

## Medical treatment for pulmonary fibrosis: current trends, concepts, and prospects

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During the past decade, significant advances have been made in the clinical understanding of diffuse fibrotic lung disease. There now is a standardized diagnostic algorithm for idiopathic pulmonary fibrosis and a clearer approach to the other idiopathic interstitial pneumonias [1]. These new diagnostic criteria have improved knowledge of the natural history of fibrotic interstitial lung disease (ILD), with recent studies providing long-term outcome data. An important concept that has emerged from these new studies is that the presence of lung fibrosis is a powerful and, in some instances, the most potent predictor of prognosis [2–4]. This information has helped focus ongoing biologic investigation, and significant advances have been made in understanding cells, cytokines, receptors, and matrix factors responsible for the initiation and progression of lung fibrosis. Unfortunately, the progress made in understanding the clinical and biologic features of these diseases has not been matched by similar advances in treatment, and in some minds has served only to highlight the poor prognosis and lack of a clearly beneficial therapy. Today, the prognosis of interstitial pulmonary fibrosis (IPF), with its underlying histopathologic pattern of usual interstitial pneumonia (UIP), differs only slightly from that of lung cancer (Table 1) [5]. The poor prognosis and lack of an efficacious therapy have resulted in despair on the part of physicians and desperation on the part of their patients.

Fifty years ago, Silverman and Talbot predicted the difficulty in identifying a useful treatment in the fibrosing lung disorders when they wrote, “Unfortunately, the rarity of diffuse interstitial pulmonary fibrosis, the extreme difficulty in establishing an early diagnosis and, finally, the complete ignorance as to its etiology present almost insurmountable obstacles in evaluating any form of therapy in this bizarre condition” [6]. New data may prove their pessimism premature. Basic investigation into the pathogenesis of lung fibrosis has offered new therapeutic targets, and the completion of large-scale, multicentered, placebo-controlled late-phase trials and the initiation of controlled early-phase treatment trials have proved that not only are definitive trials possible but also discoveries made in the laboratory can be translated into a clinical trial in relatively short order. This article offers a look at the future possibilities for treatment of IPF and other fibrosing lung disorders.

### Natural history of untreated pulmonary fibrosis

To understand the benefits of any therapy, it is necessary to understand the natural history of the untreated disease. This type of information assists the clinician in determining when and in whom to initiate treatment and helps define a therapeutic response. More importantly, it dictates the primary end points, number of subjects, and necessary length of any controlled trials. Recent and past clinical investigations can help understand what happens to these patients over time.

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Table 1  
Mortality: interstitial pulmonary fibrosis versus other lung diseases

Disease	Median survival
IPF	50% at 3 years 65% at 5 years
Chronic obstructive pulmonary disease FEV <sub>1</sub> < 30% predicted	50% at 3 years
Lung cancer	85% at 5 years

Data from Mason RJ, Schwarz MI, Hunninghake GW, Musson RA. NHLBI Workshop Summary. Pharmacological therapy for idiopathic pulmonary fibrosis. Past, present, and future. Am J Respir Crit Care Med 1999;160:1771–7.

In 1978, Carrington et al described 53 patients who had surgical lung biopsy–proved UIP [7]. Some of the patients had either a collagen vascular disease or drug toxicity, whereas the majority seemed to have had an idiopathic fibrosing lung disease illness consistent with IPF. Forty-eight patients were untreated for at least 1 year. When evaluated by a combination of clinical, physiologic, and radiographic criteria, no patient improved and 85% showed disease progression. In a more recent study from Spain, patients who met current American Thoracic Society (ATS) criteria for the diagnosis of IPF were investigated. Forty-three subjects were enrolled; 29 initially were treated with corticosteroids with or without azathioprine and 14 initially received no therapy [8]. Therapy was begun in untreated patients if progressive dyspnea occurred. Of the untreated patients, 7 of 13 required therapy for an average of 12 months after enrollment. The remaining 6 patients (15% of the total cohort) remained clinically and physiologi-

cally stable and without therapy after 2 years of follow-up. No significant differences in physiology or gas exchange were noted between the treated and untreated subjects at the end of the study, and no spontaneous improvements were described.

Regarding survival, in 1980, Turner-Warwick et al described 220 patients who met the prevailing definition of IPF, 118 of which had had biopsy or autopsy confirmation of their disease [9]. Seventy-seven of these patients were untreated and survived a median of 54 months. Current interpretation of this study allows us to appreciate the enrollment of patients who had known autoimmune disease and the assured presence of patients who had pathologic nonspecific interstitial pneumonia (NSIP). More recent data, using current definitions of IPF, is available from a retrospective study from the Mayo Clinic. Of the nearly 500 patients identified, 157 received no treatment. Median survival was 3.2 years for all patients. These and other studies [2,10,11] de-

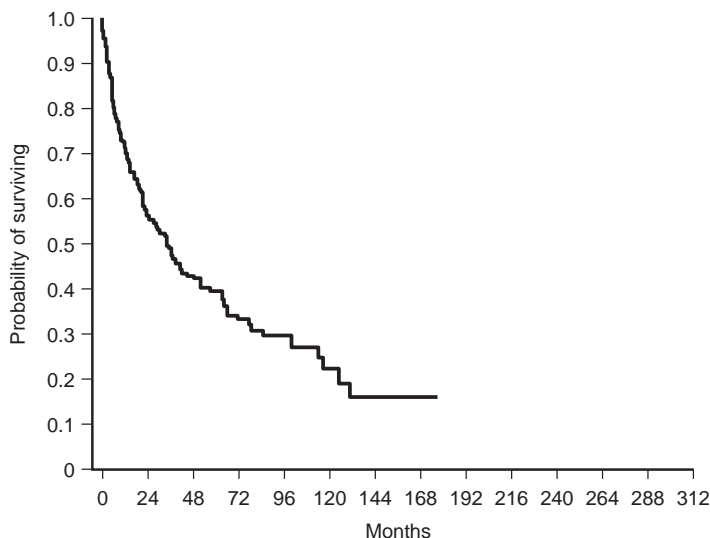


Fig. 1. Kaplan-Meier plot of survival of 238 surgical lung biopsy–proven cases of IPF from time of initial visit. Median survival 35.2 months. (Modified from King Jr TE, Tooze JA, Schwarz MI, Brown KR, Cherniak RM. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. Am J Respir Crit Care Med 2001;164:1171–81; with permission.)

scribe the untreated natural history of IPF as marked by progressive symptomatic and physiologic deterioration with a median survival of approximately 3 years (Fig. 1).

### Predictors of outcome and definitions of response to therapy

To interpret the available and anticipated data regarding response to therapy, an understanding of the clinical features used to define a response is essential. As no therapy has been proved to prolong survival, improve functional status and quality of life, or decrease the health care burden associated with IPF, analysis of available data often rests on interpretation of surrogate markers of disease activity. Recent investigation offers insight into easily measured and clinically relevant disease features that can help predict the short- and long-term outcome of patients who have IPF. These same features also can help evaluate the results of treatment trials and an individual patient's response to therapy (Box 1).

#### Baseline demographic variables

Although baseline demographic variables rarely are modifiable, they may have a significant impact on outcome. There is a relationship with age: the older the patient, the poorer the outcome [12]. The median survival of a 70-year-old patient who is diagnosed with IPF is less than 2 years, whereas that of a 50-year-old patient is more than 5 years [10]. In Turner-Warwick et al's study of the relationship between clinical features and survival in patients who have IPF, males had a poorer outcome [9]; other studies with stricter diagnostic criteria seem to support this [12]. Alternatively, when IPF is confirmed

by the identification of UIP on surgical lung biopsy, no difference in outcome between genders has been appreciated [10]. Neither race nor ethnicity is noted to have an impact on outcome. Cigarette smoking is identified as a potential risk factor for the development and progression of IPF [13], although recent data suggests that active cigarette smoking may provide an unexplained protective effect [10,14]. Given this new information, the explanation of the role of cigarette smoking in IPF requires further study.

#### Dynamic clinical variables

Dyspnea ultimately develops in all patients who have IPF. The severity [9,15] and the duration of dyspnea before diagnosis are associated with outcome, greater degrees of breathlessness, and a longer duration of symptoms associated with a poorer prognosis [16]. Improvements in dyspnea can occur, however, in response to treatment [17] and this improvement is associated with longer survival [18].

In patients who have IPF, progressive restrictive physiology occurs and the severity of this restriction correlates with prognosis. The decrease in total lung capacity and forced vital capacity (FVC) and an increase in the ratio of forced expiratory volume in 1 second (FEV<sub>1</sub>) to FVC all have been associated with a poorer prognosis [13,19]. The change in these measures, however (in particular the FVC), over time has proved more useful than baseline values in predicting survival. Progressive restriction, as measured by a falling FVC, particularly during a 6- to 12-month period, portends an extremely poor prognosis, whereas an increasing FVC during the same time frame predicts prolonged survival [18,20,21]. Measures of gas exchange reveal that a decreased diffusing capacity of the lung for carbon monoxide (DLCO) [12] and a progressive rise in the alveolar-arterial (A-a) gradient similarly predict a poor outcome [18], as does desaturation with a timed walk test [22].

Radiographic features on the plain chest radiograph and high-resolution CT (HRCT) scan have prognostic significance. More severe disease, as quantified by the International Labor Organization criteria on chest radiograph, is associated with a worse prognosis [9,23]. The pattern and extent of changes on HRCT scan also are useful. The presence or absence of three features (ground glass opacity, reticular opacities, and honeycombing) and their extent are shown to correlate with outcome. The ground glass opacity predominant pattern, rarely encountered, is associated with longer survival, whereas a predominance of reticular abnormality or

#### Box 1. Predictors of outcome

- Age
- Duration, severity, and change in symptoms over time
- Radiographic features and their extent
- Severity of pulmonary physiologic abnormalities and their change over time
- Severity of gas exchange abnormalities and their change over time
- Initial response to therapy

the presence of honeycombing is an accurate predictor of shorter survival [24,25]. Extensive reticulation or honeycombing is a particularly poor prognostic sign and may be more predictive of outcome than physiologic, histologic, or combination scoring systems [26–28]. Changes in the radiographic pattern or extent over time have yet to prove useful [21].

### Limitations of traditional immunosuppressive therapy

After reviewing the relevant world literature on IPF, in 1999 a panel of experts came to the following conclusions: (1) no data adequately document that any of the current treatment approaches improves survival or the quality of life for patients who have IPF and (2) there is insufficient clinical evidence to conclude that any treatment improves survival or the quality of life for patients who have IPF [1]. To provide guidance to clinicians while acknowledging that the supporting data on which therapeutic decisions can be made was limited, however, this international consensus conference provided therapeutic recommendations for those patients who have IPF in whom treatment is considered appropriate. They suggested a combination of corticosteroids with a cytotoxic agent, either cyclophosphamide or azathioprine, used for a minimum of 6 months if no intolerable side effects occur. The rationale for this approach rests on the inflammation hypothesis: the progressive fibrosis that characterizes IPF and other fibrosing lung diseases results from chronic persistent inflammation of the lung parenchyma; this chronic inflammation precedes and promotes the development of lung fibrosis; and aggressive suppression of this inflammation blocks new and may reverse established fibrosis. Although there are data to support this approach, interpretation of this older information requires care, as these older studies did not have the benefit of current strict clinical and histologic criteria for diagnosis and assuredly included idiopathic and secondary interstitial pneumonias known to respond to anti-inflammatory therapy. Moreover, the absence of placebo controls, variability in study duration, and inconsistent and nonvalidated criteria for assessment of response to therapy make any firm conclusions suspect.

In 1980, Turner-Warwick et al [17] described 143 patients who had IPF and received corticosteroids and showed that 57% had significant subjective and 17% had objective physiologic improvement after 4 to 6 weeks of therapy. The median survival of the

responders (7 years) was significantly greater than of nonresponders. As the improved survival also correlated with the amount of lymphocytic cellularity on biopsy [17], it is likely that many of those patients now would be classified pathologically as having NSIP. Cytotoxic therapy was added to minimize corticosteroid complications for those patients at high risk for these complications and for those nonresponsive to corticosteroids alone. Johnson and coworkers [29] noted that the addition of cyclophosphamide to corticosteroids as first-line therapy offered benefit beyond that noted with corticosteroids alone. After 3 years of follow-up, 10 of 22 patients in the prednisolone alone arm had died compared with 3 of 21 in the prednisolone plus cyclophosphamide group. The patients were not matched by disease severity at entry, however, and the prednisolone alone arm contained 9 of the 12 patients who had the most severe restrictive disease. As all 12 of these patients died or showed progressive disease within 2 years on their initially assigned therapy, the benefit assigned to cyclophosphamide must be viewed with caution.

Subsequent investigations have addressed the limitations of previous studies. Although several retrospective studies failed to show a benefit from immunosuppressive therapy [18,30,31], a more recent study prospectively evaluated the response to therapy and complications of corticosteroid treatment in 41 patients who had previously untreated, biopsy-proved IPF [32]. Twenty-seven percent responded to therapy, as defined by a standardized clinico-radiographic-physiologic score after 3 months, whereas 46% remained stable. Those who showed progression of disease had a significantly shortened survival. The benefit achieved was accompanied by a substantial risk of clinically significant side effects, however. Regarding cytotoxic therapy, Raghu and colleagues published the first prospective, double-blind, randomized, placebo-controlled trial of IPF evaluating the benefits of prednisone with and without the addition of azathioprine [33]. All patients had the diagnosis confirmed by surgical or transbronchial lung biopsy, were untreated, and had shown clinical evidence of disease progression at the time of enrollment. During the 9-year follow-up period, 6 of 14 patients in the azathioprine group died compared with 10 of 13 in the prednisone alone group. This difference, although not significantly different, suggests a potential survival advantage for combination therapy and highlights the importance of further study.

In summary, although 10% to 30% of patients may show subjective or objective improvement in response to treatment, this benefit is accompanied by a high risk of clinically important side effects.

Therefore, the results of an aggressive anti-inflammatory approach to treatment remain unsatisfactory for the majority of IPF patients.

### Rationale for novel antifibrotic treatments

Current research points to the excessive deposition of extracellular matrix (ECM), failure of the normal remodeling mechanisms and abnormal angiogenesis as important features of IPF. An active central hypothesis to account for these changes is that the progressive lung fibrosis that characterizes these disorders is a result of recurrent or ongoing tissue injury and aberrant wound healing. Given this new hypothesis and years of disappointing results from immunosuppressive therapy, the current therapeutic emphasis is on antifibrotic agents. Given that a generic antifibrotic approach using colchicine [34] or D-penicillamine [35] failed to show a clinical response or survival benefit in prospective treatment trials, a more targeted approach using current understanding of specific pathways is appropriate. The therapeutic targets in the fibrosing lung diseases should have the following features: the target must be proved important in producing the disease; it should be expressed differentially in the presence and absence of the disease; and modifying its level or activity should effect the development or course. Characteristics of an antifibrotic drug includes an ability to decrease lung fibroblast and myofibroblast proliferation and increase their apoptosis, decrease ECM synthesis and deposition, and promote ECM remodeling and the restoration of normal tissue architecture. The following sections review potential new therapies based on current understanding of the biology of lung fibrosis.

### Antagonism of transforming growth factor- $\beta$

There is considerable evidence that transforming growth factor (TGF)- $\beta$  plays a central role in the development of fibrosis in multiple organs. In rodents, pulmonary fibrosis induced by bleomycin, radiation, asbestos, and silica are associated with an upregulation of TGF- $\beta$  gene expression and protein production [36–38]. Bleomycin-induced lung fibrosis in the rodent can be blocked by the delivery of TGF- $\beta$ –signaling inhibitors, blocking antibodies, or an excess of chimeric TGF- $\beta$  receptors [39–41]. In human lung, TGF- $\beta$  is present in the healthy state; however, in several clinical conditions associated with fibrosis, including radiation, asbestosis, pneu-

moconiosis, and IPF [42,43], increases in message and protein are described. Together, these data support TGF- $\beta$  as a key mediator of tissue, in particular lung fibrogenesis.

### *Antibodies to transforming growth factor- $\beta$ and connective tissue growth factor*

Given TGF- $\beta$ 's central role in the elaboration of fibrosis, anti-TGF- $\beta$  antibodies are attractive therapeutic agents. There are biologically active antibodies that show themselves capable of modifying the response to fibrogenic agents [44] and a phase I trial of an anti-TGF- $\beta$  antibody is in the planning stages. Connective tissue growth factor (CTGF) is shown to mediate several of TGF- $\beta$ 's fibrogenic effects and induces collagen synthesis in cultured fibroblasts [45]. It is expressed highly in the lungs of patients who have IPF [46]. CTGF is induced primarily, but not exclusively, through a TGF- $\beta$  response element in the CTGF promoter, and failure to activate CTGF gene expression seems to offer relative protection against TGF- $\beta$  overexpression–induced pulmonary fibrosis [47]. A phase I trial of an anti-CTGF antibody currently is under way.

### *Interferons*

The interferons are cytokines that as a class have many of the characteristics of a prototypical antifibrotic agent, including the ability to inhibit fibroblast proliferation [48], reduce collagen gene expression and synthesis [49], and collagen contraction [50]. There are two types of interferons. Interferons (IFNs)- $\alpha$ , - $\beta$ , - $\omega$ , and - $\tau$  are type I with IFN- $\gamma$  the sole type II interferon. The primary differences between I and II are in their cells of origin: type I interferons are produced in almost all cell types, whereas IFN- $\gamma$  is produced in T lymphocytes and natural killer cells [51].

The ability of type I interferons to modulate pulmonary fibrosis has been explored in animal models and humans. The capacity of IFN- $\alpha$ 2a to inhibit bleomycin-induced lung fibrosis in the mouse recently was evaluated [52]. Unfortunately, treatment with IFN- $\alpha$ 2a seemed to enhance bleomycin-induced injury. In a pilot trial of IFN- $\beta$  in patients who had advanced, histologically proved IPF and who had evidence of progression while on corticosteroids, were treated with IFN- $\beta$  3 times a week [53]. One-half of the patients showed evidence of persistent improvement based on improved performance status, physiology, or gas exchange. IFN- $\beta$  for IPF subsequently was studied in a large multicentered,



randomized, double-blind, placebo-controlled dose-ranging trial. Unpublished results of this trial were presented at the 2001 International Conference of the ATS [54]. No evidence of therapeutic benefit was noted in the treated subjects, as survival and clinically important surrogate measures of disease activity, such as FVC, A-a gradient, and dyspnea, were unaffected by treatment.

The type II interferon, IFN- $\gamma$ , has been shown to limit fibroblast proliferation [48], inhibit the transcription of collagen mRNA and the synthesis of protein in normal and isolated IPF fibroblasts [55–57], abrogate the increased collagen synthesis associated with TGF- $\beta$  stimulation [58], reduce issue myofibroblast numbers [59], and increase the expression of matrix metalloproteinase-1 message [60]. A small prospective, randomized study of IFN- $\gamma$  for the treatment of IPF suggests substantial therapeutic benefit with a significant improvement in lung function and gas exchange in actively treated subjects [61]. Two subsequent open-label nonrandomized studies, however, failed to confirm this benefit [62,63]. Subsequently, a 330-patient multicenter, double-blind, placebo-controlled treatment trial was performed. The primary endpoint was progression-free survival time, defined as any of the following: (1) a decrease in FVC greater than 10%; (2) an increase in the resting room air A-a gradient of 5 mm Hg or more; or (3) death. Active therapy with IFN- $\gamma$  had no effect on the primary endpoint. In post-hoc analysis, a mortality benefit in patients who had mild-to-moderate disease as measured by physiology could not be excluded. Of the 254 patients who had an FVC greater than 55% of predicted, 6 of 126 died in the treated group (4.8%), whereas 21 of 128 died in the placebo group (16.4%) ( $P = 0.004$ ). Because of the equivocal mortality results of this study, a larger, hopefully definitive, 600-patient prospective randomized trial with mortality as the primary endpoint currently is enrolling patients in Europe and the United States.

### *Pirfenidone*

The investigational pyridone molecule (5-methylphenyl-2 [1H]-pyridone), or pirfenidone, also has been shown to have antifibrotic activity *in vitro* and *in vivo*. Although the precise mechanism of its anti-inflammatory and antifibrotic effects is unknown, it inhibits the TGF- $\beta$ -induced collagen synthesis in isolated IPF-derived lung fibroblasts and the mitogenic effects of other profibrotic cytokines on lung fibroblasts. It inhibited collagen production, reduced

BAL inflammatory cell numbers, and decreased lung cell transcription of TGF- $\beta$  and platelet-derived growth factor (PDGF) in a rodent model of bleomycin-induced lung fibrosis [64,65]. In a recent phase II, open-label study of IPF treatment [66] in patients who previously had shown disease progression on immunosuppressive therapy, 29 of 54 showed evidence of stability or improvement of the FVC at 6 months. No effect on survival could be determined given the study design. Although a majority of the patients developed significant side effects (rash or upper gastrointestinal discomfort), only a handful had to discontinue therapy.

Other studies subsequently have reported similar results in patients who had IPF [67] and in patients who had pulmonary fibrosis associated with Hermansky-Pudlak syndrome [68]. Prompted by such encouraging results, a multicenter, prospectively randomized phase II clinical trial recently has been completed. The full results of this clinical trial were presented at the 2004 ATS International Conference [69]. Although these results provide further supporting evidence for the potential beneficial use of pirfenidone, it is hoped that the recently initiated phase III multicenter clinical trial in Japan and future clinical trials worldwide will determine the efficacy of pirfenidone in IPF. Currently, pirfenidone is an investigational drug and not available by prescription.

### **Antioxidants**

Recent evidence suggests an imbalance between the oxidation products and antioxidants in the lungs of patients who have IPF [70]. Large amounts of reactive oxygen species are identified in the lower respiratory tract of patients [71]. Glutathione functions as an antioxidant and in its reduced form can regulate the process of oxidant-induced cellular damage [72]; its levels are reduced in the epithelial lining fluid of patients who have IPF. TGF- $\beta$  has been shown to inhibit glutathione biosynthesis by suppression of its rate-limiting enzyme. N-acetyl-L-cysteine (NAC), a metabolic precursor of glutathione, has been shown to increase glutathione levels in the alveolar lining fluid in patients who have IPF [70]. In a rodent model of bleomycin-induced lung fibrosis, aerosolized delivery of NAC reduced BAL and tissue infiltration by inflammatory cells and collagen deposition. Oral NAC has been tested in a proof of concept study. Twenty patients who had IPF had oral NAC (600 mg three times a day) added to their conventional immunosuppressive regimen for 12 weeks.

Neither symptoms nor examination findings changed; a significant improvement in DLCO, however, was appreciated [70]. A multicenter treatment trial in Europe, Idiopathic Pulmonary Fibrosis International Group Exploring NAC-1 Annually, has investigated the efficacy NAC in a placebo-controlled trial. Patients were treated with prednisone and azathioprine with or without NAC. Initial results presented at the 2004 International Conference of the American Thoracic Society (ATS) and European Respiratory Society (ERS) suggest a potential benefit with addition of NAC to prednisone and azathioprine. Further data from this trial are awaited. Given that NAC is available over the counter as a supplement/antioxidant, it will not be surprising for patients to include NAC as an added supplement to their daily regimen with supplements (multivitamins, antioxidants, herbal extracts, etc).

### Tumor necrosis factor- $\alpha$

Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is a pleiotropic cytokine that has been variously described as profibrotic, antifibrotic, and proinflammatory. It has been shown to stimulate fibroblast proliferation and collagen gene upregulation through a TGF- $\beta$  or PDGF pathway [73], although it also has been shown to suppress collagen gene expression [74]. TNF- $\alpha$  gene expression rises in the rodent lung after the administration of bleomycin [75], whereas animals missing TNF- $\alpha$  receptors [76] or treated with soluble TNF- $\alpha$  receptors are relatively resistant to bleomycin-induced fibrosis [77]. In the human lung, alveolar macrophages from patients who have IPF or asbestosis produce increased amounts of TNF- $\alpha$  [78], and the hyperplastic type II cells of IPF patients contain significant amounts of the protein by immunohistochemistry [79]; new data suggests an association between TNF- $\alpha$  promoter polymorphisms and an increased risk of developing IPF [80]. This and similar data led to a recent open label pilot study that prospectively tested the use of the TNF- $\alpha$  blocker, etanercept, in the treatment of nine consecutive patients who had IPF [81]. After an average of 9 months of treatment with twice-weekly etanercept and 10 mg of prednisone, one patient died, although improvements in FVC (3 patients), DLCO (4 patients), and A-a gradient (5 patients) were noted. A prospective, multicentered, double-blind, placebo-controlled trial of etanercept for the treatment of IPF has been enrolled fully, with initial results anticipated in mid-2005.

### Endothelin

Endothelins are a family of 21 amino acid peptides with vasoactive, mitogenic, bronchogenic, and immunomodulatory activity. There are three isoforms, endothelin (ET)-1, -2, and -3, with ET-1 the most abundant and best characterized. The lung contains significant levels of ET-1, secreted by cells of the airway (epithelial and smooth muscle cells), vasculature (endothelial and smooth muscle cells), and hematopoietic system (platelets and macrophages) [82]. Along with inducing TGF- $\beta$  [83], ET-1 stimulates fibroblast proliferation, migration, and their conversion to myofibroblasts [84]. It stimulates collagen synthesis while decreasing collagen breakdown [85] and increases fibronectin production [86]. In an animal model of bleomycin-induced lung fibrosis, increased ET-1 expression was seen in alveolar macrophages and the epithelium, with the increase preceding the development of the fibrosis [87]. Mice engineered to constitutively overexpress human ET-1 develop age-dependent pulmonary fibrosis and glomerulosclerosis [88]. Although blocking endothelin receptors has been shown to decrease fibroblast proliferation and collagen production in vitro, blocking them in vivo has produced mixed results [89,90]. In human systems, BAL and plasma levels of ET-1 are increased in IPF [91,92], with increased protein seen in airway epithelial cells and type II pneumocytes [93]. Stimulated fibroblast proliferation induced by BAL from patients who have scleroderma-related lung disease is inhibited by ETA receptor blockers [94,95]. Based on these data, multicentered double-blind placebo-controlled trials of the endothelin receptor antagonist, bosentan, have been initiated for the treatment of IPF and scleroderma-associated fibrotic lung disease.

### Type 1/Type 2 cytokine imbalance

Although named after the cytokines produced by subsets of CD4 T helper lymphocytes [96], other cells, including fibroblasts, can produce these ubiquitous proteins. Type I cytokines include interleukin (IL)-2, IL-12, IL-18, TNF- $\beta$ , and IFN- $\gamma$ . Type II cytokines include IL-4, IL-5, IL-10, IL-13, and monocyte chemoattractant protein-1 (MCP-1). Traditionally, type I responses are associated with cell-mediated and type II humoral immunity. Newer information suggests, however, that the pattern of cytokine expression plays an important role in the response to tissue injury, with type I cytokines

responsible for repair and the restoration of normal architecture. A type II response, alternatively, seems to promote fibrosis with ECM deposition. IL-4, the prototypic type II cytokine, promotes collagen gene expression and protein production [97] and the prototypic type I cytokine, IFN- $\gamma$ , has the opposite effect. In vivo, upregulation of IL-4, IL-5, and MCP-1 occur in the rodent model of bleomycin-induced lung injury [98,99]. Neutralizing antibodies to IL-5 or MCP-1 limit fibrosis in this model [100]. Murine IL-10 knockouts are protected from silica-induced pulmonary fibrosis, whereas wild type mice show increased type 2 responses [101,102]. In humans, isolated lung cell from subjects who have fibrotic lung disease produce an excess of IL-4, IL-5, IL-13, and MCP-1. Increases in some of the type II cytokines also are present in BAL and serum of patients who have IPF [103–106], with excess numbers of type II cytokine producing mononuclear cells also found [107,108]. The weight of these data supports an imbalance between types I and II cytokines in IPF, with an excess of type II cytokines associated with the development of tissue fibrosis. Therapeutic approaches that decrease the level of fibrogenic cytokines or increase the level of type I cytokines may prove beneficial.

### Eicosanoids

Eicosanoids are lipid mediators derived from the cyclooxygenase and lipoxygenase metabolic pathways of arachidonic acid [109]. Almost all eicosanoids have some physiologic function in the lung. The lipoxygenase pathway ends in the generation of leukotrienes that possess proinflammatory effects and the ability to promote fibroblast migration, proliferation, and the production of ECM proteins [110,111]. Increased levels of leukotriene B<sub>4</sub> and leukotriene C<sub>4</sub> are found in lungs of patients who have IPF [112]. Recent evidence suggests that 5-lipoxygenase deficient mice have a decreased capacity to produce cysteinyl leukotrienes and produce less inflammation and lung fibrosis in a bleomycin lung injury model [113].

Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) has potent bronchodilatory, immunomodulatory, and antifibrotic effects. In mice engineered to be deficient in cyclooxygenase-1, PGE<sub>2</sub> levels are reduced in BAL and are noted to have an increased acute inflammatory response and enhanced bronchoconstriction in response to an inhaled allergen [114]. More interestingly, when cyclooxygenase-2 is knocked out, mice respond to

bleomycin administration with an increased amount of lung fibrosis [115]. In patients who have IPF, isolated lung fibroblasts seem to have an intrinsic defect in their ability to upregulate cyclooxygenase-2 and biosynthesize PGE<sub>2</sub> [116]. Based on this data, attempts to decrease specific leukotriene production and increase PGE<sub>2</sub> availability have a biologic rationale.

### Renin–angiotensin–aldosterone system

Systemic activation of the renin–angiotensin–aldosterone system (RAAS) is important for regulation of blood pressure and maintenance of the intravascular volume. There also is a local tissue RAAS that normally is quiescent after birth but can be reactivated in response to tissue injury [117]. Locally elaborated angiotensin II initiates tissue repair through stimulation of TGF- $\beta$  [118]; as in vitro and in vivo studies show, angiotensin II stimulates TGF- $\beta$  gene and protein expression and can directly induce cell growth and matrix accumulation in the kidney [119]. The angiotensin-converting enzyme inhibitor (ACEI) captopril is shown to inhibit radiation-induced lung fibrosis in a rodent model [120]. It also is believed that angiotensin II can inhibit the degradation of matrix by creating an imbalance in the plasminogen/plasmin system. The primary fibrinolytic enzyme in the body is plasmin. Plasminogen activator inhibitor–1 (PAI-1) is the major physiologic inhibitor of tissue-type plasminogen activator and urokinase-like plasminogen activator, both of which activate plasminogen to plasmin, promoting fibrinolysis, proteolysis, and activation of latent matrix metalloproteinases. PAI-1 also encourages the migration of matrix-producing cells into damaged tissue [121]. PAI-1 is upregulated in bleomycin-induced pulmonary fibrosis and the fibrosis generated by bleomycin is worse in PAI-1–overexpressing mice [122], and PAI-1 levels in bronchoalveolar lavage fluid are considerably elevated in patients who have IPF [123].

Although observational data suggest that the use of ACEI prolongs survival in IPF [124], this information is contradicted by other investigators [125], and treatment with captopril, when tested in a prospective trial, does not seem to provide a survival benefit in patients who have biopsy-proved IPF [126] (M. Selman, MD, personal communication, 2003), nor does the use of ACE inhibitors seem to protect against radiation-induced lung injury [127]. A role for ACEI in limiting or reducing fibrosis awaits further investigation.



## Statins

The intrinsic pattern of programmed cell death (apoptosis) built into nearly every cell and the proliferative response of cells play critical roles in normal wound healing. Both of these responses are active in the epithelium and mesenchymal cells of the lung after an injury. Fibroblast and myofibroblasts are required to undergo both processes in sequence in order for wound healing to proceed appropriately. In human lung, there is evidence of significant cellular apoptosis in the fibromyxoid Masson bodies of subjects who have bronchiolitis obliterans organizing pneumonia, an ILD with an excellent clinical outcome, whereas the fibroblastic foci of UIP contain few if any apoptotic cells [128]. Lovastatin is shown to induce apoptosis in isolated normal lung and fibrotic lung fibroblasts and reduce granulation tissue formation and induce fibroblast apoptosis in a rodent wound chamber model [129]. A recent abstract suggests that treatment with a statin prolongs life in patients who have IPF [130]; however, this was not confirmed in a follow-up post hoc analysis of a recent treatment trial [125]. Determination of the appropriate role, if any, of statin therapy in IPF requires additional study.

## Others

Agents that decrease lung fibroblast proliferation, synthesis and deposition of ECM, and increase degradation of aberrantly deposited ECM can be considered antifibrotic agents. Because there are several currently and soon to be available drugs that can modulate fibrotic cascade and repair mechanisms, it should be anticipated that a number of these may be used in future clinical studies of IPF. These include interleukin receptor antagonists, antibodies against specific growth factors (eg, platelet-derived growth factor), thalidomide analogs, mycophenolate, rapamycin, suramin, relaxin, imatinib and others.

## Summary

A diagnosis of pulmonary fibrosis, in particular IPF, carries a poor prognosis. Although significant advances have been made in understanding the clinical and biologic features of the disorder, these advances have not been matched by similar advances in therapy. No therapy has been demonstrated to

improve survival or quality of life for these patients. Fortunately, several early- and late-phase treatment trials are under way, and the results of some will likely be available within the next year. These novel therapies are taking advantage of insights into the pathobiology of the disease. Because the complex cascade of biologic events associated with inflammation and fibrosis seems to be ongoing in a concurrent manner in the local milieu, it is likely that the to-be-determined effective treatment regimen will be a combination therapy or a pleiotropic single agent (with multipotent biologic functions) that modulates the process in a multitargeted manner. Newer therapies, focusing on defined specific molecular targets responsible for the development and progression of lung fibrosis, likely are forthcoming.

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