¹¹⁷. Thy-1 interacts with integrin $\alpha_v\beta_5$ with a RGD-like motif, RLD⁹⁴. Lung fibroblasts null for Thy-1 or expressing non integrin-binding Thy-1 (RLD mutated to RLE) respond to endothelin-1-induced contraction with increased latent TGF- β 1 activation, whereas lung fibroblasts expressing wild-type Thy-1 do not⁹⁴, suggesting that Thy-1 interruption of integrin-LAP interactions blocks mechano-induced latent TGF- β 1 activation. Together, these studies support the concept that targeting the mechanotransduction machinery involved in latent TGF- β 1 activation represents another approach to inhibiting persistent myofibroblast contraction/activation in IPF.

D. Modulation of Oxidative Stress

The established link between IPF and increased oxidant burden provides strong rationale for the investigation of antioxidants as a therapeutic strategy⁶⁶. A deficiency in the tripeptide antioxidant, glutathione (GSH), in the lung epithelial lining fluid, has been thought to play a role in the pathogenesis and progression of IPF^{168–169}. Therefore, it is reasonable to envision that therapeutic interventions aimed at increasing GSH levels may be a viable strategy for inhibiting the progression of IPF. N-acetyl-cystine (NAC) exerts its function as an antioxidant via its main metabolite, cysteine, a precursor of GSH biosynthesis. The anti-fibrotic effects of NAC have been documented in animal models of lung fibrosis. Furthermore, a study of high-dose NAC therapy in patients with NAC demonstrated clinical efficacy, and is one of the few Phase III trials to meet the pre-determined primary end-point of the study¹⁹. In this clinical trial (IFIGENIA), patients were treated with NAC, an antioxidant, in addition to the standard treatment regimen (prednisone, azathioprine). Patients demonstrated preservation of forced vital capacity (FVC) and diffusing capacity for carbon monoxide (DLCO) in the NAC-treated group. Despite limitations of this clinical trial, the development of therapeutic strategies targeting oxidative stress pathways remains promising. An ongoing NIH IPFnet-sponsored clinical study (PANTHER) will evaluate the effectiveness of NAC alone or in combination with prednisone and azathioprine vs. placebo control (www.clinicaltrials.gov: NCT00650091).

A member of the NADPH oxidase (NOX) family of enzymes, NOX4, has been recently implicated in lung fibrogenesis¹⁷⁰; in this study, NOX4-dependent generation of ROS (specifically, H₂O₂) was found to be required for TGF- β 1-induced myofibroblast differentiation, extracellular matrix production, and contractility of lung myofibroblasts. NOX4 is upregulated in lungs of human subjects with IPF, and genetic or pharmacologic

targeting of NOX4 attenuated lung fibrogenesis in two different murine models of lung injury¹⁷⁰. Therapeutic strategies that more directly target the source(s) of ROS generation, including pharmacological inhibitors of NOX4, may prove to be more specific and effective in comparison to antioxidant interventions for IPF.

VII. CONCLUSION: PERSONALIZED MEDICINE FOR IPF

Many of the evolving treatments that we have discussed are targeted towards halting the fibrotic process. Even if an effective therapeutic is identified, patients must be identified early in the disease process and the drug must be “personalized” for the individual, ideally with reliable biomarkers that identify at-risk patients and targeted use of available therapeutic agents. A major problem with IPF is that diagnosis is often delayed until the lungs are severely scarred and the resulting loss of lung volume reserve and gas-exchange capacity eventually manifests in clinical symptoms. Another major problem is the heterogeneity of the patient population. We have already discussed that there are likely multiple possible environmental risk factors that predispose a patient to pulmonary fibrosis; since not all patients with these exposures develop pulmonary fibrosis, there must be individual genetic predispositions that confer susceptibility to individual patients. To make a significant impact in IPF, focus must be on early diagnosis, risk factor modification, and the identification of distinct subpopulations most likely to benefit from specific therapeutic interventions.

There has been much attention given to the idea of personalized medicine. The guiding principle behind personalized medicine is that an individual’s genetic makeup can provide the information necessary to make more informed decisions on disease treatments by minimizing toxicities to drugs and choosing drugs that will target patient specific molecular phenotypes of a disease¹⁷¹. There are two main goals of personalized medicine, to use genomics to determine susceptibility of disease for prevention and early treatment, and to diagnose and treat diseases by their molecular profiles, and not just the usual signs and symptoms¹⁷¹. IPF is an ideal disease for the application of personalized medicine, since this approach is likely to work best in disorders that are caused by a combination of genetic and environmental factors, and in diseases that tend to be chronic.

In order for personalized medicine to be effective in diagnosis and/or treatment of IPF, we must understand the underlying genetic alterations and molecular pathways that lead to this disease; therapeutic agents targeting these pathways must be discovered and developed into the clinic. Several recent studies have suggested multiple biomarkers that might be useful in distinguishing IPF patients from other lung diseases and healthy controls such as MHC polymorphisms¹⁷²; MMP-1^{173–174}; MMP-7¹⁷⁴; oxidative stress¹⁷⁵, and telomere shortening^{62–63}, among others. However, none of these biomarkers have been prospectively studied to determine if they predict early disease. Other studies have demonstrated that some biomarkers may be useful to identify patients who have a more rapid progression and earlier mortality. Serum surfactant protein A (SpA)¹⁷⁶, serum CCL18 levels¹⁷⁷, and TGF- β 1 polymorphisms¹⁷⁸ have all been suggested as potential biomarkers of disease progression. Clinical trials could be focused on those patients predicted to rapidly progress, and those more likely to gain benefit from a particular therapeutic agent. While personalized medicine holds great promise in the future management of IPF, efforts must continue to gain better understanding of disease pathogenesis, to identify reliable biomarkers of disease activity/progression, and to develop novel anti-fibrotic agents that can be tested in well-designed, randomized, and “personalized” clinical trials.

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Figure 1. Pathogenesis of IPF

Host-environmental interactions result in the generation of abnormal cell types and interactions between these cells of the lower respiratory tract. Host factors include age, genetic/epigenetic influences, and co-morbidities such as gastroesophageal reflux and diabetes. Environmental factors include noxious agents (e.g. cigarette smoke) and infectious agents (e.g. viruses). Ultimately, altered communication between aberrant epithelial and mesenchymal cells leads to a loss of tissue homeostasis, culminating in tissue remodeling, contraction, and fibrosis.

Table 1

Recently completed randomized controlled clinical trials in IPF

Study	Drug	Entry criteria	Primary endpoint	n	CS allowed	Results ^(reference)
GIPF 001	Interferon γ 1b	FVC > 50, D _L CO > 25	Progression Free Survival	330	Yes	Negative ⁽¹⁷⁹⁾
Azuma	Pirfenidone	Age, SpO ₂	Δ SpO ₂ 6MWT	109	Yes	Negative ⁽²¹⁾
IFIGENIA	Pred/Aza $\pm$ NAC	FVC 80, D _L CO < 80	Abs. Δ FVC, D _L CO	182	Yes	Less deterioration in FVC and D _L CO with triple therapy ⁽¹⁹⁾
Kubo	CS $\pm$ Anticoagulant	Decline with CS	Survival/time to death	56	Yes	Better survival in the anticoagulant group ⁽²⁰⁾
BUILD 1	Bosentan	FVC 50, D _L CO 30	6MWD	158	Yes	Negative ⁽¹⁸⁰⁾
Raghu	Etanercept	FVC 45, D _L CO 25	Δ %FVC, D _L CO, A-a	88	No	Negative ⁽¹⁸¹⁾
INSPIRE	Interferon γ 1b	FVC 55, D _L CO 35	Survival	826	Yes	Negative ⁽¹⁷⁾
Daniels	Imatinib mesylate	FVC 55, D _L CO 35	Disease progression/death	119	No	Negative ⁽¹⁸²⁾
PIPF004	Pirfenidone	FVC 50, D _L CO 35	Δ %FVC	435	Yes	Lower decline in FVC*
PIPF006	Pirfenidone	FVC 50, D _L CO 35	Δ %FVC	344	Yes	Negative*
Taniguchi	Pirfenidone	SpO ₂ on 6MWT	Δ FVC	275	Yes	Lower decline in FVC for the group randomized to the higher dose of pirfenidone ⁽²²⁾
STEP-IPF	Sildenafil	D _L CO < 35	6MWD	180	Yes	Negative ⁽¹⁸³⁾

FVC: Forced Vital Capacity; D_LCO: Diffusion capacity for carbon monoxide; SpO₂: peripheral saturation of oxygen; NAC: N-acetylcysteine; CS: Corticosteroids; 6MWT: six-minute walk test; 6MWD: six-minute walk distance

* unpublished; data available at: <http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMaterials/Drug/Pulmonary-AllergyDrugsAdvisoryCommittee/UCM206399.pdf>