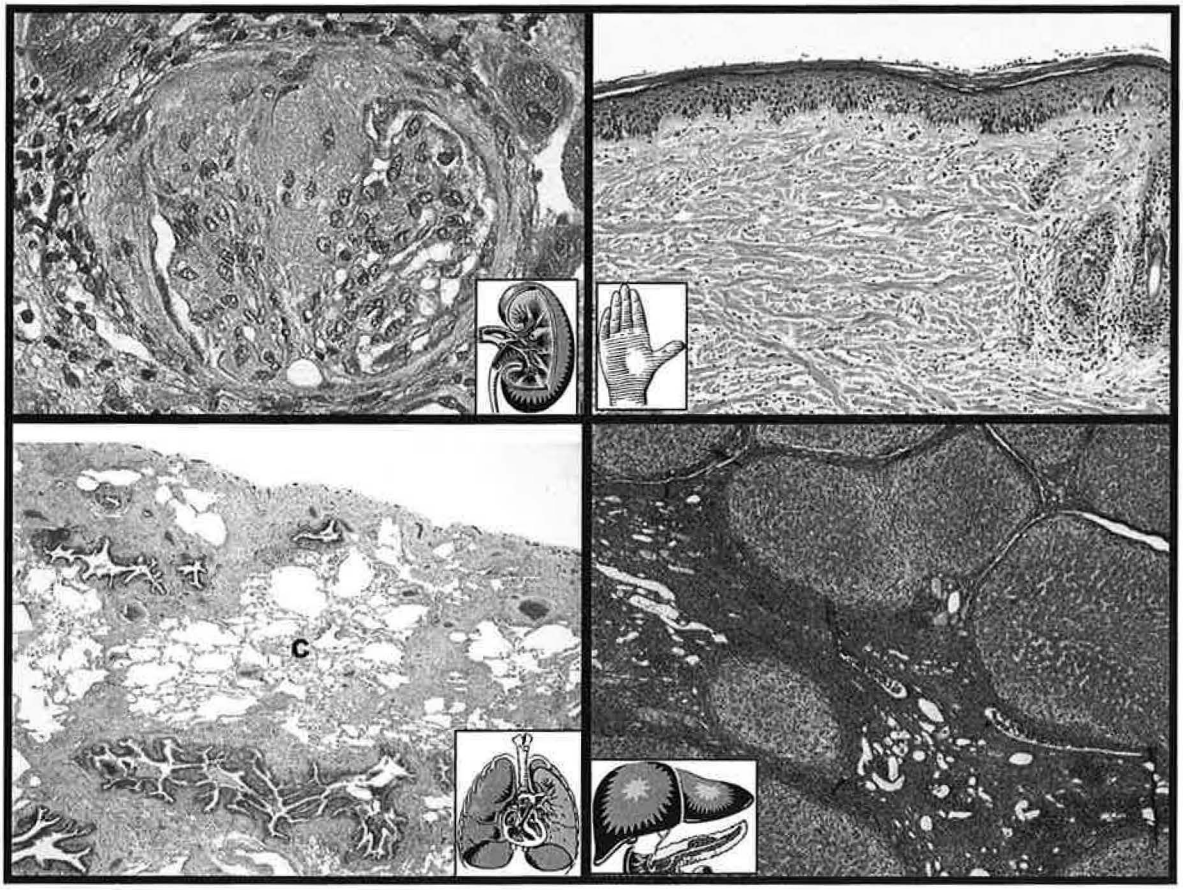
Anti-Fibrosis:
The Future of Medicine



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Internal Medicine Grand Rounds
University of Texas Southwestern Medical Center
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INTRODUCTION:

The future of medicine should continue to focus on disease screening and prevention. Significant strides have been made in reducing morbidity and mortality from cardiovascular, cerebrovascular, and oncological diseases. Despite preventive medicine, fibrotic diseases, such as hypertensive cardiomyopathy, progressive systemic sclerosis (PSS), idiopathic pulmonary fibrosis (IPF), and liver cirrhosis, will continue to be important causes of morbidity and mortality. In the United States, an estimated 45% of deaths are due to organ dysfunction caused by chronic fibrotic diseases[1, 2]. Fibrosis is the final common pathway for repetitive epithelial and endothelial injury and is characterized by excessive connective tissue accumulation and distortion of normal organ architecture and function (see Figure 1)[1, 2]. At this time, there are no FDA-approved medications for the treatment of organ fibrosis. Recently, a large body of scientific research has elucidated the important mechanisms for tissue and organ fibrosis[1-5]. As potential targets for therapy are identified, the future appears much brighter.

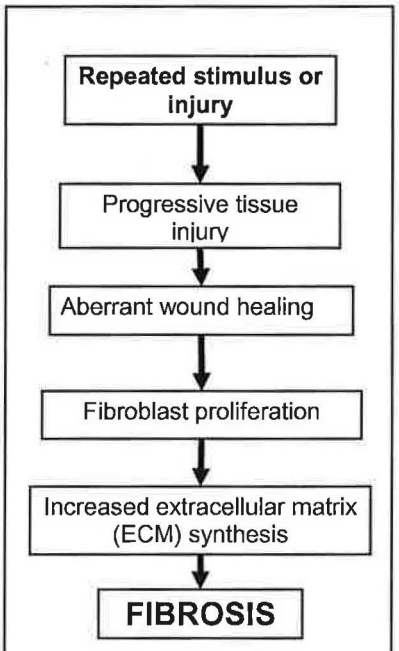

Figure 1: Common mechanism for the development of organ or tissue fibrosis. Adapted from Samuel C.S. Clinical Medicine and Research 2005;3:241-9.

Each medical specialty faces chronic diseases that progress to fibrosis with few therapeutic options available. Common fibrotic pathways have been identified in most organ systems (i.e. skin, joints, heart, lung, liver, kidney, pancreas, bone marrow), thus allowing for future collaboration in therapeutic drug design[1, 2, 4]. As the fibrotic mechanisms are uncovered, a subspecialty within Internal Medicine called "Antifibrosis Medicine" could recruit bench researchers, clinical researchers, and clinicians with this common goal.

This review will attempt to describe the common and specific pathways of the various fibrotic diseases and discuss possible therapeutic targets. This discussion will attempt to clarify and challenge some of the following common **misconceptions**:

- Fibrosis is driven by acute and chronic inflammation and therefore, treating the inflammation with corticosteroids or anti-inflammatory agents should prevent fibrosis.
- The fibrotic process is organ- or tissue-specific (for example, studying the mechanism of fibrosis in the liver will not help identify potential treatments for pulmonary fibrosis).
- Organ fibrosis is irreversible.

MAGNITUDE OF THE PROBLEM:

Liver cirrhosis[5], nephrosclerosis[6], progressive systemic sclerosis (PSS)[7], and idiopathic pulmonary fibrosis (IPF)[8] are some of the most common and studied fibrotic diseases. In 2004, chronic kidney disease and liver cirrhosis accounted for the 9th and 12th leading cause of death in the United States, respectively[9]. Globally, chronic kidney disease leads to 850,000 deaths each year and a cumulative 15,010,167 disability-adjusted life years[6]. These diseases commonly progress without warning making preventive measures no longer helpful. Scleroderma (PSS) and IPF are much rarer diseases but are associated with rapid decline in health and death. Unfortunately, PSS and IPF do not have identifiable triggers and prevention is not an option. Patients with PSS and IPF face an aggressive disabling disease with a high predicted mortality and no known successful treatment.

THE FIBROTIC PATHWAY: Aberrant wound healing and repair

Tissue or organ injury usually heals via a regenerative wound repair that replaces dead or injured cells with no residual functional damage. This requires control of the repair process to prevent progression to an unregulated fibrotic phase[2, 3, 10]. Organ and tissue fibrosis represents an exuberant response to repetitive injury and inflammation. Its hallmarks are the proliferation of fibroblasts, accumulation of connective tissue, and expansion of the extracellular matrix[1, 2, 7]. As connective tissue accumulates, organizes, and contracts, the organ or tissue loses function and its architecture is altered[2, 5, 11].

Recently, a new perspective or hypothesis has been generated regarding the initiation and perpetuation of the fibrotic process. Although inflammation is considered to be an important trigger of the fibrotic response, this process may perpetuate in the absence of persistent injury or active inflammation[11-15]. As evident by histology, fibrotic diseases, such as cirrhosis, scleroderma, pulmonary fibrosis, and glomerulosclerosis, are relatively free of acute inflammation. A persistent and unabated fibrotic response appears to be driven by locally synthesized chemokines and cytokines[5, 13] and cellular crosstalk between resident and recruited cells and the extracellular matrix (ECM)[5, 7, 16]. It is now clear that targeting acute and chronic inflammation with corticosteroids and/or cytotoxic therapy does not appear to significantly impact the progression of fibrotic diseases[2, 7, 15, 17, 18].

There are various components of the fibrotic response to injury. Most of the pathways are universal although there are some organ or tissue-specific responses (see Figure 2). In order to prepare a background for the study of potential anti-fibrotic drugs, this discussion will focus on the following mechanisms:

1) Epithelial damage and basement membrane breach
2) The role of effector cells (resident and circulating)
3) Chemokine and cytokine responses: The role of TGF-ß
4) The activated fibroblast and myofibroblast
5) Extracellular matrix (ECM) deposition

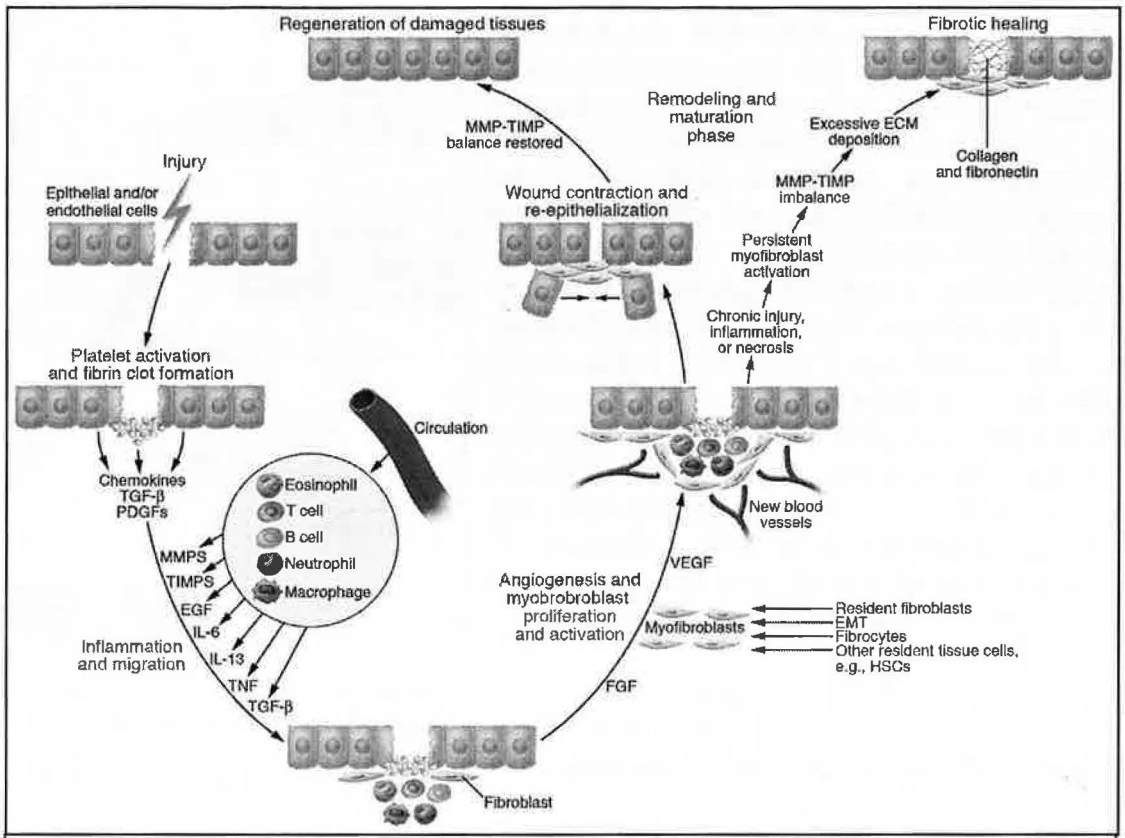

Figure 2: Diagram detailing the various steps of wound healing and repair. The reparative and fibrotic processes are depicted with their various compartments, cytokines, chemokines, and effector cells. From Wynn TA. J Clin Invest 2007;117(3):524-9.

A) Epithelial damage and disruption of the basement membrane:

Multiple repetitive insults to the epithelium lead to damage and activation of epithelial cells[2, 11, 16, 19]. For the most common fibrotic diseases, various etiologic agents have been identified and are listed in Table 1.

Table 1: Most prevalent fibrotic diseases and their most common cause(s):	
Liver cirrhosis	Chronic active hepatitis, alcohol, non-alcoholic hepatic steatosis, parasitic infections, and inborn errors of metabolism[4, 5]
Renal fibrosis	Hypertension[20], diabetes, glomerulonephritis, and aging[21]
Hypertensive heart disease	Hypertension[22]
Chronic pancreatitis	Alcohol abuse, pancreatic duct obstruction, metabolic disorders, hereditary, cystic fibrosis, and idiopathic[23]
Progressive systemic sclerosis (scleroderma)	Unknown[24]
Idiopathic pulmonary fibrosis	Unknown[8, 17]

Epithelial cells are in close contact with the basement membrane and interact with the extracellular matrix[16]. When activated, epithelial cells secrete a number of inflammatory mediators leading to deposition of fibrin and matrix metalloproteinases (MMP's) that digest the basement membrane permitting passage of inflammatory cells into the site of injury (Figure 3)[2]. During repetitive injury, epithelial cells also secrete a number of cytokines and chemokines that promote fibroblast and myofibroblast proliferation. In normal organ and tissue homeostasis, epithelial cells have an important role in the reparative process with re-epithelialization through cell proliferation and differentiation. As an example, in pulmonary fibrosis, the alveolar type II cell differentiates into the flat type I cell in an attempt to re-epithelialize the alveolar epithelium[19, 25]. Also, bronchial epithelial cells proliferate into the alveolus in an attempt to repair the epithelial breach (Figure 4)[19].

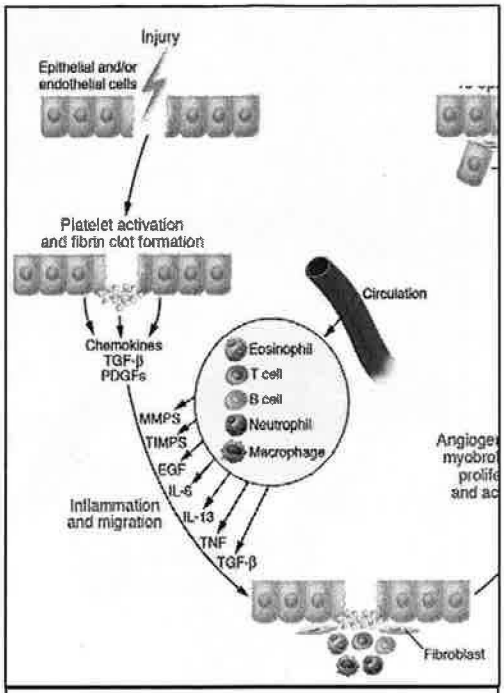

Figure 3: Diagram illustrating the role of epithelial cells in early response to repetitive injury. From Wynn TA. J Clin Invest 2007;117:524-9.

B) <u>The role of resident and circulating effector cells</u>:
When epithelial cells proliferate, they secrete chemokines and cytokines that activate resident and circulating cells (Figure 3). Early in wound healing, circulating neutrophils and resident macrophages enter the injured tissue and amplify the inflammatory response by secreting proteolytic enzymes in an attempt to clear pathogens and dying cells[2]. Furthermore, activation of T cells leads to secretion of pro-inflammatory and pro-fibrotic cytokines[4].

C) <u>Chemokine and cytokine responses: The role of TGF-ß</u>:
There are many important chemokines and cytokines secreted during fibrosis, among them interleukin-13 (IL-13), IL-6, platelet-derived growth factor (PDGF), tumor necrosis-alpha (TNF-α), connective tissue growth factor (CTGF), epidermal growth factor (EGF), and insulin-like growth factor-1 (IGF-1)[2, 4, 13, 15, 26]. Transforming growth factor- beta (TGF-ß) is regarded the most potent fibrogenic cytokine and a key element in the development of chronic fibrotic diseases[5, 7, 15]. Originally identified in 1983 as "sarcoma growth factor", this potent cytokine has broad effects on cellular function and homeostasis of the extracellular matrix[27]. TGF-ß is secreted by activated epithelial cells, neutrophils, macrophages, and T cells[5, 7, 10, 12, 28] and it participates in organ and tissue embryogenesis, development, angiogenesis, apoptosis, immunosuppression, and cellular differentiation[27]. Thus, it is not surprising that drug development has focused on TGF-ß inhibition[13]. Of interest, TGF-ß has opposite and conflicting functions, such as pro-inflammatory vs. anti-inflammatory, proliferative vs. anti-proliferative, or reparative vs. fibrogenic, depending if the cells, tissues, and/or organs are

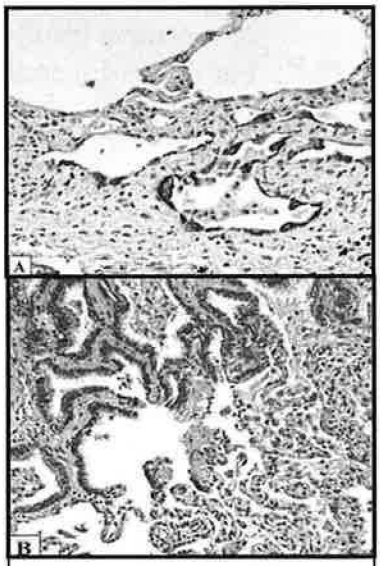

Figure 4: A) Reactive and flattened alveolar epithelial cells responding to alveolar injury. B) Atypical bronchial cells extending into a honeycomb cyst caused by alveolar collapse. From Selman M, Pardo A. Proc Am Thorac Soc 2006;3(4):364.

undergoing normal homeostasis, tumorigenesis, or wound injury and repair[7, 20, 27].

In various animal and *in vitro* models of cirrhosis, nephrosclerosis, and IPF, the addition or overexpression of TGF-ß leads to a fibrotic response[20, 27-32]. In fact, mice overexpressing TGF-β develop pulmonary fibrosis in the absence of an inflammatory insult or injury[15, 33]. Furthermore, high levels of TGF-ß are universally seen in human fibrosis. The histologic lesion of IPF demonstrates abundant cytokine expression in the epithelium, endothelium, fibroblasts, and end-stage honeycomb. Bronchoalveolar lavages of patients with idiopathic pulmonary fibrosis (IPF) have higher levels of TGF-ß than other benign interstitial lung diseases[26, 34].

Much work has focused on the TGF-ß family, its receptor, and post-receptor signaling. TGF-ß binds to cell membrane type I and type II serine/threonine kinase receptors with phosphorylation of its two main downstream signals, *Smad2* and *Smad3* (Figure 5). The phosphorylated *Smad2/3* then binds to *Smad4* and enters the nucleus where the complex binds to promoter regions of TGF-ß-responsive genes[7, 13, 20]. Hundreds of genes may be activated by this cytokine and its receptor, but tissue and organ specificity is directed by organ-explicit transcription factors[27]. The intracellular signaling is regulated by *Smad7* and other Smad antagonists, *SnoN*, *Ski*, and *TGIF*[7, 20]. TGF-ß provides for a powerful signal that, if not checked by inhibitory molecules, leads to the activation and differentiation of resident fibroblasts into activated myofibroblasts[2, 7, 10, 19].

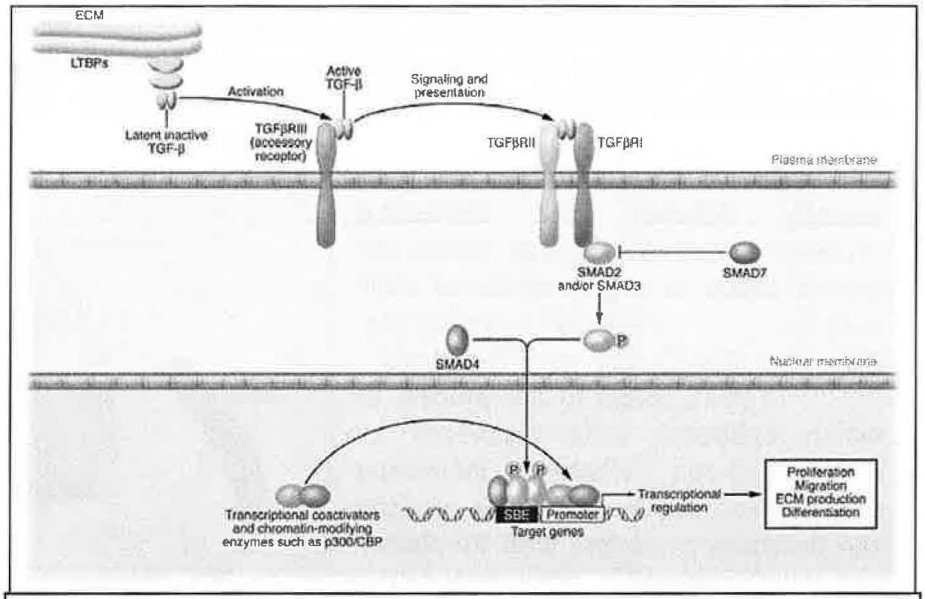

Figure 5: Illustration of TGF-β binding to its receptor and intracellular signaling through Smad-dependent signal transduction. Smad2/3 complex binds to Smad4 entering the nucleus and binding to promoter regions of pro-fibrotic target genes. From Varga J, Abraham D. *J Clin Invest.* Mar 2007;117:557-567.

D) <u>The activated fibroblast and myofibroblast:</u>

The hallmark of the fibrotic response is the proliferation and activation of tissue and organ fibroblasts and myofibroblasts within the extracellular matrix (ECM)[5, 7]. This matrix of connective tissue is composed of collagen, fibrillins, proteoglycans, and adhesion molecules. Within the ECM, resident tissue and organ fibroblasts respond to epithelial injury and to stimulatory cytokines by proliferating and migrating to the wound[7, 16]. During stimulation, the fibroblasts differentiate into activated myofibroblasts that have enhanced capacity to migrate, secrete important fibrillins and collagens, and activate tissue contraction in an attempt to repair the epithelial breach[2, 5, 24]. Fibroblast differentiation and myofibroblast activation is induced by a variety of cytokines and chemokines including TGF-β, CTGF, endothelin-1, interleukins, and

platelet-derived growth factor (PDGF)[10, 24]. The myofibroblast is a distinct cell that has markers and features of fibroblasts and smooth muscle cells, including smooth-muscle actin (SMA), cytoskeletal stress proteins, vimentin, desmin, and collagen[7, 10, 35]. Furthermore, these cells are also potent secretors of TGF-β[4]. In order to achieve normal wound healing, the myofibroblast response is checked with activation of cell death (apoptosis) and cell clearance[36]. Uncontrolled fibrosis is due to persistent activation of myofibroblasts through inhibition of apoptosis[36] and secretion of excessive extracellular matrix[5, 7, 37].

An abundant body of scientific research into the pathogenesis of fibrotic diseases has focused on the origin of the activated myofibroblast. Elegant studies have demonstrated that most tissues or organs contain resident fibroblasts, specialized cells, and/or epithelial cells with the capacity to differentiate into activated myofibroblasts. Although the progenitor cell for the myofibroblast cells may be different from tissue to tissue, the cellular and molecular mechanisms appear to be similar and provide a common theme[2]. In liver cirrhosis, the hepatic stellate cell (HSC) appears to be an important source of myofibroblasts in the fibrotic response, as recently reviewed by Dr. Don Rockey in his Internal Medicine Grand Rounds[4]. These resident cells have the potential for activation, proliferation, and differentiation into myofibroblasts capable of enzymatic digestion of the extracellular matrix (ECM) making them the key effector cells of cirrhosis[4, 5, 38].

The origin of myofibroblasts has been recently debated with increasing evidence demonstrating that within the injured tissue or organ, epithelial cells undergo epithelial-mesenchymal transition (EMT) into myofibroblasts[15, 35, 37, 39]. EMT refers to the process in which epithelial cells transform by losing cell-cell adhesion, increasing motility, and acquiring cellular markers and functions consistent with fibroblasts and myofibroblasts[19]. This provocative idea suggests that a large pool of recruitable myofibroblast progenitor cells exists within the injured tissue or organ and counters the dogma of a terminal differentiated state for epithelial cells[19]. Epithelial to mesenchymal transition (EMT) has been documented in mesangial and tubular epithelial cells of the kidney[20], biliary epithelial cells and hepatocytes[5, 40], and alveolar type II cells[19, 35, 39, 41, 42]. In chronic renal fibrosis, it is estimated that 33-50% of all mesenchymal cells are from epithelial origin[15, 39].

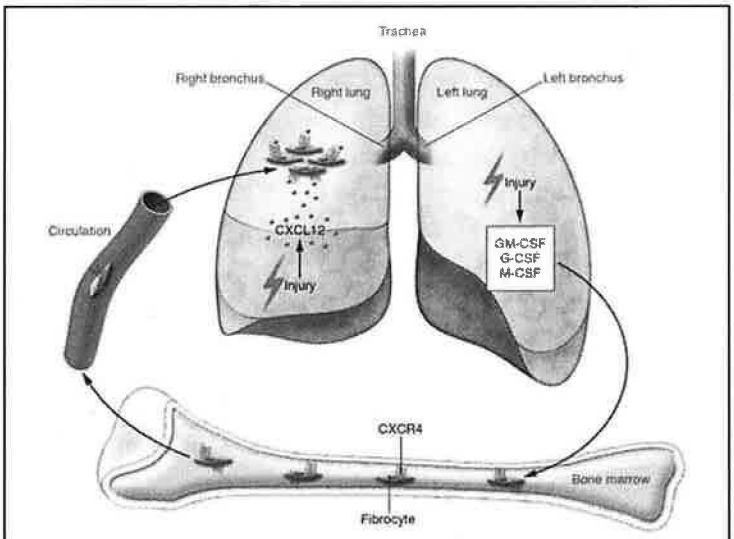

Figure 6: Illustration of circulating bone marrow progenitor cells or fibrocytes recruited into the lung during fibrosis. The recruitment may be directed by CXC chemokine family (CXCR4 and CXCL12). From Strieter RM *J. Clin. Invest.* 2007;117(3):549-556.

Recently, bone marrow-derived cells called fibrocytes have been reported as a potential source for activated myofibroblasts during renal, hepatic, and lung fibrosis[2, 5, 11, 20, 25, 43]. These cells are suspected to be mesenchymal stem cells and have been detected in the fibrotic scar[5, 43]. Forbes

and colleagues reviewed a series of male recipients of liver transplants from female donors, who later developed post-transplant liver fibrosis due to graft-versus-host disease. Using Y chromosome staining and immunohistochemistry for myofibroblast markers (i.e. α-SMA), co-localization was seen in cells located within areas of active fibrosis in the transplanted livers[44]. This suggests that progenitor cells from the bone marrow are recruited into tissue and organ fibrosis. A recent review suggests that circulating fibrocytes or progenitor cells express chemokine receptors that are believed to help them hone in to areas of fibrotic wound or organ repair where resident epithelial or endothelial cells are releasing important chemokines, such as CXCR4 and CXCL12 (See Figure 6)[25, 45]. Further studies are required to determine the importance of this mechanism in human fibrosis.

E) Changes in the extracellular matrix (ECM):

The extracellular matrix is a vibrant and dynamic structure consisting of a meshwork of connective tissue containing resident and infiltrating cells[7]. The ECM undergoes constant turnover requiring a delicate balance between matrix degradation and synthesis[7, 15, 16, 22]. Resident fibroblasts are influenced by the composition of the ECM and may become activated or differentiate into myofibroblasts as directed by cell-matrix interactions[5]. The fibrotic diseases are caused by either excessive extracellular matrix deposition by fibroblasts and myofibroblasts or by impaired degradation[3, 15, 16]. In cirrhosis, the hepatic stellate cells are able to secrete metalloproteinases (MMPs) or tissue inhibitors of metalloproteinases (TIMPs) disrupting the balance to either degradation or secretion of essential proteins of the ECM. A similar pattern is seen in hypertensive heart disease[22], IPF[16], scleroderma[7], and renal fibrosis[20]. Future studies will help determine whether antifibrotic agents could shift the balance towards degradation of excessive ECM.

CASE STUDY: A patient with IPF treated with a putative antifibrotic drug: Hope or despair?

Mr. W.M. is a 72 y/o man who presented to UTSW for a second opinion after developing progressive cough and dyspnea over the last year and having abnormal chest radiographs and CT scans. He did not have a history of asthma and was a non-smoker. He denied symptoms of heart failure, chronic infection, or connective tissue disease. An extensive environmental and occupational history was negative for significant inhalational exposures. Cardiovascular workup was negative for congestive heart failure. Examination revealed bibasilar "velcro-like" crackles in half of the lung fields and mild clubbing. Pulmonary function tests demonstrated a restrictive process with severe impairment in diffusion capacity with an FEV_1 70% of predicted, FVC 63% of predicted, and a DL_{CO} 35% of predicted. Radiographs were consistent with a diagnosis of idiopathic pulmonary fibrosis (Figure 7).

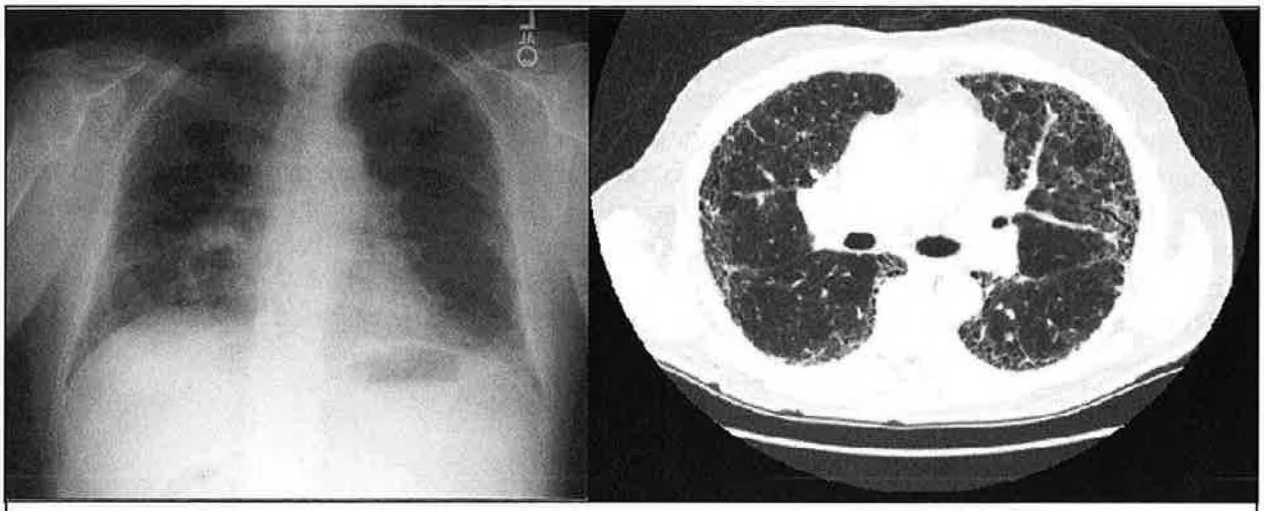

Figure 7: Chest radiograph revealing small lung volumes, subpleural and basilar reticular opacities, areas of honeycombing, and enlarged pulmonary arteries. High-resolution CT scan with the characteristic pattern of IPF: subpleural honeycombing, traction bronchiectasis, and intra- and interlobular septal thickening.

Video-assisted thoracoscopic biopsy confirmed a histological pattern of "usual interstitial pneumonia (UIP)" consistent with the clinical diagnosis of idiopathic pulmonary fibrosis (IPF). The patient was enrolled in an open-label trial utilizing Pirfenidone, an investigational antifibrotic agent, with stability of disease for 2 years. Discontinuation of this agent led to decline in lung function suggesting a treatment benefit with Pirfenidone (Figure 8)[46]. Nine months after Pirfenidone discontinuation, the patient was admitted to Zale-Lipshy University Hospital with an acute IPF exacerbation leading to respiratory failure and death.

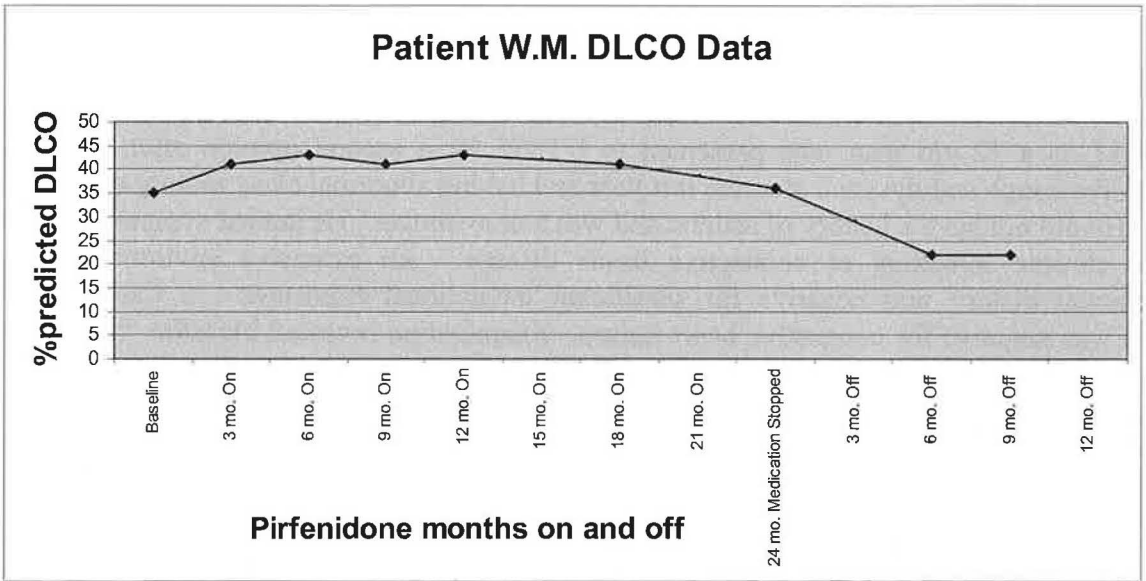

Figure 8: DLCO data on and off pirfenidone in a patient with idiopathic pulmonary fibrosis. Decline in DLCO was noted after discontinuation of this agent.

IDIOPATHIC PULMONARY FIBROSIS: An unrelenting fibrotic disease.

Idiopathic pulmonary fibrosis accounts for approximately 50% of the Idiopathic Interstitial Pneumonias. The prevalence of IPF is 27-29 cases per 100,000 persons[47]. In contrast with hypertensive heart disease, liver cirrhosis, and renal fibrosis, IPF has no known trigger or identifiable repetitive injury[12]. 2% of cases of IPF are familial in origin[25, 48]. When compared to the general population, certain professions or lifestyles, such as farming and raising livestock, exposure to wood dust, metals, stone, silica, and cigarette smoking are associated with double the risk for IPF[8, 49]. Most patients with IPF die within 3-8 years from onset of symptoms with a 5-year survival of ~70%, a figure comparable with lung cancer[25, 47]. The course may be variable with either stable disease for years or with a progressive decline in lung capacity[17, 50]. Approximately 50% of patients die after a 4-week period of deterioration consistent with a diagnosis of acute exacerbation of IPF[50, 51].

Recent publications have proposed various hypotheses for the pathogenesis of IPF[11, 17, 25, 52]. It is now understood that the IPF lesion has very little acute inflammation and that its pathogenesis is best explained by the "epithelial-fibroblastic" hypothesis[14, 53, 54]. Repetitive insults to the bronchial and alveolar epithelium leads to unregulated repair with activation of fibroblasts and differentiation into myofibroblasts[25, 52, 53]. The fibrotic mechanisms in IPF appear to be similar to those described in previous sections with epithelial cell death, fibroblast proliferation, and deposition of excess extracellular matrix[17, 34, 55, 56]. This aberrant repair leads to architectural distortion with alveolar collapse, interstitial thickening, and formation of the end-stage "honeycomb" lung. The collagen deposition occurs in the lung interstitium, widening the alveolo-capillary interface leading to impaired gas exchange and hypoxemia[8, 25].

In IPF, the "fibroblastic foci" is the focal center of fibrotic activity. These foci contain intense mesenchymal cell proliferation and collagen deposition overlying or in close proximity to sites of epithelial injury[13, 34]. The fibroblastic foci appear to be "leading edge" of an organized fibrotic matrix extending throughout the interstitium (Figure 9)[25]. They contain fibroblasts expressing markers of myofibroblasts, such as α-smooth muscle actin (α-SMA), within an extracellular collagen and fibronectin matrix[34]. Furthermore, increased TGF-β1 expression has been demonstrated within these foci in lung biopsies of patients with IPF[15, 29]. Interestingly, the identification of an increased number of fibroproliferative foci in surgical lung biopsies directly correlates with increased mortality[34, 57].

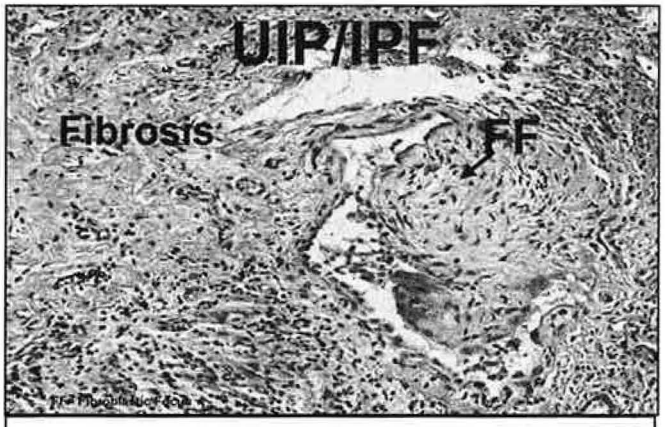

Figure 9: Fibroblastic foci: The leading edge of fibrosis with fibroblast and myofibroblast infiltration and collagen deposition. From Strieter RM. Chest 2005;128(5 suppl 1):526S-32.

Recent work with familial IPF has proposed a supplemental mechanism for the "epithelial-fibroblastic" hypothesis focusing on alveolar cell turnover, apoptosis, and death. In familial IPF, mutations in telomerase, an essential enzyme that maintains the normal length of the

chromosome ends (telomeres) during cell division, was demonstrated[48, 58]. As DNA telomeres shorten, arrest of the cell cycle occurs and apoptosis is activated. It is now suspected that the alveolar epithelium undergoes rapid and extensive turnover. During injury, acquired or inherited telomerase mutations may lead to premature epithelial cell death or apoptosis forcing repair into an unregulated fibrotic response characterized by fibroblast and myofibroblast activation[48, 58].

CONVENTIONAL THERAPY FOR IPF AND OTHER FIBROTIC DISORDERS:

Steroids and immunomodulatory therapy- The wrong strategy!

For the last 50 years, the therapy of IPF and the most common fibrotic disorders has focused on anti-inflammatory and immunomodulatory therapy with little effect on disease progression[2, 7, 17, 18]. Prednisone therapy reduces inflammatory cell influx into tissues, immune complex formation, and resident macrophage functions with little effect on progression of disease[18]. In IPF and other fibrotic lung diseases, the inflammatory hypothesis has been challenged by a new paradigm of fibrosis as an exuberant and unregulated wound repair response[11], perhaps independent or relatively free of inflammation and thus, poorly responsive to steroids and immunomodulators[17, 18].

In IPF, recent reviews have demonstrated the inefficacy of prednisone and cytotoxic therapy, such as azathioprine, and cyclophosphamide[8, 17, 18, 47]. In IPF, a placebo-controlled trial with prednisone has not been performed and experts recommend against its use as monotherapy[18]. In fact, some experimental data suggests that prednisone may have profibrotic effects in lung repair after injury. A recent *in vitro* model of fibrosis by Wen and colleagues demonstrated synergistic enhancement of TGF-β and corticosteroids on lung fibroblast collagen gel contraction[59]. Azathioprine in combination with the anti-oxidant N-acetyl cysteine (NAC) was recently evaluated in a randomized, controlled clinical trial for the treatment of IPF, the INFIGENIA study[60]. This study was criticized for its lack of a "no treatment" group for comparison[18, 61]. When compared to the "no treatment" arm of two recent placebo-controlled clinical studies, the use of azathioprine and prednisone did not appear to impact the decline in forced vital capacity (FVC) (Figure 10)[18]. A recommendation for the use of azathioprine in IPF is not justified by available clinical data and a comparison study with a "no treatment" group is needed[18, 61].

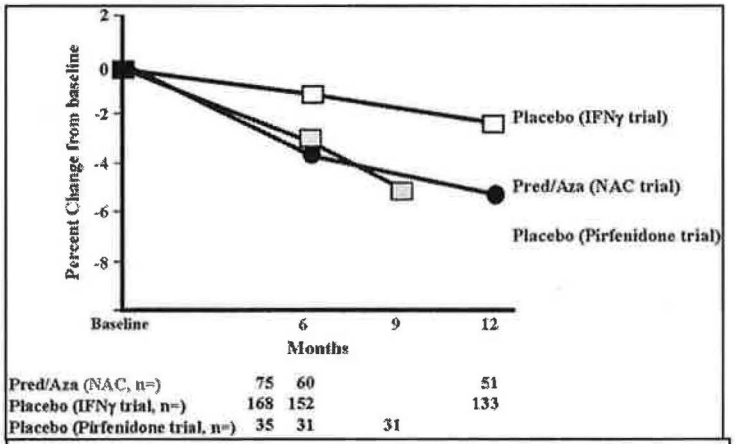

Figure 10: A comparison of the results of 3 different studies in IPF suggests no treatment benefit for prednisone and azathioprine. From Walter, N. *Proc Am Thorac Soc.* 2006;3:330-338.

Cyclophosphamide, an alkylating chemotherapy agent with potent immunomodulatory and immunosuppressive effects[18] has been used as second-line therapy in IPF[47] despite no available rigorous randomized controlled clinical trials. The best data comes from a large registry of IPF

comparing 82 patients treated with cyclophosphamide and prednisone versus 82 untreated patients demonstrating no difference in survival days at 1,431 and 1,665, respectively (p=0.58)[18, 62]. In progressive systemic sclerosis (PSS), the Scleroderma Lung Study Research group (2006) demonstrated modest improvements in FVC, DLCO, dyspnea scores, and skin thickness with cyclophosphamide, but at the expense of increased toxicity[63].

In summary, the use of corticosteroids or cytotoxic therapy does not appear to be justified in patients with idiopathic pulmonary fibrosis (IPF). In patients with progressive systemic sclerosis or scleroderma, cyclophosphamide leads to modest improvements in markers of disease progression. Some patients with liver disease, such as autoimmune and severe alcoholic hepatitis respond favorably to corticosteroid treatment with retardation of cirrhosis. For a large number of patients, the development of organ fibrosis is inevitable despite treatment for the inciting fibrotic insult or agent. The future of medicine rests on designing therapeutic agents capable of positively intervening with the fibrotic pathway[2, 7, 47, 64].

ANTIFIBROTIC AGENTS: Targeting unregulated wound healing and repair.

As knowledge of the fibrotic mechanisms expands, potential pathways for therapeutic targets are being identified[7, 65]. Animal studies have been promising[3, 20] but early work with human disease has been slow and somewhat disappointing. Currently, only a small number of clinical studies with potential antifibrotic agents are underway[2]. This might be due to the fact that the fibrotic process is a slow one with an expected protracted course for clinical studies and high expense. Furthermore, the activation of fibrosis has alternate and redundant pathways so that inhibition of one fibrotic cell, cytokine, chemokine, or receptor may be bypassed by other signals. Effective antifibrosis therapy will likely require more than one drug targeting the various compartments of the fibrotic pathway[47]. The perfect antifibrotic drug should have:

- Oral bioavailability
- Little organ toxicity
- Potent anti-fibrotic effect at the areas of unregulated repair
- Preservation of normal organ and tissue homeostasis and wound repair
- Low cost[65]

The potential toxicity of antifibrotic drugs must not be underestimated. Healthy wound healing and repair depends on the same mechanisms producing aberrant fibrosis. Two examples of this phenomenon come from the study of TGF-β inhibition and the interference of extracellular matrix (ECM) turnover. The fibrotic effect of TGF-β in animal models is well-known. Paradoxically, this potent cytokine also has prominent anti-inflammatory effects and continued inhibition could lead to unopposed inflammation[20]. In another example, development of drugs designed to inhibit metalloproteinase activity was hampered by the development of tendonitis due to healthy connective tissue degradation[22]. Thus, caution must be taken during the clinical application and study of current and future antifibrotics. This section will discuss potential antifibrotic agents (Table 3), focusing primarily on IPF.

Table 3: Antifibrotic Agents- available, tested, or under active investigation:
(Classified by major fibrotic pathway or mechanism)

Anti-inflammatory:
D-penicillamine[47]
IL-10[64, 65]

Inhibition of Reactive Oxygen Species (ROS):
N-acetyl cysteine (NAC)[18, 60]
Vitamin C and E[65]
Saiko-keishi-to (herbal preparation)[23]
Polyenylphosphatidylcholine[4, 65]
Superoxide dismutase mimetics[12]

Inhibition of fibrin clot formation:
Systemic anticoagulation[66, 67]
Nebulized heparin[15]

Immunomodulator:
Interferon gamma-1b[18, 68, 69]
Sirolimus (Rapamycin)[18]
Rituximab (B cell depletion)

Angiogenesis, vascular remodeling, and vasoactivity:
Bosentan[2, 18]

Fibroblast and myofibroblast activation and proliferation:
Relaxin[70]
Pirfenidone[18, 71]
Leukotriene inhibitors (Zileuton)[47]
Interferon gamma-1b[7]
Colchicine[18]
Bosentan[18]

Cytokine/Chemokine effects:
TGF-β blockade[2]:
- Imatinib mesylate (Gleevec)[37, 72]
- TGF-β antibody[15]
- Angiotensin II inhibition[18, 73]
- Smad-7[20]
- Relaxin[1, 70]

CTGF blockade[15]:
- Monoclonal antibody (FG3019)[47]

Keratinocyte growth factor (Repifermin)[18, 74]
TNFα inhibition[15, 47]:
- Etanercept[15]
- Adalimumab
- Pentoxyfilline[18]
- Thalidomide[2]

Interleukin-10 (IL-10)
All-trans-retinoic acid (ATRA)[75]
Suramin[18]

Extracellular matrix (ECM) synthesis and turnover:
Aerosolized heparin[15]
Aerosolized urokinase
TIMP-1 inhibitor[2, 76]
Relaxin[1, 70]
Colchicine[18]
D-penicillamine[18]

Epithelial-Mesenchymal transition:
Hepatocyte growth factor[37]
TGF-β blockade[2]

1) Interleukin 10:

Interleukin-10 has important anti-inflammatory and immunomodulatory effects through reduction of pro-inflammatory Th-1 cytokines (TNF-α, interferon-γ, IL-1, and IL-2). This agent has been used in chronic hepatitis C infection with improvement in fibrotic scores but increase in viral titers significantly limiting its use[64]. The use of IL-10 in IPF remains controversial. In patients with IPF, IL-10 levels in the serum, bronchoalveolar lavage, and lung tissue are higher than those found in normal lung or other benign interstitial lung diseases[77, 78]. In fact, the use of IL-10 could shift the immune response to a predominant Th-2 response potentially promoting fibrosis[11].

2) Anticoagulation therapy:

During tissue injury and disruption, activation of platelets and fibrin clot formation occurs in an attempt to stabilize the endothelial or epithelial breach[2]. This fibrinous exudate, deposited at the site of injury, promotes mesenchymal cell proliferation, tissue remodeling, and architectural distortion[79, 80]. Mice overexpressing plasminogen activator inhibitor-1 gene, an inhibitor of fibrinolysis, are thrombophilic and are more susceptible to pulmonary fibrosis when exposed to bleomycin[79]. In human studies, the bronchoalveolar lavage of IPF patients contain high levels of inhibitors of fibrinolysis[81]. The compartmentalized delivery of anticoagulant (heparin) or thrombolytic agents (urokinase) to the lung via nebulization is effective in decreasing bleomycin toxicity in rabbits. A human Phase I toxicity study with inhaled heparin in patients with IPF is currently underway in Germany[15].

Further interest in anticoagulation for the treatment of idiopathic pulmonary fibrosis was fueled by an interesting report by Kubo and colleagues. Fifty-six IPF patients hospitalized with an acute exacerbation were given steroids and randomized either to systemic anticoagulation with low-molecular weight heparin followed by oral anticoagulation or to placebo. The major cause of death was IPF exacerbation with significantly reduced mortality in the anticoagulated patients (18%) versus the patients not receiving anticoagulation (71%, p=0.008)[66]. Autopsies and surgical lung biopsies performed in patients with IPF exacerbations have revealed an acute lung injury pattern with diffuse alveolar damage characterized by alveolar hyaline membrane deposition and microvascular *in situ* thrombosis (Figure 11)[17, 51, 66]. The mechanism for improved survival with anticoagulation for IPF exacerbations may be due to prevention of intra-alveolar and vascular fibrin clot formation. This study has been criticized by the high frequency of IPF exacerbations and the overall high mortality of the placebo group when

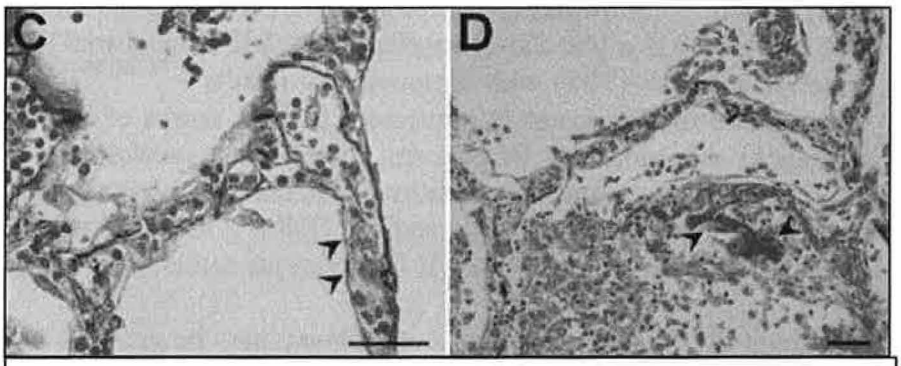

Figure 11: Photomicrograph illustrating the pattern of diffuse alveolar damage (DAD) in a patient with IPF exacerbation. Panel C demonstrates a small thrombus in a capillary structure (arrowheads). Panel D demonstrates the hyaline membranes or alveolar fibrinous exudate as stained with Elastica-Masson staining. From Kubo H, Nakayama K, Yanai M, et al. Chest 2005;128(3):1475-82.

compared to historical controls[18, 50]. This study is provocative and hypothesis-generating. The exact role of anticoagulation in IPF or other fibrotic diseases still remains unknown.

3) Endothelin receptor antagonists:
Endothelin-1 (ET-1) is secreted by endothelial and smooth muscle cells with effects on proliferation, vasoactivity, and immunomodulation[18, 47]. ET-1 also has direct effects on fibroblasts and myofibroblasts with secretion and contraction of extracellular matrix (ECM)[82]. In IPF, ET-1 is highly expressed by epithelial cells, endothelial cells, alveolar macrophages, and fibroblasts[83]. In fact, transgenic ET-1 overexpressor mice develop spontaneous pulmonary fibrosis at age 9-12 months[84, 85]. Animal models suggest that ET-1 inhibition may have antifibrotic effects[83]. Bosentan is an FDA-approved endothelin receptor A and B antagonist for the treatment of Primary Pulmonary Hypertension (PPH) and functions as a pulmonary artery vasodilator with effects on vascular remodeling. Bosentan appears to be well-tolerated in IPF without worsening of hypoxemia or increased shunt[84]. Two studies, BUILD-1 and BUILD-2, focused on IPF and pulmonary fibrosis due to progressive systemic sclerosis (PSS), respectively. In both studies, no improvement in exercise was seen, as measured by the 6-minute walk. For BUILD-1 (IPF patients), there was a trend towards reduced mortality or treatment failure at 1 year (secondary endpoints) in the Bosentan group when compared to placebo (22.5% versus 36.1%, p=0.076). In BUILD-2 (PSS patients), no improvement in secondary endpoints was seen[86]. The BUILD-1 data has led to the design of a multicenter Phase III study (called BUILD-3) in IPF and UT Southwestern will be one of the participating sites[87].

4) TGF-β inhibition:
The inhibition of TGF-β and its receptor has been a topic of intense research[7, 15, 65, 72]. As delineated in the previous sections, TGF-β appears to be the master fibrotic cytokine of most organs and tissues with effects on epithelial-mesenchymal transition, fibroblast/myofibroblast proliferation and activation, and extracellular matrix deposition[7]. Its receptor and intracellular molecular signaling are potential targets for drug development with the use of monoclonal antibodies, direct interference with TGF-β intracellular signaling (Smad protein phosphorylation), or indirectly through inhibition of non-Smad pathways[7, 20]. Various pharmaceutical companies are developing monoclonal antibodies to TGF-β. One of them, CAT-192 was tested in a placebo-controlled Phase I/II clinical trial with 45 patients with progressive systemic sclerosis (PSS) with disappointing results[20, 72, 88, 89]. The CAT-192 antibody infusions did not lead to significant improvement in skin scores or in mRNA expression of markers of fibrosis in skin biopsies. Of concern, four patients receiving the CAT-192 humanized antibody died from progression of systemic sclerosis involving the heart and lungs[88]. A second humanized monoclonal antibody against TGF-β (GC-1008) is being evaluated in a Phase I study in idiopathic pulmonary fibrosis (IPF) at various centers, including UT Southwestern[90].

Inhibition of TGF-β via a non-*Smad* pathway may be possible with the use of imatinib mesylate (Gleevec™), a drug approved by the FDA in 2001 for the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors[72, 91, 92]. Imatinib mesylate inhibits the platelet-derived growth factor (PDGF) tyrosine kinases *c-Abl* and *c-Kit*, which also block TGF-β signaling through a non-Smad pathway[72, 76, 93]. In various animal and *in vitro* fibrotic models of lung, liver, skin, bone marrow and kidney, this drug inhibited fibroblast proliferation and secretion of extracellular matrix[13, 20, 76, 91, 93]. Cultured fibroblasts from skin biopsies of patients

with systemic sclerosis are inhibited by this agent[72, 92, 94], with similar effects in animal models of autoimmune nephritis and bleomycin-induced lung fibrosis[13, 91, 93]. In 2003, a patient with long-standing rheumatoid arthritis and a new diagnosis of chronic myelogenous leukemia was treated with imatinib mesylate with suppression of rheumatoid arthritic changes[72, 95]. Furthermore, patients treated with imatinib for chronic myelogenous leukemia demonstrate improvement in bone marrow fibrosis[92].

The only human trial of imatinib mesylate as a potential antifibrotic drug is being conducted in patients with Idiopathic Pulmonary Fibrosis (IPF). This phase II randomized-controlled clinical trial has completed enrollment with results pending at this time. It has as primary endpoints either a 10% fall in FVC or death[18, 47, 91, 96]. Although there is optimism with the use of imatinib for fibrotic diseases, there is also concern about the prolonged effects of TGF-β inhibition, such as uncontrolled inflammation and tumorigenesis[20, 97]. Furthermore, the use of imatinib mesylate in chronic myelogenous leukemia has been associated with the development of congestive heart failure in 10 patients[72, 98] and paradoxical drug-induced pulmonary fibrosis[99].

5) Anti-oxidant therapy: Effects of N-acetyl cysteine (NAC) in fibrotic diseases:
Inflammation is typically associated with the production and secretion of radical oxygen species (ROS) by resident and recruitable inflammatory cells. Excess ROS have direct effect on organ and tissue epithelium, fibroblasts, protease-antiprotease balance, and extracellular matrix turnover[12]. Animal models of fibrotic disease, such as cirrhosis, hypertrophic cardiomyopathy, and IPF, have demonstrated the potential benefit of anti-oxidant therapy in maintaining the oxidant-antioxidant balance and directly inhibiting TGF-β signaling[12, 100, 101]. NAC is a sulfhydryl molecule with powerful antioxidant properties and is the precursor of glutathione. Its exogenous administration has been documented to increase glutathione levels in human lung[18].

The IFIGENIA study with NAC in IPF was recently published in the New England Journal of Medicine[60]. The design of the study had a potential flaw, the lack of a "no treatment arm", as NAC was tested as an adjunct to prednisone and azathioprine. The group receiving NAC (at levels higher than those approved by the FDA) had a relative reduction of 9% and 24% in the decline of the forced vital capacity (FVC) and D_LCO, respectively[18, 60]. In the accompanying editorial, an interesting explanation for the benefits seen with NAC focused on the reduction of azathioprine side effects, such as bone marrow toxicity[61]. This has led to the recommendation of NAC use in patients with IPF receiving azathioprine and prednisone[18].

6) Interferon gamma-1b:
In the study of antifibrosis, one of the fastest transitions from bench to clinical research focused on the hypothesis of swaying wound repair from a fibrotic cytokine Th-2 environment (IL-4, IL-5, and IL-13) to a Th-1 cytokine milieu through the administration of exogenous interferon-γ[7]. Animal models have demonstrated that interferon-γ inhibits fibroblast proliferation, TGF-β expression, and extracellular matrix deposition[7, 11, 52, 64, 69, 102]. Tissues of patients with progressive systemic sclerosis and IPF demonstrate predominant Th-2 cytokines and decreased levels of interferon-γ[7, 69].

Unfortunately in human fibrotic lung disease, the antifibrotic effects of interferon-γ have not lived up to the expected hypothetical response seen *in vitro* or in animal models. A recent

controlled clinical trial of 502 patients with chronic hepatitis C infection demonstrated no benefit in fibrosis scores with interferon-γ administration for 1-year[103]. In systemic sclerosis, interferon-γ also had disappointing results with no changes in Rodnan skin scores and increased side effects[104].

In IPF, interferon-γ1b created controversy after its off-label use (FDA-approved for the treatment of chronic granulomatous disease) was sparked by a small pilot study by Ziche and colleagues demonstrating dramatic improvement in lung function[105]. Unfortunately, these preliminary results could not be reproduced in subsequent randomized, controlled clinical trials. Raghu and colleagues completed a multicenter Phase III clinical study of 330 patients with no benefit in progression-free survival, pulmonary function tests, or quality of life[69]. Subgroup analysis suggested a possible mortality benefit for patients with FVC > 55% predicted. This led to a large randomized, controlled Phase III study (called the INSPIRE trial) looking at overall mortality as primary endpoint. On March 5, 2007, the INSPIRE trial was discontinued by an independent monitoring committee after randomization of 826 IPF patients with mild-to-moderate disease found no mortality difference between placebo and interferon-γ (12.7% versus 14.5%, respectively)[106]. This latest study likely marks the end of interferon-γ in the treatment of fibrotic diseases.

7) Pirfenidone, a direct fibroblast inhibitor:
Pirfenidone is a synthetic [5 methyl-1-phenyl-2-(1H)] pyridone with antifibrotic potential and with oral formulation[106]. This agent has antifibrotic effects in animal models of lung[107], kidney[108], and liver fibrosis[109, 110]. Pirfenidone appears to decrease pro-fibrotic cytokine expression and inhibit fibroblast proliferation[47, 108]. This drug was tested in a small pilot study of chronic hepatitis C with a 30% reduction in fibrosis at 1 year and reduction in mRNA expression of fibrotic cytokines and markers, such as TGF-β and tissue inhibitor of metalloproteinase-1 (TIMP-1), in sequential liver biopsies[111]. Further randomized, controlled clinical trials are needed to confirm these findings.

An open label study with Pirfenidone in IPF demonstrated stabilization of lung function in previously deteriorating patients[112]. Recently, our group has demonstrated that discontinuation of Pirfenidone in patients with moderate-to-severe IPF leads to a more rapid decline in lung function than seen while on this agent[46]. This observation needs to be evaluated in larger studies but suggests that abrupt discontinuation of Pirfenidone may be detrimental. Azuma and colleagues in Japan conducted a phase-III randomized, controlled clinical trial in 107 IPF patients with trend towards improvement in the 6-minute walk test at 6 months when compared to placebo ($p=0.072$)[71]. Interestingly, this study was discontinued by the safety monitors before its completion after no IPF exacerbations were observed in the Pirfenidone group versus 14% in the placebo arm[71]. This data has led to two large Phase III randomized, controlled clinical trials in IPF (CAPACITY 1 and 2), which are close to completing enrollment and one of the study centers is UT Southwestern[113]. These studies plan to randomize 580 patients with primary endpoint being a significant reduction in the decline in the forced vital capacity (FVC).

8) CTGF Blockade (FG-3019 monoclonal antibody):
Connective tissue growth factor (CTGF) is a protein of the CCN protein family that functions downstream from the TGF-β/receptor complex and its intracellular signaling. CTGF

transcription is upregulated by TGF-β signaling through the *Smad* pathway and both cytokines appear to work synergistically. Depending on the tissue or cell type, CTGF gene expression affects cellular proliferation, migration, and ECM synthesis. Increased CTGF expression is seen in animal models of fibrosis[47, 114]. Perhaps by working downstream from TGF-β, chronic CTGF inhibition may have specific antifibrotic effects with potentially less toxicity than that expected with TGF-β inhibition[114]. FG-3019 is a monoclonal antibody against CTGF that has recently completed a Phase I toxicity study in patients with IPF with good safety and tolerance[115]. An ongoing Phase-1b study is currently recruiting subjects with diabetic microalbuminuria[116].

9) Rapamycin (sirolimus):
Rapamycin, a macrolide that is synthesized by *Streptomyces hygroscopicus*, has immunosuppressive activity by preventing lymphoid cell proliferation through inhibition of cytokine and growth factor signaling[47]. Rapamycin is being used as an anti-rejection drug in solid organ transplantation. Recently, the molecular target for rapamycin has been identified as an intracellular serine/threonine protein kinase called *mTOR*, an important molecule of cell proliferation and tissue/organ growth[117]. In animal models of liver, pulmonary, and renal fibrosis, this agent appears to inhibit mesenchymal cell proliferation with significant reduction in extracellular matrix deposition[47, 118, 119]. Excitement about the antifibrotic potential of rapamycin has been tempered by a report of lung toxicity in renal transplant patients[120] and increased proteinuria and worsening GFR in a Phase II study in patients with FSGS (focal segmental glomerulosclerosis)[121].

10) Relaxin:
Relaxin is a peptide hormone of the insulin family with various functions in the body[1]. It is primarily secreted in the ovaries and is an important hormone of pregnancy and childbirth. During normal parturition, the birth canal and symphysis pubis, normally composed of connective tissue and collagen, undergo collagen breakdown through the action of relaxin allowing for the dilatation of the birth canal and passage of the fetus[1]. Transgenic mice deficient in relaxin develop spontaneous interstitial fibrosis of the lungs, kidneys, heart, and skin[122, 123]. Relaxin appears to inhibit ECM synthesis and fibroblast differentiation into myofibroblasts[122]. In humans, the expression of relaxin is not limited to the reproductive system but also seen in heart failure suggesting an important role in ECM production and turnover and its effects on hemodynamics[124]. Human studies have focused on progressive systemic sclerosis (PSS) with a daily 24-week recombinant relaxin infusion being safe and associated with improved skin thickness and a trend to stabilization of FVC decline[70]. Despite the excitement of positive animal and human studies, there are no current clinical trials in patients with renal, pulmonary, or liver fibrosis.

11) Future agents:
Recent reviews have focused on newer antifibrotic targets for future drug development. The transition from bench research to clinical use for these agents is years away and depends on further discoveries of the mechanisms regulating fibrosis. A primary goal is to develop agents that target tissue and organ specific fibrosis without altering normal epithelial and tissue repair and turnover. Also, the effects of antifibrotic therapy on fostering a persistent inflammatory state or tumorigenesis need further investigation.

One example of a recent scientific breakthrough with potential for future investigation comes from two recent studies of patients with familial IPF demonstrating mutations in the genes encoding for telomerase. These affected patients have shorter telomere lengths[48, 58]. Telomere shortening leads to decreased capacity for epithelial cell division and cell death and may be the initiating factor for the unregulated fibrotic repair characterized by fibroblast and myofibroblast activation[53]. In fact, TGF-β, the potent fibrotic cytokine, leads to inhibition of telomerase gene expression via its Smad3 intracellular signaling and strengthens the interactive role of cytokines in epithelial cell senescence[125]. Enhancing telomerase activity could prevent or retard tissue and organ fibrosis[53] and could represent an important future therapeutic target. Other potential therapeutic targets of tissue and organ fibrosis are listed in Table 4.

Table 4: Potential future therapeutic antifibrotic targets:

Antifibrotic Targets:	**Specific molecules:**
TIMP inhibition	TIMP-1 antibody
TGF-β signaling inhibition	Smad7, BMP-7
TGF-β1 gene silencing	shRNA for TGF-β1
Antifibrotic chemokines	CXCL10 and CXCL11
Chemokine receptor antagonists	
Collagen synthesis inhibitors	Prolyl hydroxylase inhibitor
Integrin/adhesion molecule antagonists	ICAM-1 and VCAM-1 antibodies α1β1 and αvβ6 integrin inhibitors
Myofibroblast pro-apoptotic drugs	
MMP inhibitors	MMP2, MMP9, and MMP12 inhibitors
TIMP inhibitors	TIMP-1 antibody
Stem/progenitor cell transplantation	
Antisense therapy	Against c-Ki-*ras* protein
Keratinocyte growth factor (KGF)	
Hepatocyte growth factor (HGF)	
Bone morphogenetic protein-7	
Telomerase induction[48, 58]	hTERT, hTR, TERC
Caveolin-1 induction[53, 126]	

Adapted from Neilson EG. Nature Clinical Practice Nephrology 2006;2(2):101-8; Ask K, Martin GEM, Kolb M, Gauldie J. Proc Am Thorac Soc 2006;3(4):389-93; Wynn TA. J Clin Invest 2007;117(3):524-9; and Selman M, King TE, Jr., Pardo A. Ann Intern Med 2001;134(2):136-51.

CONCLUSION: The future of medicine?

Fibrotic diseases lead to significant morbidity and mortality despite progress in prevention and treatment of disease. Diseases with known cause such as hypertensive nephrosclerosis, hypertensive cardiomyopathy, and liver cirrhosis are best managed with primary prevention utilizing an expanding drug and immunization armamentarium. Despite this progress, some patients will not be identified in time to allow effective prevention and others may not respond to therapy with progression to unregulated fibrotic repair. In idiopathic diseases, such as IPF and systemic sclerosis, prevention is not possible and antifibrotic therapy is the only viable option.

Significant strides have been made in the identification of the fibrotic mechanisms and their possible targets for attenuation. The transition from bench to clinical studies has been slowed by the expense of drug design and study, concerns about patient safety, and lack of non-invasive markers of fibrosis regression. As previously mentioned, there are no FDA-approved drugs for the treatment of fibrosis. The "off-label" use of available drugs is not justified in an individual basis and must be carefully addressed by randomized-controlled clinical trials. Putative antifibrotic agents have demonstrated mixed results in human studies with disease response markedly below that predicted by *in vitro* and animal models. Furthermore, heightened concern about antifibrotics perpetuating inflammation, interfering with normal tissue and organ homeostasis, and promoting tumorigenesis has tempered enthusiasm. Nevertheless, control of unregulated fibrosis will clearly advance medicine into the future with protection of critical organs and tissues, maintenance of solid organ transplant function, and its anti-aging potential.

In the coming years, we will see an exponential growth in the number of antifibrotic agents moving from bench to clinical research. The mechanisms of fibrosis follow similar themes across all organs and collaboration between specialties and between basic scientists and clinical researchers will be of utmost importance. Every internist should be knowledgeable of the fibrotic pathway in order to understand the mechanism of future drug action and predict expected toxicity. Furthermore, this field yields so much promise that the establishment of a multi-disciplinary Antifibrosis center should be a major priority for academic medical centers.

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