British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2011.01247.x
www.brjpharmacol.org

Themed Issue: Respiratory Pharmacology

REVIEW

Novel therapeutic approaches for pulmonary fibrosis

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Keywords
pulmonary fibrosis; fibrosis therapy; drug targets; myofibroblast

Received
19 November 2010
Revised
10 January 2011
Accepted
12 January 2011

Pulmonary fibrosis represents the end stage of a number of heterogeneous conditions and is, to a greater or lesser degree, the hallmark of the interstitial lung diseases. It is characterized by the excessive deposition of extracellular matrix proteins within the pulmonary interstitium leading to the obliteration of functional alveolar units and in many cases, respiratory failure. While a small number of interstitial lung diseases have known aetiologies, most are idiopathic in nature, and of these, idiopathic pulmonary fibrosis is the most common and carries with it an appalling prognosis – median survival from the time of diagnosis is less than 3 years. This reflects the lack of any effective therapy to modify the course of the disease, which in turn is indicative of our incomplete understanding of the pathogenesis of this condition. Current prevailing hypotheses focus on dysregulated epithelial–mesenchymal interactions promoting a cycle of continued epithelial cell injury and fibroblast activation leading to progressive fibrosis. However, it is likely that multiple abnormalities in a myriad of biological pathways affecting inflammation and wound repair – including matrix regulation, epithelial reconstitution, the coagulation cascade, neovascularization and antioxidant pathways – modulate this defective crosstalk and promote fibrogenesis. This review aims to offer a pathogenetic rationale behind current therapies, briefly outlining previous and ongoing clinical trials, but will focus on recent and exciting advancements in our understanding of the pathogenesis of idiopathic pulmonary fibrosis, which may ultimately lead to the development of novel and effective therapeutic interventions for this devastating condition.

LINKED ARTICLES

This article is part of a themed issue on Respiratory Pharmacology. To view the other articles in this issue visit http://dx.doi.org/10.1111/bph.2011.163.issue-1

Abbreviations

5-LO, 5-lipooxygenase; A-a, alveolar-arterial; ACE, angiotensin converting enzyme; AEC, alveolar epithelial cell; AGT, angiotensinogen; ALK, activin-like kinase receptor; ANGII, angiotensin II; AT(1),angiotensin II type 1 receptor; BALF, bronchoalveolar lavage fluid; CCL2, chemokine (C-C motif) ligand 2; CCR2, C-C chemokine receptor 2; COX, cyclooxygenase; CTGF, connective tissue growth factor; CXCL12, C-X-C motif chemokine ligand-12; CXCR4, C-X-C motif chemokine receptor-4; DL_{co}, carbon monoxide diffusing capacity; ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; ET-1, endothelin-1; (F)VC, (forced) vital capacity; HGF, hepatocyte growth factor; HRCT, high resolution computed tomography; HSP, heat shock protein; IFN-γ, interferon-γ; IL-13, interleukin-13; IPF, idiopathic pulmonary fibrosis; KGF, keratinocyte growth factor; LAP, latency-associated peptide; LOXL2, lysyl oxidase-2; LPA, lysophosphaditic acid; LTB_4, leukotriene B_4; MCP-1, monocyte chemotactic protein 1; mTOR, mammalian target of rapamycin; NAC, N-acetylcysteine; NOX, NADPH-oxidase; PAR, proteinase-activated receptor; PDGF, platelet derived growth factor; PGE_2, prostaglandin E_2; PGI_2, prostacyclin; PHT, pulmonary hypertension; QoL, quality of life; ROS, reactive oxidative species; TGF-β, transforming growth factor-β; Th, T-helper; TM, tetrathiomolybdate; TNF-α, tumour necrosis factor-α; VEGF, vascular endothelial growth factor; α-SMA, α-smooth muscle actin

Introduction

Pulmonary fibrosis represents the end stage of several interstitial lung diseases, including the idiopathic interstitial pneumonias, and is characterized by the excessive deposition of extracellular matrix (ECM) within the pulmonary interstitium. Among the idiopathic interstitial pneumonias, idiopathic pulmonary fibrosis (IPF) represents the commonest and most fatal condition with a median survival of 3–5 years following diagnosis. Fibrosis in IPF is generally progressive, refractory to current pharmacological intervention and inexorably leads to respiratory failure due to obliteration of functional alveolar units. IPF affects approximately 500 000 people in the USA and Europe (Coultas *et al.*, 1994). This condition therefore represents a major unmet medical need for which novel therapeutic approaches are urgently required. This review will focus on IPF, although the paradigms and potential molecular targets described here may be relevant to a number of other fibrotic conditions, including sarcoidosis and systemic sclerosis.

IPF – incidence/aetiology/pathogenesis

Idiopathic pulmonary fibrosis has a reported incidence of 4.6 per 100 000 people in the UK, but between 1991 and 2003 the incidence increased annually by 11% (Gribbin *et al.*, 2006). Around 4000 new cases are now diagnosed each year in the UK (Gribbin *et al.*, 2006), a disease burden that is currently comparable with that of small cell lung cancer. Clinically, patients generally present with increasing dyspnoea, which may be associated with a dry cough and non-specific systemic upset. A diagnosis of IPF can be made following clinical, radiographic and histological evaluation paying particular attention to exclude secondary causes of pulmonary fibrosis.

The aetiology of IPF remains unknown, although a number of risk factors have been identified. For example, cigarette smoking has been associated with an increased risk of developing IPF, as have certain latent viral infections, including Epstein-Barr virus and herpesvirus (Kelly *et al.*, 2002; Tang *et al.*, 2003). Three per cent of IPF patients appear to have a familial form, and gene polymorphisms of tumour necrosis factor (TNF)-α and transforming growth factor (TGF)-β1, as well as mutations in surfactant protein C, appear to confer an increased risk of developing IPF (Whyte *et al.*, 2000; Xaubet *et al.*, 2003; Lawson *et al.*, 2004). However, as only a small number of those individuals exposed to known risk factors develop IPF, the aetiology is likely to be multifactorial.

The classical histopathological pattern of IPF is one of usual interstitial pneumonia characterized by evidence of patchy epithelial damage including type II pneumocyte hyperplasia, together with abnormal proliferation of mesenchymal cells, varying degrees of fibrosis and overproduction and disorganized deposition of collagen and ECM – this results in significant distortion of pulmonary architecture and honeycombing (Figure 1). Fibrotic foci are often observed within the mura of microscopic honeycomb lesions

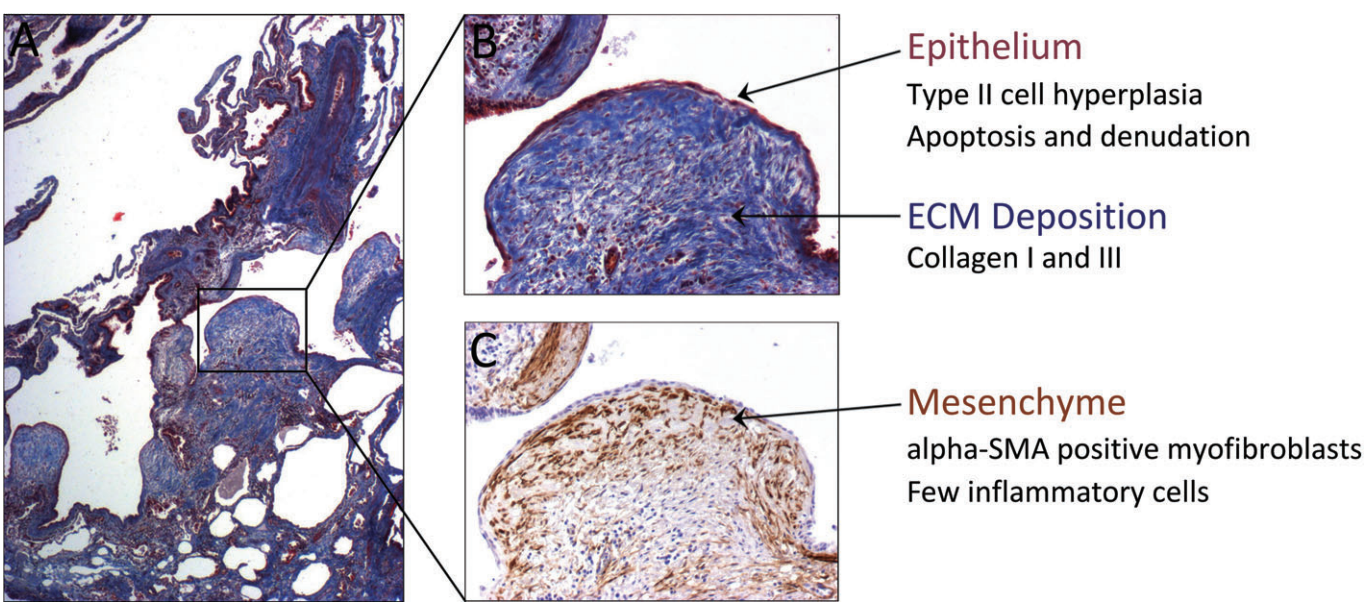

Figure 1

Fibrotic foci – a histological hallmark of idiopathic pulmonary fibrosis. (A) Histological analysis of human IPF tissue reveals the presence of dense collagen deposition within the interstitium (Martius Scarlet Blue staining; original magnification ×10). Fibroblastic foci are revealed as accumulations of fibroblasts and alpha-SMA$^+$ myofibroblasts, which are highly synthetic for collagen and have a contractile phenotype (B: Martius Scarlet Blue staining; C: immunohistochemistry for alpha-SMA. Original magnification ×20). The overlying epithelium is often hyperplastic, with frequent apoptosis and areas of denudation. The presence and distribution of fibrotic foci, together with the spatial and temporal heterogeneity of the pathology is crucial to defining a UIP pattern.

underlying injured and reparative epithelium, as well as within the interstitium – these fibrotic (or fibroblastic foci) represent accumulations of fibroblasts and myofibroblasts within organizing ECM, and their presence and distribution, together with the spatial and temporal heterogeneity of the pathology, is crucial to defining a usual interstitial pneumonia pattern (Katzenstein and Myers, 1998).

Although significant advances have been made in understanding the pathogenesis of pulmonary fibrosis, the specific cellular and molecular mechanisms that contribute to disease progression remain unclear. This article will briefly review the current status of IPF clinical trials (summarized in Table 1) but will focus on novel therapeutic approaches that are emerging from exciting recent advances in our understanding of pathogenetic mechanisms (see Figure 2).

Inflammation in IPF: clinically, an unresolved issue

Early hypotheses embraced the concept that pulmonary fibrosis represents the end stage of an inflammatory cascade initiated following alveolar injury, and that fibrogenesis following such alveolitis was mediated by a number of inflammatory and fibrogenic mediators derived from recruited inflammatory cells. However, the lack of efficacy of anti-inflammatory/immunosuppressive therapy in concert with experimental evidence suggesting that inflammation is not necessary for the progression to fibrosis has brought this hypothesis into question. Crucially, overexpression of the potent pro-fibrotic mediator, TGF-β1, leads to progressive fibrosis in mice, without any significant inflammatory component (Sime *et al.*, 1997). Conversely, it has been argued that despite the lack of clinical benefit observed following anti-inflammatory treatment in established disease, a pathogenic role for inflammation cannot be excluded in the early initiating (subclinical) stages of the disease (Strieter, 2005). In fact, the forced vital capacity (FVC) of most patients is already significantly reduced by the time of presentation (King *et al.*, 2001) indicating that fibrosis is already present. Recent work has demonstrated a potential role for the adaptive immune response to injury in fibrogenesis: peripheral CD4+ cells from IPF patients have increased effector functions (Feghali-Bostwick *et al.*, 2007), and CD4+ cells from the lymph nodes of IPF patients proliferate in co-culture with autologous lung extract, suggesting an autoimmune component to the pathogenesis of IPF (Feghali-Bostwick *et al.*, 2007). Indeed, interactions between T cells and antigen-presenting dendritic cells, critical to the development of an adaptive immune response, have recently been observed in IPF lung, in the form of tertiary lymphoid follicles, composed of reactivated T cells, B cells and locally maturing dendritic cells (Marchal-Somme *et al.*, 2007). Finally, recent gene microarray studies have demonstrated that, in addition to the expected increase in gene expression of proteins associated with ECM turnover, expression of genes traditionally associated with inflammatory processes such as cytokines and chemokines (Zuo *et al.*, 2002) is increased in IPF. These recent data have reinvigorated the argument that perhaps a more focused anti-inflammatory strategy may be of benefit in IPF.

In reality, the question of whether or not current anti-inflammatory/immunosuppressive therapy is of benefit to IPF patients remains unanswered. To date, there has only been a single completed randomized, double-blinded placebo-controlled trial evaluating the efficacy of such treatment (Raghu *et al.*, 1991), which demonstrated a marginal long-term survival benefit over a 9 year follow up in patients treated with azathioprine and prednisolone, compared with prednisolone alone. Beneficial responses to such therapy have been reported in a number of prospective non-randomized trials (Selman *et al.*, 1998; Zisman *et al.*, 2000; Flaherty *et al.*, 2001; Kondoh *et al.*, 2005), as well as several retrospective case series (Turner-Warwick *et al.*, 1980; Douglas *et al.*, 1997; 2000; Kolb *et al.*, 1998; Collard *et al.*, 2004). However, difficulty in interpreting such data is further compounded by low patient numbers and diagnostic heterogeneity. With these crucial caveats in mind, and given the lack of any detrimental effect on survival or lung function, the American Thoracic Society (ATS)/European Respiratory Society (ERS) consensus statement on the management of IPF published in 2000 (ATS/ERS, 2000) suggested combined anti-inflammatory therapy with prednisolone plus azathioprine in patients with active disease. Considering the lack of strong data to wholeheartedly support this statement, two trials are currently recruiting patients to hopefully clarify this issue. The AZAPRED trial (Thorax National Institute, Chile) is a randomized double-blinded placebo-controlled trial evaluating the efficacy of azathioprine/prednisolone, while the PANTHER trial (NHLBI, USA) will assess the efficacy of the current recommended 'gold-standard triple therapy' of azathioprine/prednisolone/N-acetylcysteine (NAC) as compared with NAC alone or placebo. While such therapy may not represent a novel advance in therapeutics in the true sense, data derived from these trials will go someway to advancing our understanding of whether the current therapy is beneficial.

The observation that steroid use may actually enhance alveolar epithelial damage by promoting apoptosis (Dorscheid *et al.*, 2001) highlights the greater importance of identifying disequilibrium in particular molecular pathways over broadly classifying IPF as a purely inflammatory condition. To this end, recent work has highlighted the potential importance of inflammasome activation, by danger signals released following lung injury, in promoting lung fibrosis. In a murine model of lung injury, mice deficient in the NALP-3 inflammasome develop an attenuated early inflammatory response to bleomycin, as well as a reduction in subsequent fibrosis, compared with wild-type controls (Gasse *et al.*, 2009); a major role for uric acid as the danger signal to the NALP-3 inflammasome following experimental lung injury has been described and the prophylactic administration of allopurinol or uricase, strategies aimed at reducing uric acid levels, attenuated bleomycin-induced fibrosis in this model (Gasse *et al.*, 2009). Moreover, the potential importance of this pathway in human disease is supported by the observation of elevated levels of uric acid in IPF lung compared with non-fibrotic control lung (Markart *et al.*, 2009). As such, selective modulation of key inflammatory pathways, such as targeting inflammasome activation by endogenous injury-induced danger signals, may be worth consideration for therapeutic development in IPF rather than a broad-based anti-inflammatory strategy.

Table 1

An overview of completed and ongoing clinical trials in IPF [modified from (Scotton and Chambers, 2007)]

Agent/treatment	Potential mechanisms of action	Example of clinical trial or retrospective series	Study design where appropriate	End points and duration of trial where appropriate/available	Outcome/comments
Anti-inflammatory/Immunosuppressive					
Corticosteroids	Immunosuppressant and anti-inflammatory	Significant lack of studies evaluating prednisolone against placebo Flaherty *et al.* (2001)	Open label study; $n = 41$	CRP score at 3 months	27% responders/46% stable/27% non-responders Adverse effects noted in all patients Cochrane Review of 2003 found no evidence for an effect of corticosteroids in IPF; no high quality prospective studies were identified as suitable for meta-analysis (Davies *et al.*, 2003)
Cyclophosphamide	Alkylating agent with anti-inflammatory properties	Collard *et al.* (2004)	Retrospective case series; cyclophosphamide + prednisolone vs. no treatment; $n = 82$ in each group	Survival at 6–12 months	No evidence for a therapeutic benefit. Significant potential adverse effects
Azathioprine	Inhibits adenine deaminase and impairs cell proliferation (particularly leukocytes) Anti-inflammatory	Raghu *et al.* (1991)	Prospective, double-blinded, randomized placebo-controlled trial; prednisolone + azathioprine ($n = 14$) vs. prednisolone + placebo ($n = 13$)	Primary end points: $\Delta FVC/DL_{co}$/A-a gradient at 1 year; survival at 9 years	Marginally significant survival benefit in azathioprine/ prednisolone group only after age-adjustment No significant improvement in remaining parameters
Etanercept	See text	Raghu *et al.* (2008)	Prospective, double-blinded, randomized placebo-controlled trial; etanercept ($n = 34$) vs. placebo ($n = 31$)	Primary end points: Δ% pred FVC/% pred DL_{co}/ΔA-a gradient over 48 weeks	No significant difference observed between treatment groups. Etanercept therapy resulted in a non-significant reduction in disease progression in several physiological, functional and QoL end points

Table 1

Continued.

Agent/treatment	Potential mechanisms of action	Example of clinical trial or retrospective series	Study design where appropriate	End points and duration of trial where appropriate/available	Outcome/comments
Azathioprine/ prednisolone	As above	Thorax National Institute, Chile	Prospective, double-blinded, randomized placebo-controlled trial; currently recruiting patients, total planned n = 100	Primary end point: progression free survival at 2 years	Results awaited
Azathioprine/ prednisolone/ N-acetylcysteine (NAC)	In addition to above, please refer to text for NAC	NHLBI, USA	Prospective, double-blinded, randomized placebo-controlled trial; currently recruiting patients, total planned n = 390	Primary end point: ΔFVC at 60 weeks	Results awaited
Anti-fibrotic/Anti-angiogenic					
anti-TGFβ (1/2/3) antibody (GC1008)	See text	Genzyme and Cambridge Antibody Technology, UK	Non-randomized, open label, single group assignment Phase I study; n = 25	Primary end points: safety and tolerability Secondary end points: potential clinical outcomes up to 3 years	Results awaited
Anti-$\alpha_v\beta_6$ integrin (STX-100)	See text	Stromedix, USA	Phase I studies completed (Stromedix) – awarded orphan drug status (USA) and Phase II studies planned		Results awaited
LPA, antagonist (AM152)	See text	Amira, USA	Phase I clinical study initiated in healthy individuals	Safety and pharmacokinetic profiles to be analysed	Results awaited

Table 1

Continued.

Agent/treatment	Potential mechanisms of action	Example of clinical trial or retrospective series	Study design where appropriate	End points and duration of trial where appropriate/available	Outcome/comments
Pirfenidone	See text	Taniguchi *et al.* (2010)	Prospective, double-blinded, randomized placebo-controlled trial; high dose pirfenidone (n = 108) vs. low dose pirfenidone (n = 55) vs. placebo (n = 104)	Primary end point: ΔFVC at 52 weeks	Significant reduction in FVC decline in high dose treatment arm. However, change in end point during trial, handling of missing data and absence of patient reported outcome means it is difficult to draw firm conclusions at this time
		CAPACITY 1 (awaiting publication) (Intermune, USA)	Prospective, double-blinded, randomized placebo-controlled trial; high dose pirfenidone (n = 171) vs. placebo (n = 173)	ΔFVC at 72 weeks	No significant difference in FVC decline between treatment groups
		CAPACITY 2 (awaiting publication) (Intermune, USA)	Prospective, double-blinded, randomized placebo-controlled trial; high dose pirfenidone (n = 174) vs. low dose pirfenidone (n = 87) vs. placebo (n = 174)	ΔFVC at 72 weeks	Significant reduction in FVC decline in pirfenidone groups
Imatinib mesylate (Gleevec)	See text	Daniels *et al.* (2010)	Prospective, double-blinded, randomized placebo-controlled trial; imatinib (n = 60) vs. placebo (n-61)	Primary end point: time to disease progression (>10% decline in % pred FVC) or death over 92 weeks	No change in primary end point between treatment and placebo
FG-3019	See text	Fibrogen, USA	Phase I open label study; n = 21	1–12 months	FG-3019 is safe and well-tolerated. Future trials will assess therapeutic potential

Table 1

Continued.

Agent/treatment	Potential mechanisms of action	Example of clinical trial or retrospective series	Study design where appropriate	End points and duration of trial where appropriate/available	Outcome/comments
Zileuton	See text	Investigator led (University of Michigan)	Randomized, open label, active control, parallel assignment Phase II study; $n = 140$	Primary end point: $\Delta[LTB_4]$ in BALF at 6 months Secondary end points include progression free survival and change in physiology	Results awaited
Iloprost	See text	Krowka *et al.* (2007)	Prospective, double-blinded, randomized placebo-controlled Phase II study; iloprost ($n = 26$) vs. placebo ($n = 25$); recruited patients with IPF and elevated pulmonary arterial pressures	Primary end point: safety Secondary end points included dyspnoea (Borg Scale) and 6MWD at 12 weeks	Patients diagnosed with IPF and PAH. Iloprost was well tolerated though no significant differences observed in secondary end points'
Anti-IL-13 antibody (QAX576)	See text	Novartis, Switzerland	Open label Phase II study; $n = 50$	Primary end point: IL13 serum levels Secondary end point: change in designated serum biomarkers over time with treatment for 4 weeks	Results awaited
IFNγ1b	See text	King *et al.* (2009)	Prospective, double-blinded, randomized placebo-controlled trial; interferon ($n = 551$) vs. placebo ($n = 275$)	Primary end point: survival from time of randomization Primary end points: safety and tolerability	Trial ended prematurely as overall survival had crossed predefined boundary at planned interim stage analysis (64 weeks); however, no difference between treatment and placebo arms
		National Centre for Research Resources, USA	Non-randomized open label single interventional study with nebulized interferon-γ Recruiting patients, planned $n = 12$	Secondary end points: lung function trends and BALF [IFN-γ] at 1 year	Results awaited

Table 1

Continued.

Agent/treatment	Potential mechanisms of action	Example of clinical trial or retrospective series	Study design where appropriate	End points and duration of trial where appropriate/available	Outcome/comments
Endothelin antagonists					
Bosentan (dual ET-1 receptor antagonist)	See text	King *et al.* (2008)	Prospective, double-blinded, randomized placebo-controlled trial; bosentan (n = 74) vs. placebo (n = 84)	Primary end point: 6MWD at 12 months	No effect on primary outcome between treatments arms; *post hoc* analysis demonstrated trend in delayed time to disease progression or death in the bosentan arm of IPF patients who had undergone lung biopsy
		BUILD-3 (Actelion, Switzerland)	Prospective, double-blinded, randomized placebo-controlled trial; total n = 616, bosentan : placebo 2:1 recruitment complete;	Primary end points: time to disease progression or death over 8–32 months	BUILD-3 trial designed to evaluate efficacy of bosentan in the subgroup of patients with IPF diagnosed at lung biopsy
Ambrisentan (selective $ET\text{-}1_A$ receptor antagonist)	See text	ARTEMIS-IPF (Gilead, USA)	Prospective, double-blinded randomized placebo-controlled trial; ambrisentan vs. placebo, currently recruiting, total planned n = 600	Primary end points: time to disease progression or death, event driven over 4 years	Terminated at interim analysis stage due to lack of efficacy
Macicentan	See text	MUSIC (Actelion, Switzerland)	Prospective, double-blinded randomized placebo-controlled trial; total n = 178; macicentan vs. placebo, recruitment complete	ΔFVC over 12 months	Results awaited

Table 1

Continued.

Agent/treatment	Potential mechanisms of action	Example of clinical trial or retrospective series	Study design where appropriate	End points and duration of trial where appropriate/available	Outcome/comments
All antagonists (Losartan)	See text and refer to sildenafil below	Losartan in Treating Patients with IPF (National Cancer Institute, USA)	Open label interventional study; recruiting patients; planned $n = 25$	Primary end point: FVC response at 1 year	Results awaited
		Targeting Vascular Reactivity in Idiopathic Pulmonary Fibrosis (University of Iowa, USA)	Prospective, double-blinded, randomized placebo-controlled trial; currently recruiting; planned total $n = 40$	Primary end points: 6MWD and QoL score	This trial is designed to evaluate the effect of losartan ± sildenafil on exercise-induced oxygen desaturation in IPF patients
Minocycline	See text	Investigator led – University of California, USA	Prospective, double-blinded, randomized placebo-controlled trial; patient numbers not disclosed	Primary end points: safety and efficacy	Results awaited
Angiokinase inhibitor (BIBF 1120)	See text	Boehringer Ingelheim Pharmaceuticals, UK	Prospective, double-blinded, randomized placebo-controlled Phase II study; BIBF1120 vs. placebo; total $n = 400$; recruitment complete	Primary end point: ΔFVC over 1 year	Results awaited
Tetrathiomolybdate	See text	Investigator led – University of Michigan, USA	Non-randomized, open label, uncontrolled, single group assignment Phase I/II; $n = 20$	Primary end point: safety Secondary end points: Δlung function tests	Results awaited
Antioxidant					
N-acetylcysteine (NAC)	See text	Demedts *et al.* (2005)	Prospective, double-blinded, randomized placebo-controlled trial; NAC + azathioprine + prednisolone ($n = 92$) vs. placebo + azathioprine + prednisolone ($n = 90$)	Primary end points: absolute ΔFVC and DL_{co} at 12 months	Reduction in FVC and DL_{co} decline over 1 year in NAC arm, though no change in mortality

Table 1

Continued.

Agent/treatment	Potential mechanisms of action	Example of clinical trial or retrospective series	Study design where appropriate	End points and duration of trial where appropriate/available	Outcome/comments
Anti-coagulation/pro-fibrinolytic					
Warfarin	See text	Kubo *et al.* (2005)	Randomized open label trial; prednisolone + warfarin/low molecular weight heparin(n = 31) vs. prednisolone + placebo (n = 33);	Primary end points: time to death and hospitalization-free time over 1 year	Anti-coagulant therapy resulted in a significant increase in survival of patients with IPF and a significant improvement in survival associated with acute exacerbations of IPF
	See text	NHLBI – Duke University, USA	Prospective, double-blinded, randomized placebo-controlled trial; warfarin vs. placebo; currently recruiting, planned total n = 256	Primary end points: time to death or disease progression over 48 weeks	Results awaited
Heparin	See text	Markart *et al.* (2010)	Open label exploratory study evaluating safety of nebulized heparin in IPF; n = 21	Study designed to assess safety and tolerability	Adequate local anticoagulation achieved with no significant adverse effects. Future trials planned to evaluate efficacy.
Other					
Sildenafil	Phosphodiesterase 5 inhibitor. Causes vasorelaxation by stabilizing cGMP	IPF Clinical Research Network, USA (Zisman *et al.*, 2010)	Prospective, double-blinded, randomized placebo-controlled trial :sildenafil (n = 89) vs. placebo (n = 91)	Primary end points: 6MWD Double-blinded over initial 12 weeks, followed by open label extension for 12 weeks with all patients receiving sildenafil	This trial enrolled patients with advanced IPF No significant improvement in primary end point in treatment arm, but significant improvement in secondary end points in sildenafil arm, including DL_{co} and quality of life score

Table 1

Continued.

Agent/treatment	Potential mechanisms of action	Example of clinical trial or retrospective series	Study design where appropriate	End points and duration of trial where appropriate/available	Outcome/comments
Anti-CCL2 antibody (CNTO 888)	See text	Centocor, USA	Prospective, double-blinded, randomized placebo-controlled Phase II trial; CNTO 888 ± usual therapy vs. placebo ± usual therapy; currently recruiting patients, planned total $n = 120$	Primary end points: safety and performance at lung function tests	Results awaited
Somatostatin analogues	See text	Institut National de la Santé Et de la Recherche Médicale, France	Non-randomized open label single interventional study with octreotide; $n = 25$	Monitoring of FVC; DL_{co}; HRCT fibrosis score; 6MWD over 48 weeks	Results awaited
Thalidomide	See text	Investigator led – John Hopkins University, USA	Non-randomized open label single interventional study designed for patients who have failed or are unsuitable for immunosuppressive therapy; currently recruiting, planned total $n = 19$	Primary end point: safety Secondary end points: Δlung function over 1 year	Results awaited

A-a, alveolar:arterial; BALF, bronchoalveolar lavage fluid; CRP, clinical-radiographic-physiological; DL_{co}, carbon monoxide transfer factor; pred, predicted; FVC, forced vital capacity; HRCT, high resolution computer tomography; IL-13, interleukin 13; IFN-γ, interferon-gamma; LTB_4, leukotriene B_4; 6MWD, 6 min walk test distance; QoL, quality of life.

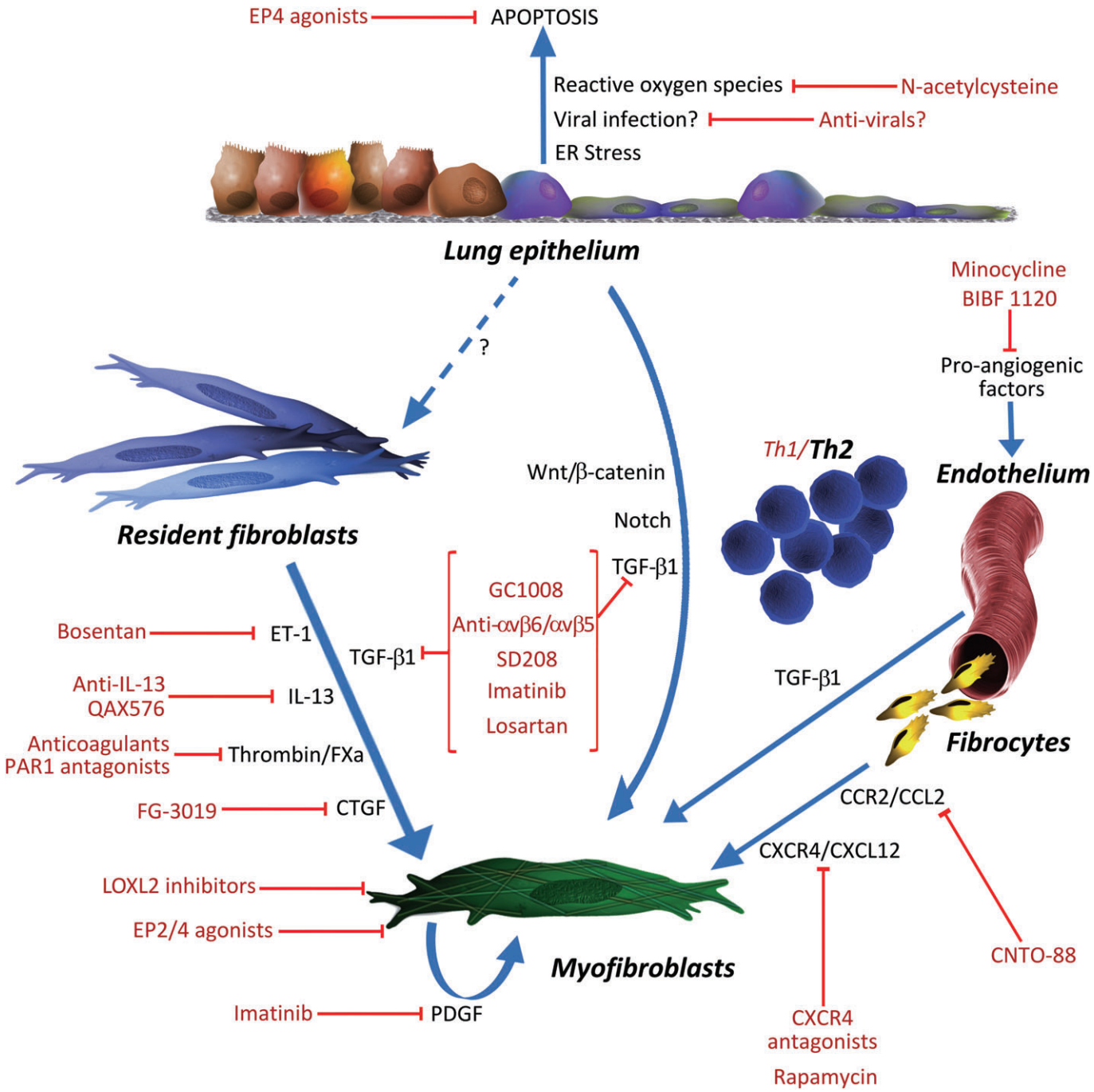

Figure 2

Key mediators in the pathogenesis of IPF. The pathobiological mechanisms underlying the development of IPF are highly complex. Recurring damage to the epithelium (possibly due to reactive oxygen species, endoplasmic reticulum stress or viral infection) results in an abnormal wound healing response characterized by dysregulated epithelial–mesenchymal crosstalk and the accumulation of myofibroblasts (the key effector cells in IPF fibrogenesis). The proposed cellular origin of these cells includes resident fibroblasts, epithelial/endothelial–mesenchymal transition or the recruitment of circulating fibrocytes. The fibrotic micro-environment may be skewed towards a pro-angiogenic and Th2-oriented profile, where multiple cytokines, growth factors and signalling pathways mediate the pro-fibrotic responses. Some of the potential anti-fibrotic strategies (shown in red) are highlighted and these are described further in the text.

Dysregulated epithelial – mesenchymal crosstalk in IPF

Irrespective of the current uncertainty regarding the precise contribution of inflammation to the initiation and/or progression of IPF, the more recent hypothesis that IPF arises as a result of a highly aberrant wound healing response following repetitive epithelial injury in susceptible individuals (Selman and Pardo, 2002) is gaining increasing recognition. According to this hypothesis, IPF is an 'epithelial-fibroblastic disease', that is, a fibroproliferative disorder preceded by alveolar epithelial injury and activation, with fibrotic foci representing the primary sites of injury and aberrant repair. The underlying mechanisms leading to the emergence of fibrotic foci are still unclear; current evidence suggests roles for local proliferation and differentiation of resident fibroblasts, recruitment of circulating stem cells and epithelial–mesenchymal transition (EMT), with a prominent role identified for the overlying, highly reactive and hyperplastic epithelium. These notions will be explored in greater detail below. Myofibroblasts in turn provoke basement membrane disruption and alveolar epithelial cell (AEC) apoptosis, perpetuating the damage and preventing appropriate re-epithelialization. The final result is the excessive deposition of ECM proteins with the destruction of the alveolar-capillary units and the formation of cystic fibrotic spaces or honeycombing. Unravelling the molecular basis for this aberrant epithelial–mesenchymal crosstalk in IPF is currently an area of intense investigation. This effort has heralded major advances in disease understanding with much current interest focused on elucidating the pathways involved in myofibroblast accumulation and differentiation in the hope that this might lead to the identification of novel molecular targets for therapeutic intervention.

The epithelium in IPF

Crucial to normal wound healing following injury is the re-establishment of an intact epithelium. Recruitment and activation of mesenchymal cells to the site of injury initiates limited deposition of ECM into the wound space – this provisional matrix acts as a scaffold for normal tissue repair. Subsequent contraction of activated fibroblasts/myofibroblasts within this matrix approximates the epithelial margins to allow re-epithelialization and wound closure.

An early and consistent feature of pulmonary fibrosis in humans is a change in the phenotype of the AEC, suggesting that ongoing AEC injury is a critical step in the pathogenesis of pulmonary fibrosis (Kasper and Haroske, 1996; Chilosi *et al.*, 2002). These changes include apoptosis (Kuwano *et al.*, 1996; Plataki *et al.*, 2005), regenerative hyperplasia (Corrin *et al.*, 1985), bronchiolarization (Sutinen *et al.*, 1980; Kawanami *et al.*, 1982) and proliferation (Katzenstein, 1985). AEC apoptosis is a well-recognized histological finding in IPF (Kuwano *et al.*, 1996; Uhal *et al.*, 1998; Barbas-Filho *et al.*, 2001; Maeyama *et al.*, 2001; Plataki *et al.*, 2005). The underlying mechanisms involved are unclear, but numerous mediators/mechanisms have been proposed, including TGF-β (Lee *et al.*, 2004), Fas activation (Maeyama *et al.*, 2001), angiotensin II (ANGII) and reactive oxygen species (Waghray *et al.*, 2005). More recently, the alveolar epithelium of patients with IPF has been shown to express markers of endoplasmic reticulum stress and the unfolded protein response (Korfei *et al.*, 2008; Lawson *et al.*, 2008). Activation of these pathways may result from altered surfactant protein processing or chronic herpesvirus infection. The persistent apoptosis and dysregulated proliferation of epithelial cells impairs adequate epithelial reconstitution, and also drives the inappropriate crosstalk between the epithelium and mesenchyme. For instance, the injured epithelium can contribute to fibrogenesis through the generation of pro-fibrotic cytokines such as TGF-β (Xu *et al.*, 2003), and the targeting of such epithelial-derived mediators as potential therapeutic strategies in IPF will be discussed in later sections.

The myofibroblast response in IPF

The myofibroblast has long been regarded as a major cell type involved in normal wound healing, and as the key effector cell in fibrogenesis. Myofibroblasts are highly synthetic for collagen and other ECM components, and are characterized by the *de novo* expression of α-smooth muscle actin (α-SMA) [reviewed in (Scotton and Chambers, 2007)]. The presence of myofibroblasts in fibrotic lesions in animal models of fibrosis correlates with the development of active fibrosis, and their persistence and localization to fibrotic foci in human disease is associated with disease progression (Kuhn and McDonald, 1991; Zhang *et al.*, 1994a). Myofibroblasts isolated from the lungs of IPF patients also exhibit an enhanced migratory phenotype (Suganuma *et al.*, 1995) and are capable of releasing numerous pro-fibrotic mediators (Moodley *et al.*, 2003). In addition, when cultured *ex vivo* they are more resistant to apoptosis (Ramos *et al.*, 2001; Moodley *et al.*, 2004) – this failure of apoptosis may explain the persistence of these highly activated cells at sites of injury.

Originally thought to be derived from the local proliferation and differentiation of resident fibroblasts in the presence of a highly pro-fibrotic cytokine milieu (Zhang *et al.*, 1994b; Phan, 2002), recent pioneering research demonstrated that myofibroblasts in pulmonary fibrosis can be derived from several other cellular sources. First, there is now growing evidence that myofibroblasts can be derived from the epithelium via EMT. During this process, epithelial cells lose their characteristic markers (e.g. E-cadherin and zona occludens-1) and acquire mesenchymal markers (e.g. fibroblast-specific protein-1 and α-SMA) (Grunert *et al.*, 2003). The concept of EMT has been recognized for over 20 years, and evidence is now accumulating to support a role for EMT in IPF. AECs *in vitro* undergo EMT in response to prolonged exposure to major fibrogenic mediators (e.g. TGF-β1) when cultured on a provisional wound matrix (Willis *et al.*, 2005). Elegant lineage tracing studies have also provided strong support for EMT as a potential source of myofibroblasts during lung fibrogenesis (Kim *et al.*, 2006; 2009). In terms of human disease evidence, the notion of EMT is supported by the observation that cells in IPF biopsy samples co-express epithelial and mesenchymal markers (Kim *et al.*, 2009) although this was not a universal finding (Yamada *et al.*, 2008). The molecular pathways underlying the development of EMT are coming to light and may

present novel avenues for therapeutic intervention. Current evidence suggests key roles for TGF-β1, Wnt and Notch signalling pathways. This will be explored in greater detail in future sections.

A second hypothesis regarding the origin of (myo)fibroblasts in lung fibrosis proposes that these cells may be derived from circulating fibrocytes (Lama and Phan, 2006). Fibrocytes were originally identified as collagen I + /CD34+/CD45RO+ cells that are likely derived from hematopoietic stem cells (Bucala *et al.*, 1994). Support for a pathogenic role for fibrocytes in lung fibrosis has been provided from studies showing that blockade of fibrocyte recruitment is protective following experimental lung injury in rodents (Phillips *et al.*, 2004; Moore *et al.*, 2005). A major role has been identified for the CXCR4/CXCL12 axis in the recruitment of fibrocytes (Phillips *et al.*, 2004) although several chemokines have been shown to be capable of recruiting fibrocytes *in vivo*. Whether fibrocyte-derived fibroblasts are capable of differentiating into fully activated myofibroblasts, especially in patients with IPF, remains the subject of an interesting debate, although recent evidence suggests that about 10% of fibrocytes express α-SMA in the bleomycin model (Mehrad *et al.*, 2009). Moreover, CXCL12 levels are increased in both plasma and bronchoalveolar lavage fluid (BALF) from patients with IPF and CXCR4/fibrocyte/mesenchymal marker co-expression studies support the notion that circulating fibrocytes may contribute to the expansion of the fibroblast/myofibroblast population in IPF (Andersson-Sjoland *et al.*, 2008) Finally, a recent report has shown that a >5% blood fibrocyte count is associated with poor survival in IPF (Moeller *et al.*, 2009).

In addition to the above cellular sources, there is very recent experimental evidence that lung capillary endothelial cells may also give rise to fibroblasts through endothelial–mesenchymal transition in a bleomycin-induced lung fibrosis model (Hashimoto *et al.*, 2010). Finally, myofibroblasts can also be derived from pericytes. In the liver, pericytes, or hepatic stellate cells, are the principal collagen-producing cell in hepatic fibrosis [reviewed in (Gressner and Weiskirchen, 2006)]. In animal studies of skin and kidney fibrosis (Humphreys *et al.*, 2010; Liu *et al.*, 2010b), pericytes have also been shown to represent a source of myofibroblasts. However, despite the suggestion that the pericyte may contribute to the myofibroblast population in lung fibrosis (Adler *et al.*, 1989), the role of this cell type in IPF remains uncertain.

Although the relative contribution of each of these potential cellular sources of fibroblasts/myofibroblasts to fibrogenesis in IPF remains unclear, the realization that fibrogenic cells may be derived from multiple cellular sources, in addition to resident fibroblasts, has opened up a myriad of new possibilities for therapeutic intervention. Molecules felt to be important in this regard will be addressed in subsequent sections.

Recently completed major placebo-controlled phase III trials in IPF

IFNγ1b

Interferon (IFN)-γ is an immunoregulatory cytokine that is crucial in both the innate and acquired immune responses. It is predominantly generated by natural killer cells and activated T-helper (Th) 1 cells (Murphy *et al.*, 2000), which led to the suggestion that it may have a therapeutic benefit in IPF by redressing the perceived dominance of Th2 cytokines in this disease (Wynn, 2004). However, IFN-γ also plays a role in counter-regulating TGF-β expression and signalling responses (Ulloa *et al.*, 1999). Moreover, IFN-γ limits fibroblast proliferation and collagen synthesis directly (Rosenbloom *et al.*, 1986; Elias *et al.*, 1987; 1990) and IFN-γ administration attenuates bleomycin-induced fibrosis in mice (Gurujeyalakshmi and Giri, 1995). IFN-γ has been extensively investigated as a novel therapy for IPF following an initial preliminary trial suggesting that lung function improved in patients with IPF treated with IFN-γ (Ziesche *et al.*, 1999). *Post hoc* analysis of a second, similarly designed trial (Raghu *et al.*, 2004), suggested that patients with relatively well-preserved lung function may have a survival benefit with IFN-γ treatment. The most recent trial investigating the efficacy of IFN-γ (Intermune, USA) used overall survival time from randomization as its primary end point (King *et al.*, 2009). However, this study was terminated prematurely at a planned interim analysis stage. Results showed that overall survival had crossed the predefined boundary for lack of benefit; in fact, among the randomized patients, there was no significant difference between treatment arms in overall mortality. Interest persists in IFN-γ as a potential therapeutic agent in IPF. To this end, a phase I pilot study is currently recruiting patients to evaluate the safety and efficacy of nebulized IFN-γ in IPF, which may help address concerns about the most appropriate mode of administration of this cytokine in this condition.

Pirfenidone

Pirfenidone is an orally available pyridine derivative that has recently received much interest in IPF in view of its anti-fibrotic (Gurujeyalakshmi *et al.*, 1999; Iyer *et al.*, 1999; Hewitson *et al.*, 2001; Di Sario *et al.*, 2002), anti-inflammatory (Iyer *et al.*, 2000; Hale *et al.*, 2002; Nakazato *et al.*, 2002; Oku *et al.*, 2002) and antioxidant properties (Giri *et al.*, 1999). Its potential role in this disease is the subject of an excellent review by Maher (Maher, 2010). Briefly, pirfenidone has been shown to inhibit fibroblast proliferation and collagen synthesis *in vitro* (Hewitson *et al.*, 2001; Di Sario *et al.*, 2002) as well as inhibiting TGF-β induced heat shock protein HSP47 expression, a molecular chaperone of collagen, the synthesis of which is known to correlate with fibroblast ECM deposition. *In vivo* pirfenidone attenuates bleomycin-induced lung fibrosis when dosed either prophylactically or therapeutically (Iyer *et al.*, 1995; Kakugawa *et al.*, 2004), and this attenuation is associated with a reduction in lung platelet derived growth factor (PDGF) and TGF-β levels (Gurujeyalakshmi *et al.*, 1999; Iyer *et al.*, 1999). Its anti-inflammatory properties are manifested by an attenuation in TNF-α and IFN-γ levels in experimental models of inflammation (Iyer *et al.*, 2000; Nakazato *et al.*, 2002; Oku *et al.*, 2002). However, the precise molecular mechanism of action of pirfenidone remains unknown. Nonetheless, in light of promising data derived from animal models of fibrosis, pirfenidone has been the subject of a number of trials in IPF. The most recently published of these, a randomized double-blinded, placebo-controlled trial (Shionogi, Japan) demonstrated a significant reduction in decline in vital capacity in the treatment arm

compared with the placebo arm (Taniguchi *et al.*, 2010). However, the change in end point during the course of the trial has been highlighted as problematic in terms of drawing any firm conclusions regarding the use of pirfenidone in IPF patients (Collard, 2010). The results of two other Phase III trials (Intermune, USA) have, to date, been presented in abstract form only at international meetings and as such the results have yet to be subjected to rigorous peer review. Briefly, however, it appears from the presented data that pirfenidone treatment resulted in a significant reduction in FVC decline compared with placebo in the CAPACITY 2 trial at 72 weeks, although no such significance was reached in the CAPACITY 1 trial. Clearly, pirfenidone represents a potentially important advance in IPF therapy, and we look forward to the publication of data derived from these studies.

Etanercept

The long-standing interest in TNF-α as a target in IPF reflects no shortage of evidence indicating that expression of this master cytokine is increased in the lungs of patients with lung fibrosis (Nash *et al.*, 1993; Piguet *et al.*, 1993; Ziegenhagen *et al.*, 1998), with expression localizing in particular to epithelial cells and macrophages. Moreover, functional polymorphisms of TNF-α are associated with an increased risk of developing IPF (Whyte *et al.*, 2000). A causative role for TNF-α in the pathogenesis of pulmonary fibrosis is suggested by observations that blocking TNF-α signalling attenuates bleomycin-induced fibrosis (Piguet *et al.*, 1989; Zhang *et al.*, 1997; Ortiz *et al.*, 1998; Oikonomou *et al.*, 2006). Furthermore, local pulmonary overexpression of TNF-α results in fibroblast accumulation and increased deposition of ECM proteins in the pulmonary interstitium (Miyazaki *et al.*, 1995; Sime *et al.*, 1998). More recently, the importance of soluble TNF-α in mediating the transition from bleomycin-induced inflammation to fibrosis, a transition accompanied by lymphocyte recruitment, has been demonstrated in mice (Oikonomou *et al.*, 2006). This latter observation highlights the potential importance of TNF-α in influencing the adaptive immune system [reviewed in (Kollias *et al.*, 1999)] and potentially, the polarization of the Th immune response to lung injury. However, in contrast to these promising preclinical studies, the soluble TNF-α receptor antagonist Etanercept proved disappointing in a subsequent randomized, double-blinded, placebo-controlled trial (Wyeth, USA) in IPF patients with no significant improvement in lung function parameters observed (Raghu *et al.*, 2008). However, a non-significant trend towards improvement in a composite of these indices was noted following secondary analysis.

Imatinib

There has been long-standing interest in the potent fibroblast mitogen and chemoattractant, PDGF, as a target in fibrosis, including lung fibrosis (Antoniades *et al.*, 1990). Although PDGF has been shown to induce procollagen production by fibroblasts *in vitro* (Lepisto *et al.*, 1995), it may play a greater role in expanding the fibroblast accumulation at sites of injury (Clark *et al.*, 1993). Most attention has been focused on the two PDGF isoforms, PDGF-A and –B, which homo- and heterodimerize, and stimulate tyrosine kinase signalling via interaction with the PDGF-α or -β receptors. The tyrosine kinase inhibitor, Imatinib mesylate (Novartis, Switzerland) has activity against the PDGF receptor, but the anti-fibrotic potential of this drug may reflect multiple potential modes of action. For example, imatinib inhibits signalling pathways directly downstream of TGF-β, in part through inhibition of c-Abl tyrosine phosphorylation (Daniels *et al.*, 2004). It also inhibits the stem cell factor/c-kit axis (Wang *et al.*, 2000b) and collagen-induced Discoidin Domain Receptor-1 activation (Day *et al.*, 2008), two pathways recently implicated in the development of bleomycin-induced fibrosis in mice (Avivi-Green *et al.*, 2006; Ding *et al.*, 2010).

Preclinical studies demonstrated that imatinib reduces collagen deposition and mesenchymal cell proliferation in the bleomycin model when dosed prophylactically (Daniels *et al.*, 2004; Aono *et al.*, 2005), but this was not the case when imatinib was administered in a therapeutic schedule (day 14 post bleomycin onwards) (Aono *et al.*, 2005). In a recent multi-centre, randomized, placebo-controlled trial (Novartis, Switzerland) of patients with mild to moderate IPF followed for 96 weeks (Daniels *et al.*, 2010) imatinib did not affect survival or lung function. The use of other tyrosine kinase inhibitors will be discussed in brief in later sections.

Endothelin receptor antagonists

Endothelin-1 (ET-1) expression is up-regulated in IPF (Giaid *et al.*, 1993; Saleh *et al.*, 1997b). Aside from promoting fibroblast proliferation (Peacock *et al.*, 1992; Shahar *et al.*, 1999), collagen synthesis (Xu *et al.*, 1998) and differentiation into myofibroblasts (Shahar *et al.*, 1999; Shi-Wen *et al.*, 2004), it is an extremely potent mitogen for endothelial cells (Pedram *et al.*, 1997) and vascular smooth muscle cells (Komuro *et al.*, 1988), thus potentially contributing to neovascularization. In a rodent model of fibrosis, the administration of bosentan, a non-selective ET-1(A) and ET-1(B) receptor antagonist, attenuates bleomycin-induced fibrosis (Park *et al.*, 1997), although this was not a universal finding (Mutsaers *et al.*, 1998). The BUILD-1 trial (Actelion, Switzerland) evaluated the effect of bosentan administration in patients with IPF but no evidence of severe pulmonary hypertension (PHT) (King *et al.*, 2008). Although no significant difference between the bosentan and placebo arms was observed in the primary end point of 6 minute walk test distance, a trend in favour of bosentan was observed in the secondary end point of time to death or disease progression. *Post hoc* analysis of data pertaining to these secondary end points, however, did demonstrate a significant benefit in the bosentan arm in those IPF patients who had undergone a lung biopsy to reach a diagnosis of IPF. The BUILD-3 trial (Actelion, Switzerland), which has finished recruiting patients, is a randomized double-blinded placebo-controlled trial, designed to explore the effect of bosentan on disease progression in this subset of patients. The ET-1(A) receptor antagonist, ambrisentan, is Food and Drug Administration approved for the treatment of PHT, and its potential in delaying disease progression in IPF patients without PHT was recently the subject of a prospective, double-blinded randomized placebo-controlled trial (ARTEMIS-IPF; Gilead, USA). Unfortunately, this trial was terminated at an interim analysis stage due to lack of efficacy. An additional trial investigating the efficacy of endothelin antagonists in IPF is currently ongoing: the MUSIC trial (Actelion, Switzerland) is a randomized double-blinded placebo-controlled trial designed

to examine the effect of the dual endothelin receptor antagonist, macicentan, on FVC, and has finished recruiting patients.

NAC

Under normal conditions, lung epithelial cells are protected from damage by reactive oxidative species (ROS) by antioxidants, such as glutathione (Reddy *et al.*, 2007). However, these defences may be inadequate in the face of excessive ROS generation. Oxidative stress is a feature of the IPF lung (Jack *et al.*, 1996; Montuschi *et al.*, 1998) and extracellular (Cantin *et al.*, 1989; Behr *et al.*, 1995; Beeh *et al.*, 2002; Montaldo *et al.*, 2002) and intracellular (Behr *et al.*, 2002) glutathione levels are reduced in IPF. Aside from being directly injurious to epithelial cell macromolecules and DNA, excess ROS can influence several pro-fibrotic cellular processes; for instance, myofibroblasts derived from IPF lung generate hydrogen peroxide (H_2O_2), which may serve as a paracrine signal inducing apoptosis in the overlying epithelial cells (Waghray *et al.*, 2005).

NAC, a derivative of cysteine, augments the synthesis of glutathione both *in vitro* (Phelps *et al.*, 1992) and *in vivo* (Borok *et al.*, 1991a), thus contributing to the replenishment of glutathione stores and bolstering epithelial cell antioxidant defence. NAC is one of the few agents whose success in attenuating experimentally-induced fibrosis in animal models (Shahzeidi *et al.*, 1991) has been translated, to some degree at least, into a clinical benefit in IPF (Demedts *et al.*, 2005). The IFIGENIA trial (ZambonSpA, Italy) demonstrated a significant reduction in the rate of decline of FVC and transfer factor (DL_{CO}) in the NAC treatment arm, although this effect did not translate into increased survival at one year. These data have supported the recent addition of NAC to standard therapy in IPF, despite a number of concerns regarding the trial that have been highlighted elsewhere (Behr and Noble, 2009). In particular, as the treatment and placebo arms of the trial were add-on therapies to prednisolone and azathioprine, it is difficult to be sure that the beneficial effect of NAC is only observed in those patients on such therapy. The results of the PANTHER trial, outlined earlier, should help clarify these questions.

Current drug targets in IPF

TGFβ1

TGF-β exists as one of three isoforms in humans, TGF-β1–3, and there continues to be overwhelming evidence that, of these isoforms, TGF-β1 plays a major mechanistic role in fibrogenesis in numerous fibrotic disorders, including IPF. Although all isoforms are potent stimulators of lung fibroblast procollagen production *in vitro*, only TGF-β1 gene expression is increased in murine lung following bleomycin challenge (Coker *et al.*, 1997), and immunohistochemical analysis of lungs from patients with pulmonary fibrosis demonstrates strong immunoreactivity for TGF-β1, but not for TGF-β2/3 (Khalil *et al.*, 1996). Transient overexpression of TGF-β3 in rat lungs is capable of inducing a fibrotic response, but this is less severe and progressive than that which results from TGF-β1 overexpression (Ask *et al.*, 2008). Paradoxically, in other models of fibrosis, including dermal and liver fibrosis, TGF-β3 appears to be anti-fibrotic (Shah *et al.*, 1995; Zhang *et al.*, 2010), and recombinant TGF-β3 is currently being evaluated in Phase II trials as a tool to promote scar-free healing following skin injury. The exact mechanism of action remains unclear, although modulation of macrophage infiltration (Shah *et al.*, 1995) and the promotion of an MMP-dominant microenvironment (Zhang *et al.*, 2010) have been postulated. However, no such data currently exist to demonstrate a similar effect in lung fibrosis, and the remainder of this section will focus on strategies targeting TGF-β1 signalling.

TGF-β1 is the most potent inducer of fibroblast ECM production characterized to date (Raghow *et al.*, 1987; Overall *et al.*, 1989; McAnulty *et al.*, 1991), and promotes fibroblast to myofibroblast differentiation (Chambers *et al.*, 2003; Subramanian *et al.*, 2004). Overexpression of TGF-β1 is sufficient to drive progressive fibrosis in mice (Sime *et al.*, 1997) and TGF-β1 has more recently been shown to drive either epithelial cell apoptosis (Yanagisawa *et al.*, 1998) or EMT (Kim *et al.*, 2009) (Willis *et al.*, 2005), depending on the composition of the ECM. A number of strategies aimed at interfering with TGF-β1-induced cellular responses have been developed, although there remains concern that TGF-β1 plays essential roles in regulating inflammation and acts as a tumour suppressor in certain contexts. If these strategies interfere with TGF-β1's homeostatic roles, this may carry the liability of highly undesirable side effects; especially in light of the fact that many IPF patients will have a previous smoking history and will already be at heightened risk of developing lung cancer (Ozawa *et al.*, 2009).

TGF-β inhibition

Both pan-TGF-β and TGF-β1, -β2 and -β3 isoform-specific antibodies are in development for multiple indications, including IPF. Cambridge Antibody Technology (UK) and Genzyme (UK) have recently completed a Phase I clinical trial in IPF patients with GC1008 – a neutralizing antibody that targets all three mammalian isoforms. We await the publication of the results of this study with interest.

Inhibition of TGF-β signalling

Approaches aimed at inhibiting active TGF-β signalling have also been intensely investigated in several disease indications with much of this effort focused on the development of inhibitors of the high-affinity serine/threonine kinase receptor TGF-βRI (activin-like kinase receptor-5). Orally active activin-like kinase receptor-5 kinase inhibitors, e.g. SB-525334 (GlaxoSmithKline, UK) have been shown to attenuate bleomycin-induced pulmonary fibrosis (Higashiyama *et al.*, 2007) and have also been shown to be effective in blocking fibrotic progression in TGF-β1 lung overexpression studies (SD-208; Scios Inc., USA) (Bonniaud *et al.*, 2005).

Blockade of TGF-β activation

As mentioned above, direct TGF-β blocking strategies may carry high liabilities with respect to interfering with key homeostatic functions of TGF-β. Another approach that has gained much favour is to develop strategies aimed at blocking TGF-β at the level of activation. TGF-β bioactivity is con-

trolled on a number of levels, with latent TGF-β activation representing one of the key rate-limiting steps. This is dependent on the dissociation of TGF-β from the TGF-β latency-associated peptide (LAP). Depending on cell type, TGF-β activation is mediated by proteolytic cleavage or by the proteolytic-independent interaction of LAP with integrins or the matrix glycoprotein thrombospondin-1 (reviewed in [Murphy-Ullrich and Poczatek, 2000; Wipff and Hinz, 2008)]. In the context of lung fibrosis, integrin-dependent mechanisms are felt to be particularly important. Integrins $\alpha v\beta 6$, $\alpha v\beta 5$, $\alpha v\beta 3$, and an as yet unidentified $\beta 1$ integrin have been shown to activate latent TGF-$\beta 1$ independently from any proteolytic activity – they all recognize the RGD sequence of LAP-TGF-$\beta 1$ as part of the ECM-bound large latent complex. When the large latent complex is covalently bound to a mechanically resistant ECM (as would be the case in fibrosis), cell traction forces exerted on LAP-TGF$\beta 1$ result in a conformational change of the latent complex and liberates active TGF-$\beta 1$ [reviewed in (Wipff and Hinz, 2008)]. There is strong *in vitro*, experimental animal and IPF patient immunohistochemistry data to support a key role for the epithelial-restricted integrin, αvβ6, in the activation of TGF-β1 (Munger *et al.*, 1999; Jenkins *et al.*, 2006). Expression of this integrin is low in normal epithelial tissues and is significantly up-regulated in injured and inflamed epithelia (Breuss *et al.*, 1995) including the activated epithelium in IPF (Horan *et al.*, 2008). Targeting this integrin therefore reduces the theoretical possibility of interfering with wider TGF-β homeostatic roles. Partial inhibition of the αvβ6 integrin by antibody blockade has been shown to prevent pulmonary fibrosis without exacerbating inflammation (Horan *et al.*, 2008). A humanized monoclonal antibody (STX-100) has recently been evaluated in a completed Phase I clinical trial (Stromedix, USA) – phase II trials are planned and this molecule has recently been granted orphan drug status in the USA.

In addition to the epithelial integrin αvβ6, the more widely expressed αvβ5 integrin has also received much recent attention in the context of TGF-β activation by myofibroblasts in fibrosis [reviewed in (Wipff and Hinz, 2008)]. We have recently shown that this integrin is co-expressed by α-SMA positive myofibroblasts within IPF fibrotic foci (Scotton *et al.*, 2009) but αvβ5 staining was weak or absent on hyperplastic epithelial cells within the same tissue samples. This raises the possibility that this integrin may play a role in the activation of TGF-β within fibrotic foci while the $\alpha v\beta 6$ integrin is involved in the activation of TGF-β by the activated epithelium. Although dual β3 and β5 integrin deficient mice have recently been reported not to be protected from developing bleomycin-induced lung fibrosis (Atabai *et al.*, 2009) it is worth bearing in mind that this model does not usually lead to the development of the typical fibrotic foci seen in patients with IPF.

Other strategies to inhibit TGF-β signalling

Of the three known human isoforms of TGF-β, TGF-β1 is thought to be the most important in human fibrotic lung disease (Khalil *et al.*, 1996), and strategies to modulate TGF-β-mediated process have reflected this. However, therapies to influence TGF-β2 activity have resulted in clinical benefits to patients with pathologies in which this isoform is perhaps more dominant. These include the use of antisense oligonucleotides to block TGF-β2 expression in patients with high grade gliomas (Schlingensiepen *et al.*, 2008) and it is conceivable that such strategies may be applicable to the TGF-β1 isoform in the future. Synthetically derived peptides have also been used to inhibit the TGF-β pathway. P144 (DigNA Biotech, Spain) is one such 14 mer peptide derived from the TGF-β1R3 sequence, which blocks binding of TGF-β1 to TGF-βR1 and has been demonstrated to attenuate experimentally induced liver fibrosis in rats (Ezquerro *et al.*, 2003). A Phase II clinical study for the treatment of skin fibrosis in systemic sclerosis with topical application of P144 is currently recruiting patients.

Connective tissue growth factor (CTGF)

There has been a long-standing interest in the role of CTGF, a prototypic member of the CCN protein family, as a potential target in fibrosis, including lung fibrosis. CTGF was originally thought to be a specific downstream mediator of the pro-fibrotic effects of TGF-β, with a particular role in stimulating fibroblast matrix production and myofibroblast differentiation (Leask and Abraham, 2003). Its cell surface receptor and downstream signalling pathways have yet to be fully determined and there is now increasing support for the notion that CTGF may not act as a classical autocrine growth factor. In addition, it is now clear that CTGF is induced by a number of other pro-fibrotic mediators, including thrombin (Chambers *et al.*, 2000). Despite the uncertainties about mechanisms of action, CTGF remains an interesting target in the context of a number of fibrotic disorders, including systemic sclerosis and IPF [reviewed in (Leask, 2009)]. CTGF expression is increased in IPF (Allen *et al.*, 1999), and although adenoviral overexpression induces only mild and transient fibrosis in rats (Bonniaud *et al.*, 2003), overexpression in mice confers susceptibility to bleomycin-induced fibrosis in the fibrosis-resistant Balb/c mouse strain (Bonniaud *et al.*, 2004). Moreover, selective expression of CTGF in fibroblasts *in vivo* has recently been shown to promote systemic tissue fibrosis, including in the lung (Sonnylal *et al.*, 2010). A Phase I clinical trial assessing a neutralizing antibody directed against CTGF (FG-3019; FibroGen, USA) was recently completed; the results demonstrate that this antibody is safe and well-tolerated. Further studies are required to assess potential therapeutic benefits of this antibody in IPF.

IL-13

There is growing evidence that the cytokine and chemokine response to an inciting agent determines whether the injury response is resolved or progresses to fibrosis. The Th hypothesis of fibrosis proposes that progressive pulmonary fibrosis results from a maladaptive immune response, dominated by Th2 cytokines, such as interleukin (IL)-13, to a persistent inciting agent [reviewed in (Wynn, 2004)]. Therapies aimed at redressing this imbalance may represent attractive anti-fibrotic strategies.

IL-13 is the most extensively studied Th2 cytokine in the context of several fibroproliferative diseases, including IPF. IL-13 levels are increased in BALF from patients with pulmonary fibrosis (Hancock *et al.*, 1998) and IL-13 promotes fibroblast collagen production (Oriente *et al.*, 2000; Saito *et al.*, 2003) and fibroblast to myofibroblast differentiation (Saito

et al., 2003) *in vitro*. These effects may be direct or dependent on secondary mediators such as TGF-β (Fichtner-Feigl *et al.*, 2006) and/or Found in Inflammatory Zone-1 (Liu *et al.*, 2004). Mice deficient for IL-13 are protected from fluorescein isothiocyanate-induced lung fibrosis (Kolodsick *et al.*, 2004) and IL-13 targeted therapies have proved successful in attenuating bleomycin-induced fibrosis in mice (Belperio *et al.*, 2002; Jakubzick *et al.*, 2003). More recently, IL-13 has also been shown to promote epithelial cell apoptosis *in vitro* (Borowski *et al.*, 2008) and may therefore play a role in the abnormal epithelial–mesenchymal crosstalk in IPF. Taken together, these observations support the notion that IL-13 may represent an attractive target for therapeutic intervention in IPF and other fibrotic lung diseases. An open label, non-randomized phase II trial investigating the effect of an anti-IL13 antibody (QAX576; Novartis, Switzerland) on IL-13 production in IPF has recently been concluded. Publication of the results is eagerly anticipated.

CCL2

There has long-standing interest in the major monocyte chemoattractant, CCL2/MCP-1, in pulmonary fibrosis based on the observation that this chemokine is elevated in BALF from IPF patients (Baran *et al.*, 2007). Moreover, serum CCL2 levels may correlate with the clinical course of IPF (Suga *et al.*, 1999). CCR2 knockout mouse studies (Moore *et al.*, 2005) and CCL12 neutralizing antibody studies (Moore *et al.*, 2006) in wild-type mice support a causal role for this chemokine axis in animal models of fibrosis. As well as being a potent chemoattractant for T cells, immature dendritic cells, mononuclear cells (Rose *et al.*, 2003) and fibrocytes (Moore *et al.*, 2005), CCL2 signalling may also promote fibrosis by inducing the expression of TGF-β (Gharaee-Kermani *et al.*, 1996). In terms of the immune response to injury, CCL2 also exerts immunomodulatory effects, which in turn may contribute to the development of a Th2 cytokine dominated phenotype (Karpus *et al.*, 1997; Hogaboam *et al.*, 1998; Gu *et al.*, 2000). A randomized double-blinded placebo-controlled trial to evaluate the safety and efficacy of the anti-CCL2 antibody, CNTO 888 (Centocor Inc, USA) is currently recruiting. Patients will be maintained on their current therapy and the primary end point is performance at lung function testing.

CXCR4 and CXCL12

As outlined earlier, there has been much recent interest in the role of chemokines in recruiting fibrocytes to the injured lung. Although both the CCR2/CCL12 and CXCL12/CXCR4 axes have been shown to play important roles in murine models, in human IPF greater focus has been placed on the CXCL12/CXCR4 axis (Phillips *et al.*, 2004). As such, there has been much interest in the development of CXCR4 antagonists for a number of indications, including cancer. Such agents may also be worth considering in the context of IPF. Alternative strategies targeting this axis include inhibition of CXCR4 expression – hypoxia- and growth factor-induced CXCR4 expression in fibrocytes is attenuated by inhibition of mTOR, and administration of the mTOR inhibitor rapamycin to rodents significantly inhibited bleomycin-induced lung collagen deposition (Simler *et al.*, 2002; Mehrad *et al.*, 2009).

Angiotensin converting enzyme (ACE) and angiotensin II (ANG II)

Angiotensin II is derived from the conversion of angiotensinogen (AGT) by ACE. ANG II is a potent inducer of epithelial apoptosis (Wang *et al.*, 1999) and there is *in vitro* and *in vivo* evidence that these effects are mediated by the ANGII receptor subtype, AT(1) (Li *et al.*, 2003a,b). ANG II is also a potent inducer of procollagen production by human lung fibroblasts, at least in part via the autocrine action of TGF-β (Marshall *et al.*, 2004). Recent studies have also provided evidence for the existence of an ANGII/TGF-β 'autocrine loop': human lung myofibroblasts derived from human IPF lung constitutively express more AGT and active TGF-β than control fibroblasts; in turn, induction of fibroblast to myofibroblast differentiation by TGF-β is associated with increased AGT expression (Uhal *et al.*, 2007).

ACE inhibitors such as captopril (Wang *et al.*, 2000a), and AT(1) receptor antagonists such as losartan (Marshall *et al.*, 2004) attenuate bleomycin-induced lung fibrosis. This response is associated with a reduction in epithelial cell apoptosis (Wang *et al.*, 2000a; Li *et al.*, 2003a) and TGF-β expression (Otsuka *et al.*, 2004).

In human disease, increased levels of ANGII are observed in IPF lung compared with non-fibrotic controls, localizing to apoptosing epithelial cells and myofibroblasts in fibrotic foci (Li *et al.*, 2006), Moreover, ACE insertion/deletion polymorphisms are associated with susceptibility and outcome in acute respiratory distress syndrome (Marshall *et al.*, 2002).

In light of these observations, two trials to evaluate the efficacy of losartan in IPF are currently recruiting. The first will focus on vascular reactivity in IPF and is beyond the scope of this review. The second trial is a pilot intervention study (University of South Florida) evaluating the FVC response to losartan after 12 months treatment. The estimated completion date for this study is March 2012.

Targeting the coagulation cascade

There is compelling evidence for a role for the coagulation cascade in driving the fibroproliferative response to lung injury [reviewed in (Chambers, 2008)]. Tissue factor is highly expressed on the hyperplastic epithelium in IPF (Imokawa *et al.*, 1997) and thrombin levels are increased in BALF from patients with fibrotic lung disease (Hernandez-Rodriguez *et al.*, 1995). Moreover, we have provided evidence that the upstream coagulation zymogen, factor X, is locally produced and activated in the intra-alveolar compartment of patients with IPF and in the bleomycin model (Scotton *et al.*, 2009). Anticoagulants are highly effective in attenuating fibrosis in experimental animal models when given either prophylactically (Howell *et al.*, 2001; Scotton *et al.*, 2009) or therapeutically (Gunther *et al.*, 2003).

While the coagulation cascade may contribute to pulmonary fibrosis by promoting the deposition and persistence of fibrin, current evidence suggests that the direct receptor-mediated cellular effects elicited by activation of the major high-affinity thrombin receptor, proteinase-activated receptor (PAR)-1) may play a central role [reviewed in (Chambers, 2008)]. Recent work has also highlighted a potentially key role for PAR-2 in pulmonary fibrosis (Borensztajn *et al.*, 2010).

PAR-1 is expressed by numerous cell types, including fibroblasts, epithelial cells and macrophages; and activation of this receptor leads to the release of potent pro-inflammatory and pro-fibrotic mediators [reviewed in (Chambers, 2008)]. In terms of pro-fibrotic responses, PAR-1 signalling in fibroblasts promotes their proliferation via the autocrine production of PDGF (Blanc-Brude *et al.*, 2005) and drives their differentiation into myofibroblasts via αvβ5-dependent TGF-β activation (Scotton *et al.*, 2009). On epithelial cells, PAR-1 activation similarly leads to TGF-β activation but this is mediated via the epithelial-restricted αvβ6 integrin (Jenkins *et al.*, 2006). PAR-1 signalling also induces the production and release of CTGF by lung fibroblasts (Chambers *et al.*, 2000) and thrombin up-regulates the expression of the fibrinolysis inhibitor, plasminogen activator inhibitor-1 in this cell type (Hayakawa *et al.*, 1995). Finally, studies in our laboratory with PAR-1 knockout mice provide strong support for a role for this receptor in mediating inflammatory and fibrotic responses to lung injury (Howell *et al.*, 2005).

In terms of demonstrating a causal role for the coagulation cascade in human disease, a recently completed trial investigating the effect of anticoagulation in IPF provided some support for this notion (Kubo *et al.*, 2005). In this non-blinded prospective randomized trial, patients with IPF who were admitted to hospital were randomly assigned to receive prednisolone or prednisolone and anticoagulation in the form of heparin or warfarin. Increased survival at 3 years was observed in the anticoagulation arm compared with the non-anticoagulation arm (63% vs. 25% respectively). Furthermore, mortality associated with acute exacerbations of IPF was reduced in the anticoagulation arm compared with those treated with prednisolone alone. Despite these promising data, concerns about the non-blinded nature of the trial as well as the diagnostic criteria used to confirm the diagnosis of IPF mean that the role of anticoagulation in IPF remains unclear. However, the AntiCoagulant Effectiveness in Idiopathic Pulmonary Fibrosis (ACE-IPF) trial (NHLBI, USA), currently recruiting patients, will hopefully shed important light on this issue. This double-blinded randomized placebo-controlled study will evaluate the efficacy of warfarin treatment on time to death or disease progression in IPF, and the results are eagerly awaited. It has also been proposed that nebulized administration of anticoagulant to patients might represent a means of achieving local anticoagulation without undesired systemic effects, and a recent open label exploratory study demonstrated that nebulized heparin was safe and well-tolerated in IPF patients (Markart *et al.*, 2010). Finally, PAR-1 antagonists are currently being developed as novel anti-thrombotic agents and several large-scale trials have recently been completed in the setting of cardiovascular disease. The scientific rationale for testing such antagonists in the setting of lung fibrosis is gaining strength.

Eicosanoid imbalance

There is good experimental evidence that re-establishing an intact epithelium following injury may serve to suppress excessive fibroblast activation. Because fibrosis may result from an imbalance in the relative levels of pro- and anti-fibrotic mediators, there has been much interest in the potential anti-fibrotic role of the cyclooxygenase (COX)-2 dependent prostanoid, prostaglandin E_2 (PGE_2). PGE_2 is the main prostanoid produced in the lung and is secreted by several cell types, including fibroblasts and epithelial cells (Maher *et al.*, 2010a). PGE_2 exerts major anti-fibrotic effects by suppressing fibroblast responses, including proliferation (Bitterman *et al.*, 1986; Lama *et al.*, 2002), differentiation into myofibroblasts (Kolodsick *et al.*, 2003) and collagen synthesis (Goldstein and Polgar, 1982). In support of an anti-fibrotic role for COX-2 in lung fibrosis, COX-2-deficient mice develop increased fibrosis following bleomycin-induced injury (Keerthisingam *et al.*, 2001). These findings are supported by the observation that PGE_2 levels are decreased in IPF lung, whereas levels of leukotrienes derived from the 5-lipooxygenase pathway, including LTB_4, are increased (Borok *et al.*, 1991b; Wilborn *et al.*, 1996). Several groups have shown that primary lung fibroblasts derived from patients with IPF are unable to up-regulate COX-2 in response to pro-inflammatory and pro-fibrotic mediator activation, including TGF-β (Keerthisingam *et al.*, 2001) (Wilborn *et al.*, 1995; Xaubet *et al.*, 2004).

Aside from exerting major anti-fibrotic effects, PGE2 has recently been implicated as playing a central role in promoting the 'apoptosis paradox' of IPF. According to this paradox, IPF is characterized by (and possibly the result of) excessive epithelial cell apoptosis and (myo)fibroblast resistance to apoptosis. Recent studies from our centre have shown that the lack of PGE_2 may provide a mechanistic explanation for increased resistance of myofibroblasts to apoptosis, in comparison with increased epithelial cell apoptosis (Maher *et al.*, 2010a). PGE_2 has also been shown to play a central role in mediating the anti-fibrotic effects of plasminogen activation (Bauman *et al.*, 2010). The EP receptors involved in mediating the anti-fibrotic and apoptotic responses of PGE_2 are coming to light with roles identified for both EP2 (Kolodsick *et al.*, 2003) and EP4 (Maher *et al.*, 2010b). Activation of these receptors with selective agonists (e.g. butaprost, ONO-A1-329) may offer promise as novel anti-fibrotic agents.

Further evidence to suggest that an eicosanoid imbalance contributes to a pro-fibrotic microenvironment stems from the observation that 5-LO knockout mice are protected from bleomycin-induced fibrosis (Peters-Golden *et al.*, 2002). In light of these data, a recent study has completed recruitment of IPF patients. This trial is an open label phase II trial (University of Michigan) comparing the 5-LO inhibitor, zileuton, to azathioprine and prednisolone: the primary end point is BALF LTB_4 levels, but secondary end points include performance at lung function testing and progression free survival.

Prostacyclin (PGI_2) is another arachidonic acid metabolite derived via the COX-2 pathway. Similar to PGE2, PGI_2 possess a number of anti-fibrotic properties, and COX-2 derived PGI_2 has been shown to play an important role in limiting the development of bleomycin-induced lung fibrosis (Lovgren *et al.*, 2006). Recent work has demonstrated that

intraperitoneal administration of iloprost, a PGI_2 analogue, attenuates the fibrosis seen in this model (Zhu *et al.*, 2010). The use of this drug in a nebulized form in PHT suggests that further investigation in IPF might be warranted.

Targeting the redox equilibrium

The recent addition of NAC to prednisolone and azathioprine as an adjunct therapy for IPF reflects the growing belief that targeting the increased oxidative burden in IPF (MacNee and Rahman, 1995; Rahman *et al.*, 1999) will result in a clinical benefit for these patients.

However, recent work has demonstrated that similar benefits may be observed when redressing this redox imbalance from the opposite side of the equation: NADPH oxidase (NOX)-4 catalyses the reduction of O_2 to ROS, and genetic and pharmacological targeting of NOX-4 attenuates experimentally induced fibrosis, possibly by interfering with TGF-β induced myofibroblast activity (Hecker *et al.*, 2009). Moreover, in addition to ROS there is evidence for nitric-oxide driven nitrosative stress in IPF (Saleh *et al.*, 1997a) and the administration of aminoguanidine, a specific inhibitor of inducible nitric oxide synthase, attenuates bleomycin-induced fibrosis in mice (Giri *et al.*, 2002). The development of such agents, as well as other novel antioxidant therapies such as superoxide dismutase mimetics, which inactivate ROS, may offer alternative therapeutic strategies to redress this redox imbalance, although the successful translation of these latter agents from animal to human studies has yet to be realized. Moreover, the identification of the redox-sensitive transcription factor Nrf2 as a regulator of antioxidant enzyme and defence protein genes [reviewed in (Walters *et al.*, 2008)], together with evidence of increased susceptibility of Nrf2 knockout mice to bleomycin-induced fibrosis (Cho *et al.*, 2004), may help identify further molecular targets and pathways for therapeutic modulation in IPF.

Epithelial mitogens

As mentioned previously, appropriate epithelial cell migration, proliferation and differentiation in response to injury is central to successful wound healing and tissue repair. There is accumulating evidence that such processes are impaired in the context of abnormal fibroproliferative responses to injury (Finch *et al.*, 1989; Rubin *et al.*, 1989; Deterding *et al.*, 1996). Intratracheal administration of the epithelial mitogen, keratinocyte growth factor (KGF) before bleomycin instillation attenuates the subsequent fibrotic response (Deterding *et al.*, 1997) while TGF-β blocks KGF-induced proliferation of alveolar pneumocytes *in vitro* (Zhang *et al.*, 2004). In addition, bone marrow transplantation of haematopoietic stem cells expressing KGF significantly reduces bleomycin-induced lung injury, possibly by promoting AEC II proliferation (Aguilar *et al.*, 2009). Administration of the epithelial mitogen, hepatocyte growth factor (HGF) similarly attenuates lung collagen accumulation in animal models of pulmonary fibrosis (Dohi *et al.*, 2000), and HGF further exerts a pro-apoptotic effect on myofibroblasts via the c-Met receptor (Mizuno *et al.*, 2005). Although this raises the possibility that activation of the HGF/c-Met system in fibrotic lungs may represent a potential target in IPF, this receptor system is frequently activated in a broad spectrum of human cancers – because IPF patients are at a heightened risk of developing lung cancer (Ozawa *et al.*, 2009), the potential role of the HGF/c-Met axis in driving epithelial tumours in IPF would need to be fully explored and understood before this approach could be deemed viable.

Angiogenesis

Despite the first observation of microvascular systemic-pulmonary anastamoses in IPF lung over 40 years ago (Turner-Warwick, 1963), the role of aberrant angiogenesis in the pathogenesis of this condition remains unclear. The key issue remains whether neovascularization represents a critical pathogenetic mechanism contributing to progressive fibrosis or a compensatory mechanism to promote alveolar repair.

In line with human studies, aberrant vascular remodelling has been observed in the lungs of bleomycin-challenged rats (Peao *et al.*, 1994) suggesting a pro-angiogenic microenvironment in this model. Evidence to suggest that an imbalance between angiogenic and angiostatic chemokines is of mechanistic importance in this model stems from observations that levels of the angiostatic chemokine CXCL10 are lower in bleomycin-challenged mice compared with control lungs (Keane *et al.*, 1999). Moreover, administration of both CXCL10 and CXCL11, another angiostatic chemokine, attenuate bleomycin-induced fibrosis with a concomitant reduction in angiogenesis (Keane *et al.*, 1999; Burdick *et al.*, 2005). In humans, increased levels of the angiogenic chemokines CXCL8 and CXCL5 have been reported in IPF lung (Keane *et al.*, 1997; 2001), while IPF-derived fibroblasts constitutively express more CXCL8 than their non-fibrotic counterparts (Keane *et al.*, 1997); depletion of these chemokines from lung tissue reduced the tissue-derived angiogenic activity (Keane *et al.*, 1997; 2001).

It seems therefore that targeting the pro-angiogenic microenvironment may provide a further therapeutic avenue for IPF patients. A more global approach to achieving an angiostatic environment may be achieved using agents such as tetrathiomolybdate (TM) and minocycline. TM is a copper-chelating agent that possesses anti-angiogenic properties *in vivo* (Pan *et al.*, 2002), which may be related to transcriptional down-regulation of angiogenic growth factors such as vascular endothelial growth factor (VEGF) (Brewer *et al.*, 2004). TM administration attenuated bleomycin-induced fibrosis in mice (Brewer *et al.*, 2003) and a non-randomized control trial (University of Michigan) investigating the safety of TM in IPF has recently been completed. The secondary end point in this trial was performance at lung function testing, and we await the results with interest. Minocycline is also known to possess anti-angiogenic properties (Tamargo *et al.*, 1991) and its efficacy in treating IPF is the subject of a Phase III trial (University of California) – this trial has finished recruiting and the results are awaited. The targeting of VEGF, along with PDGF and fibroblast growth factor signalling pathways is an area of active research in tumour biology. Optimism that BIBF 1120 [Boehringer Ingelheim Pharmaceuticals (BIP), UK], an inhibitor of their respective receptor kinases, fuelled by the observa-

tion that anti-VEGF gene therapy attenuates experimentally induced fibrosis (Hamada *et al.*, 2005), could prove an effective anti-angiogenic drug in this field prompted the initiation of a double-blinded, placebo-controlled trial (BIP, UK) to evaluate the safety and efficacy of this drug in IPF. The results are currently pending.

However, recent work suggests a decrease in the extent of anastamoses (Renzoni *et al.*, 2003) between the pulmonary and systemic vasculature within the fibroblastic foci of IPF, highlighting the lack of clarity regarding the role of neovascularization in IPF – to this end, it has been proposed that aberrant neovascularization in areas of less fibrosis may represent a compensatory mechanism to the vascular ablation reported in the aforementioned work, and necessary for regeneration of alveolar septae (Renzoni, 2004). Further investigation is therefore required to elucidate the role of neovascularization in the pathogenesis of IPF to enable a rational interpretation of data derived from the aforementioned trials.

Ongoing trials of other agents in IPF

Somatostatin

Aside from the broad and overlapping therapeutic categories discussed above, a number of other novel targets potentially important in the pathogenesis of IPF have recently been identified. For example, recent work has demonstrated that expression of the somatostatin receptor, sst2, is increased in mice following bleomycin challenge (Borie *et al.*, 2008). Subcutaneous administration of a somatostatin analogue, SOM230 (Novartis, Switzerland), in this model attenuates bleomycin-induced fibrosis, and this attenuation is associated with reduced expression of TGF-β and CTGF (Borie *et al.*, 2008). The anti-fibrotic mechanism of action of somatostatin analogues remains unclear, although it may relate to inhibition of fibroblast proliferation (Borie *et al.*, 2008). Increased expression of the sst2 receptor is also observed in the lungs of patients with IPF (Antoniu, 2008), and these data prompted the initiation of a proof of concept, non-randomized open label study (Institut National de la Santé Et de la Recherche Médicale, France) to evaluate the efficacy of octreotide, a somatostatin analogue, in IPF. This study has been completed, although the results are yet to be reported.

Thalidomide

Thalidomide is a drug originally introduced as a sedative. Despite its well-known teratogenic effects, it has proven efficacious in treating a wide variety of conditions including multiple myeloma. Thalidomide possesses anti-inflammatory (Koch, 1985), immunomodulatory (Haslett *et al.*, 1998) and anti-angiogenic properties (D'Amato *et al.*, 1994), and has been demonstrated to attenuate bleomycin-induced fibrosis in mice (Tabata *et al.*, 2007). Its precise mechanism of action remains unclear, although the observed attenuation in fibrosis following experimental lung injury is accompanied by a reduction in VEGF expression (Tabata *et al.*, 2007), suggesting that inhibition of neovascularization might represent a potential mechanism. IL-6 expression has also been shown to be reduced in this model suggesting multiple possible modes of action (Tabata *et al.*, 2007). In light of these data, a non-randomized open label study (John Hopkins University) designed to evaluate the safety and efficacy of thalidomide in patients with IPF has been completed, but the results are not yet published.

Recent advances in the identification of novel targets and pathways

Lysophosphatidic acid

Recent work has identified the bioactive phospholipid derivative lysophosphaditic acid (LPA), acting via stimulation of its multiple G-protein-coupled receptors LPA_{1-5}, as an important mediator in wound repair and tissue fibrogenesis (Watterson *et al.*, 2007). The potential significance of this mediator in lung fibrosis has been highlighted by studies demonstrating a critical role for LPA_1 activation in fibroblast recruitment and vascular leak following experimentally induced lung injury in mice (Tager *et al.*, 2008). In addition, LPA induces αvβ6-mediated TGF-β activation in lung epithelial cells (Xu *et al.*, 2009) via RhoA and Rho kinase, following interaction with the LPA_2 receptor, findings consistent with previous observations that LPA is capable of mediating cellular contraction in a number of different cell types (Chrzanowska-Wodnicka and Burridge, 1996). In support of a role for LPA in human disease, BALF LPA levels and LPA_2 immunoreactivity are significantly increased in IPF patients compared with non-fibrotic control samples (Tager *et al.*, 2008; Xu *et al.*, 2009), suggesting that LPA may represent a novel therapeutic target in pulmonary fibrosis. Indeed, the success of prophylactic administration of a LPA_1 receptor antagonist, in attenuating bleomycin-induced lung fibrosis in mice, has prompted the initiation of a phase I clinical study using AM152 (Amira, USA), an alternative LPA_1 antagonist, in healthy subjects, with a view to evaluating its anti-fibrotic efficacy in IPF in the future.

Wnt signalling

The Wnt signalling pathway plays a crucial role in lung development, regulating both epithelial and mesenchymal development via autocrine and paracrine signals. A detailed discussion of this signalling pathway is beyond the scope of this article and has been recently reviewed (Kikuchi *et al.*, 2007). In brief, Wnt proteins bind to Frizzled cell surface receptors or low-density lipoprotein co-receptors. The inhibition of glycogen synthase kinase 3β results in the hypophosphorylation of β-catenin that allows translocation of this cytoskeletal protein into the nucleus. Subsequent binding of β-catenin to the LEC/TCF family of transcription factors converts them from transcriptional repressors to activators.

Support for the potential involvement of this pathway in IPF comes from observations in humans and animal models. Strong nuclear β-catenin immunoreactivity is observed in the lungs of IPF patients, localizing to fibroblasts within fibrotic foci and to proliferative bronchiolar lesions, a finding not observed in non-IPF lung (Chilosi *et al.*, 2003). β-catenin and WNT-1-inducible signalling protein (WISP-1) have been shown to promote EMT *in vitro* suggesting that dysregulated activation of β-catenin associated transcription factors could promote an expansion of the myofibroblast population in IPF

(Chilosi *et al*., 2003) (Konigshoff *et al*., 2009). Further support for the importance of the Wnt signalling pathway in the pathogenesis of IPF stems from the observation that mice deficient in matrilysin (MMP-7), a target gene of the β-catenin-LEF1 signalling pathway, are protected from bleomycin-induced fibrosis, and interestingly expression of MMP7 is significantly increased in IPF lung (Zuo *et al*., 2002)., WISP-1 is up-regulated in humans with IPF and mediates pulmonary fibrosis in mice (Konigshoff *et al*., 2009) and pharmacological inhibition of Wnt/beta-catenin/CREB binding protein signalling reverses experimentally-induced pulmonary fibrosis (Konigshoff *et al*., 2009; Henderson *et al*., 2010) The scientific rationale for interfering with Wnt signalling in IPF is therefore rapidly gaining strength.

Jagged/Notch pathway

As highlighted previously, it is increasingly recognized that in the context of a pro-fibrogenic cytokine milieu, EMT may promote the development of a myofibroblast population that significantly contributes to fibrogenesis. While TGF-β is seen as the principle driving force for EMT, recent studies have demonstrated the potential importance of integration of TGF-β and Jagged ligand/Notch receptor signalling pathways in EMT – these pathways are highly evolutionary conserved cell signalling systems that regulate cell fate specification, and siRNA targeting of components of the Notch pathway has previously been shown to block TGF-β induced EMT in kidney tubule epithelial cells (Zavadil *et al*., 2004). Recent work has demonstrated that Notch signalling plays a role in EMT both upstream and downstream of TGF-β in rat AECs *in vitro*, and that inhibition of Notch receptor activation attenuates TGF-β – induced α-SMA expression (Aoyagi-Ikeda *et al*., 2010), a finding seen also in kidney tubule epithelial cells (Nyhan *et al*., 2010). Furthermore, Notch1 co-localizes with α-SMA in bleomycin-induced pulmonary fibrosis and in patients with pulmonary fibrosis (Aoyagi-Ikeda *et al*., 2010). These exciting data suggest that inhibition of the Notch signalling pathway may offer a further therapeutic opportunity to tackle pulmonary fibrosis, one that may bypass the potential problems of a more global anti-TGF-β strategy.

Lysyl oxidase-2

Lysyl oxidase-2 (LOXL2) belongs to a family of five enzymes that play essential roles during the biogenesis of connective tissue by catalysing the first step in the formation of cross links in collagens and elastin (Kagan and Li, 2003). Recent studies have highlighted a novel role for LOXL2 in the creation and maintenance of the pathologic micro-environment of cancer and fibrotic diseases (Akiri *et al*., 2003; Erler *et al*., 2006). This enzyme is up-regulated in IPF and administration of a monoclonal anti-LOXL2 antibody (AB0023; Arresto Bio-Sciences, USA) dosed either prophylactically or therapeutically, significantly attenuated bleomycin-induced fibrosis in mice (Barry-Hamilton *et al*., 2010). This attenuation was associated with a decrease in the number of α-SMA + fibroblasts as well as a considerable improvement in cross-linked fibrillar collagen abundance and a reduction in TGF-β signalling (Barry-Hamilton *et al*., 2010). LOXL2 could therefore potentially mediate fibroblast activation *in vivo* by enzymatically catalysing the cross-linking of fibrillar collagen with a corresponding increase in local matrix tension (Wipff *et al*., 2007) resulting in activation of TGF-β1 signalling from the latent complex as outlined earlier. LOXL2 expression is relatively low in normal tissues so therapeutic targeting of this enzyme may represent an attractive target for therapeutic intervention in several fibrotic conditions, including pulmonary fibrosis.

microRNA

microRNAs (miRNAs) are short (20–24 nt) non-coding RNAs that are involved in post-transcriptional regulation of gene expression by affecting both the stability and translation of mRNA. They are known to play critical roles in organogenesis (Stefani and Slack, 2008), and dysregulation of miRNAs is increasingly implicated in a variety of disease processes. Recent microRNA microarray studies in IPF showed that a number of miRNAs are differentially expressed in IPF compared with non-fibrotic control lungs. Notable examples include the miRNA, let-7d, which is down-regulated in the IPF lung, as well as in response to TGF-β signalling. The down-regulation of let-7d in IPF and the pro-fibrotic effects of this down-regulation *in vitro* and *in vivo* suggest a key regulatory role for this microRNA in preventing lung fibrosis (Pandit *et al*., 2010). In contrast, a second study focused on miR-21 – this is up-regulated in human IPF lung specimens, as well as in the murine bleomycin model (Liu *et al*., 2010a). In this study, miR-21 was found to exert/promote pro-fibrotic responses, potentially by amplifying the fibrogenic effects of TGF-β by regulating the expression of an inhibitory Smad, Smad7. Administration of miR-21 antisense probes diminished the severity of experimental lung fibrosis in mice, even when treatment was started 5–7 days after initiation of bleomycin injury. This study raises the tantalizing possibility that future developments in miRNA therapeutics may open up novel opportunities for treating clinically refractory fibrotic diseases, such as IPF.

Conclusions and future directions

Idiopathic pulmonary fibrosis is a devastating and progressive condition with an appalling prognosis. It is clear that the fibroproliferative response to injury seen in this condition reflects an extremely complex interplay between a number of different cellular and signalling mechanisms, with an unknown degree of redundancy. Furthermore, it is increasingly appreciated that the targeting of one particular pathway only, may not have any effect on fibrosis secondary to injury; rather, lung fibrosis may be a consequence of disequilibrium in a number of different processes – epithelial and endothelial injury; inflammation and the immune response to injury; myofibroblast expansion; hypercoagulation; angiogenesis and aberrant wound repair mechanisms. The relative importance of these pathways, which share the final common pathway of fibrogenesis, may further vary across individuals, highlighting the importance of identifying subgroups of phenotypes, which may be more responsive to particular therapies.

The bleomycin model of fibrosis in mice is a useful model to delineate the relative importance to pathogenesis of these pathways but, as has been well documented, is by no means

an accurate representation of all the features of IPF (Scotton and Chambers, 2010). Aside from pirfenidone and NAC, optimism that targets derived from attenuation of experimentally induced fibrosis would translate into a clinical benefit in IPF has not yet been realized. In terms of the clinical predictability of the bleomycin model to IPF, therapeutic rather than prophylactic dosing is recommended in order to avoid interfering with the inflammatory response rather than the fibrotic response to injury (Moeller *et al.*, 2008; Scotton and Chambers, 2010).

The use of high-throughput gene expression profiling technology may be of particular benefit in understanding the complex interplays seen in pulmonary fibrosis. Microarray analysis of RNA expression in human disease samples can reveal regulatory networks and expression profiles, which underlie disease progression (see (Kaminski and Rosas, 2006) for review), but as yet, no targets identified by this means have been trialled in IPF.

Aside from challenges to understanding pathogenetic mechanisms in IPF, advances in therapeutics have been limited by a number of other factors. Importantly, the intrinsic nature of the disease, a slow burning process reflecting years of dysregulated remodelling, means that identifying patients before end-stage fibrosis has developed is problematic – it is by no means certain that an adult lung has the capacity to remodel and regain functionality from established fibrosis, and a halt to disease progression may be all that can achieved. The design of clinical trials to evaluate therapeutic strategies in IPF can also be beset by problems. For instance, selection bias and diagnostic uncertainty in this heterogeneous condition may result in patients with varying degrees of baseline disease, and therefore varying degrees of sensitivity to treatment, being inappropriately enrolled into the same trial. In addition, there remains no clear consensus as to the most appropriate end point to study in interventional studies. Clearly, mortality is the most robust outcome but large numbers of patients are required to be maintained within a trial for long periods. A 10% decline in FVC over 1 year is a widely used parameter of disease progression and increased risk of mortality (King *et al.*, 2001; Flaherty *et al.*, 2003; Latsi *et al.*, 2003), although recent work suggests that smaller changes may be of clinical significance in IPF (Zappala *et al.*, 2010) and while composite indices of lung function can predict mortality, their use has not been adopted into the design of recent clinical trials. The recent demonstration, however, that large well-conducted trials can be performed to evaluate drug treatments in IPF, together with the realization that the therapeutic targeting of multiple pro-fibrotic pathways is likely to be more successful than focusing on single pathways, offers more hope than ever before to sufferers of this devastating condition.

Acknowledgements

The authors are grateful to the following grant agencies for funding for research into the pathogenesis of pulmonary fibrosis: Wellcome Trust (A.D. Clinical Research Training Fellowship 084382/Z/07/Z), MRC (C.J.S. Career Development Award G0800340; R.C.C. CASE Award 2009–12), European Commission (R.C.C Framework 7 Programme HEALTH-F2-2007-2224; European IPF Network) and BBSRC (CASE Award BB/I532702/1).

Conflicts of interest

R.C.C has/is acting as a consultant for the following companies: Centocor, Sanofi-Aventis and GlaxoSmithKline and is currently the recipient of research funding from GlaxoSmithKline and Novartis. C.J.S. has acted as a consultant for GlaxoSmithKline and is currently the recipient of research funding from GlaxoSmithKline.

References

Adler KB, Low RB, Leslie KO, Mitchell J, Evans JN (1989). Contractile cells in normal and fibrotic lung. Lab Invest 60: 473–485.

Aguilar S, Scotton CJ, McNulty K, Nye E, Stamp G, Laurent G *et al.* (2009). Bone marrow stem cells expressing keratinocyte growth factor via an inducible lentivirus protects against bleomycin-induced pulmonary fibrosis. PloS ONE 4: e8013.

Akiri G, Sabo E, Dafni H, Vadasz Z, Kartvelishvily Y, Gan N *et al.* (2003). Lysyl oxidase-related protein-1 promotes tumor fibrosis and tumor progression in vivo. Cancer Res 63: 1657–1666.

Allen JT, Knight RA, Bloor CA, Spiteri MA (1999). Enhanced insulin-like growth factor binding protein-related protein 2 (Connective tissue growth factor) expression in patients with idiopathic pulmonary fibrosis and pulmonary sarcoidosis. Am J Respir Cell Mol Biol 21: 693–700.

Andersson-Sjoland A, de Alba CG, Nihlberg K, Becerril C, Ramirez R, Pardo A *et al.* (2008). Fibrocytes are a potential source of lung fibroblasts in idiopathic pulmonary fibrosis. Int J Biochem Cell Biol 40: 2129–2140.

Antoniades HN, Bravo MA, Avila RE, Galanopoulos T, Neville-Golden J, Maxwell M *et al.* (1990). Platelet-derived growth factor in idiopathic pulmonary fibrosis. J Clin Invest 86: 1055–1064.

Antoniu SA (2008). Somatostatin analogs for idiopathic pulmonary fibrosis therapy. Expert Opin Investig Drugs 17: 1137–1140.

Aono Y, Nishioka Y, Inayama M, Ugai M, Kishi J, Uehara H *et al.* (2005). Imatinib as a novel antifibrotic agent in bleomycin-induced pulmonary fibrosis in mice. Am J Respir Crit Care Med 171: 1279–1285.

Aoyagi-Ikeda K, Maeno T, Matsui H, Ueno M, Hara K, Aoki Y *et al.* (2010). Notch Induces myofibroblast differentiation of alveolar epithelial cells via TGF-ss/Smad3 pathway. Am J Respir Cell Mol Biol (in press).

Ask K, Bonniaud P, Maass K, Eickelberg O, Margetts PJ, Warburton D *et al.* (2008). Progressive pulmonary fibrosis is mediated by TGF-beta isoform 1 but not TGF-beta3. Int J Biochem Cell Biol 40: 484–495.

Atabai K, Jame S, Azhar N, Kuo A, Lam M, McKleroy W *et al.* (2009). Mfge8 diminishes the severity of tissue fibrosis in mice by binding and targeting collagen for uptake by macrophages. J Clin Invest 119: 3713–3722.

ATS/ERS (2000). American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med 161: 646–664.

Avivi-Green C, Singal M, Vogel WF (2006). Discoidin domain receptor 1-deficient mice are resistant to bleomycin-induced lung fibrosis. Am J Respir Crit Care Med 174: 420–427.

Baran CP, Opalek JM, McMaken S, Newland CA, O'Brien JM Jr, Hunter MG *et al.* (2007). Important roles for macrophage colony-stimulating factor, CC chemokine ligand 2, and mononuclear phagocytes in the pathogenesis of pulmonary fibrosis. Am J Respir Crit Care Med 176: 78–89.

Barbas-Filho JV, Ferreira MA, Sesso A, Kairalla RA, Carvalho CR, Capelozzi VL (2001). Evidence of type II pneumocyte apoptosis in the pathogenesis of idiopathic pulmonary fibrosis (IFP)/usual interstitial pneumonia (UIP). J Clin Pathol 54: 132–138.

Barry-Hamilton V, Spangler R, Marshall D, McCauley S, Rodriguez HM, Oyasu M *et al.* (2010). Allosteric inhibition of lysyl oxidase-like-2 impedes the development of a pathologic microenvironment. Nat Med 16: 1009–1017.

Bauman KA, Wettlaufer SH, Okunishi K, Vannella KM, Stoolman JS, Huang SK *et al.* (2010). The antifibrotic effects of plasminogen activation occur via prostaglandin E2 synthesis in humans and mice. J Clin Invest 120: 1950–1960.

Beeh KM, Beier J, Haas IC, Kornmann O, Micke P, Buhl R (2002). Glutathione deficiency of the lower respiratory tract in patients with idiopathic pulmonary fibrosis. Eur Respir J 19: 1119–1123.

Behr J, Noble PW (2009). Clinical trials in interstitial lung disease. European Respiratory Soc Monograph 46: 67–84.

Behr J, Degenkolb B, Maier K, Braun B, Beinert T, Krombach F *et al.* (1995). Increased oxidation of extracellular glutathione by bronchoalveolar inflammatory cells in diffuse fibrosing alveolitis. Eur Respir J 8: 1286–1292.

Behr J, Degenkolb B, Krombach F, Vogelmeier C (2002). Intracellular glutathione and bronchoalveolar cells in fibrosing alveolitis: effects of N-acetylcysteine. Eur Respir J 19: 906–911.

Belperio JA, Dy M, Burdick MD, Xue YY, Li K, Elias JA *et al.* (2002). Interaction of IL-13 and C10 in the pathogenesis of bleomycin-induced pulmonary fibrosis. Am J Respir Cell Mol Biol 27: 419–427.

Bitterman PB, Wewers MD, Rennard SI, Adelberg S, Crystal RG (1986). Modulation of alveolar macrophage-driven fibroblast proliferation by alternative macrophage mediators. J Clin Invest 77: 700–708.

Blanc-Brude OP, Archer F, Leoni P, Derian C, Bolsover S, Laurent GJ *et al.* (2005). Factor Xa stimulates fibroblast procollagen production, proliferation, and calcium signaling via PAR1 activation. Exp Cell Res 304: 16–27.

Bonniaud P, Margetts PJ, Kolb M, Haberberger T, Kelly M, Robertson J *et al.* (2003). Adenoviral gene transfer of connective tissue growth factor in the lung induces transient fibrosis. Am J Respir Crit Care Med 168: 770–778.

Bonniaud P, Martin G, Margetts PJ, Ask K, Robertson J, Gauldie J *et al.* (2004). Connective tissue growth factor is crucial to inducing a profibrotic environment in 'fibrosis-resistant' BALB/c mouse lungs. Am J Respir Cell Mol Biol 31: 510–516.

Bonniaud P, Margetts PJ, Kolb M, Schroeder JA, Kapoun AM, Damm D *et al.* (2005). Progressive transforming growth factor beta1-induced lung fibrosis is blocked by an orally active ALK5 kinase inhibitor. Am J Respir Crit Care Med 171: 889–898.

Borensztajn K, Bresser P, van der Loos C, Bot I, van den Blink B, den Bakker MA *et al.* (2010). Protease-activated receptor-2 induces myofibroblast differentiation and tissue factor up-regulation during bleomycin-induced lung injury. Potential role in pulmonary fibrosis. Am J Pathol 177: 2753–2764.

Borie R, Fabre A, Prost F, Marchal-Somme J, Lebtahi R, Marchand-Adam S *et al.* (2008). Activation of somatostatin receptors attenuates pulmonary fibrosis. Thorax 63: 251–258.

Borok Z, Buhl R, Grimes GJ, Bokser AD, Hubbard RC, Holroyd KJ *et al.* (1991a). Effect of glutathione aerosol on oxidant-antioxidant imbalance in idiopathic pulmonary fibrosis. Lancet 338: 215–216.

Borok Z, Gillissen A, Buhl R, Hoyt RF, Hubbard RC, Ozaki T *et al.* (1991b). Augmentation of functional prostaglandin E levels on the respiratory epithelial surface by aerosol administration of prostaglandin E. Am Rev Respir Dis 144: 1080–1084.

Borowski A, Kuepper M, Horn U, Knupfer U, Zissel G, Hohne K *et al.* (2008). Interleukin-13 acts as an apoptotic effector on lung epithelial cells and induces pro-fibrotic gene expression in lung fibroblasts. Clin Exp Allergy 38: 619–628.

Breuss JM, Gallo J, DeLisser HM, Klimanskaya IV, Folkesson HG, Pittet JF *et al.* (1995). Expression of the beta 6 integrin subunit in development, neoplasia and tissue repair suggests a role in epithelial remodeling. J Cell Sci 108: 2241–2251.

Brewer GJ, Ullenbruch MR, Dick R, Olivarez L, Phan SH (2003). Tetrathiomolybdate therapy protects against bleomycin-induced pulmonary fibrosis in mice. J Lab Clin Med 141: 210–216.

Brewer GJ, Dick R, Ullenbruch MR, Jin H, Phan SH (2004). Inhibition of key cytokines by tetrathiomolybdate in the bleomycin model of pulmonary fibrosis. J Inorg Biochem 98: 2160–2167.

Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A (1994). Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. Mol Med 1: 71–81.

Burdick MD, Murray LA, Keane MP, Xue YY, Zisman DA, Belperio JA *et al.* (2005). CXCL11 attenuates bleomycin-induced pulmonary fibrosis via inhibition of vascular remodeling. Am J Respir Crit Care Med 171: 261–268.

Cantin AM, Hubbard RC, Crystal RG (1989). Glutathione deficiency in the epithelial lining fluid of the lower respiratory tract in idiopathic pulmonary fibrosis. Am Rev Respir Dis 139: 370–372.

Chambers RC (2008). Procoagulant signalling mechanisms in lung inflammation and fibrosis: novel opportunities for pharmacological intervention? Br J Pharmacol 153 (Suppl. 1): S367–S378.

Chambers RC, Leoni P, Blanc-Brude OP, Wembridge DE, Laurent GJ (2000). Thrombin is a potent inducer of connective tissue growth factor production via proteolytic activation of protease-activated receptor-1. J Biol Chem 275: 35584–35591.

Chambers RC, Leoni P, Kaminski N, Laurent GJ, Heller RA (2003). Global expression profiling of fibroblast responses to transforming growth factor-beta1 reveals the induction of inhibitor of differentiation-1 and provides evidence of smooth muscle cell phenotypic switching. Am J Pathol 162: 533–546.

Chilosi M, Poletti V, Murer B, Lestani M, Cancellieri A, Montagna L *et al.* (2002). Abnormal re-epithelialization and lung remodeling in idiopathic pulmonary fibrosis: the role of deltaN-p63. Lab Invest 82: 1335–1345.

Chilosi M, Poletti V, Zamo A, Lestani M, Montagna L, Piccoli P *et al.* (2003). Aberrant Wnt/beta-catenin pathway activation in idiopathic pulmonary fibrosis. Am J Pathol 162: 1495–1502.

Cho HY, Reddy SP, Yamamoto M, Kleeberger SR (2004). The transcription factor NRF2 protects against pulmonary fibrosis. FASEB J 18: 1258–1260.

Chrzanowska-Wodnicka M, Burridge K (1996). Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. J Cell Biol 133: 1403–1415.

Clark JG, Madtes DK, Raghu G (1993). Effects of platelet-derived growth factor isoforms on human lung fibroblast proliferation and procollagen gene expression. Exp Lung Res 19: 327–344.

Coker RK, Laurent GJ, Shahzeidi S, Lympany PA, du Bois RM, Jeffery PK *et al*. (1997). Transforming growth factors-beta 1, -beta 2, and -beta 3 stimulate fibroblast procollagen production in vitro but are differentially expressed during bleomycin-induced lung fibrosis. Am J Pathol 150: 981–991.

Collard HR (2010). Idiopathic pulmonary fibrosis and pirfenidone. Eur Respir J 35: 728–729.

Collard HR, Ryu JH, Douglas WW, Schwarz MI, Curran-Everett D, King TE *et al*. (2004). Combined corticosteroid and cyclophosphamide therapy does not alter survival in idiopathic pulmonary fibrosis. Chest 125: 2169–2174.

Corrin B, Dewar A, Rodriguez-Roisin R, Turner-Warwick M (1985). Fine structural changes in cryptogenic fibrosing alveolitis and asbestosis. J Pathol 147: 107–119.

Coultas DB, Zumwalt RE, Black WC, Sobonya RE (1994). The epidemiology of interstitial lung diseases. Am J Respir Crit Care Med 150: 967–972.

D'Amato RJ, Loughnan MS, Flynn E, Folkman J (1994). Thalidomide is an inhibitor of angiogenesis. Proc Natl Acad Sci USA 91: 4082–4085.

Daniels CE, Wilkes MC, Edens M, Kottom TJ, Murphy SJ, Limper AH *et al*. (2004). Imatinib mesylate inhibits the profibrogenic activity of TGF-beta and prevents bleomycin-mediated lung fibrosis. J Clin Invest 114: 1308–1316.

Daniels CE, Lasky JA, Limper AH, Mieras K, Gabor E, Schroeder DR (2010). Imatinib treatment for idiopathic pulmonary fibrosis: randomized placebo-controlled trial results. Am J Respir Crit Care Med 181: 604–610.

Davies HR, Richeldi L, Walters EH (2003). Immunomodulatory agents for idiopathic pulmonary fibrosis. Cochrane Database Syst Rev (3): CD003134.

Day E, Waters B, Spiegel K, Alnadaf T, Manley PW, Buchdunger E *et al*. (2008). Inhibition of collagen-induced discoidin domain receptor 1 and 2 activation by imatinib, nilotinib and dasatinib. Eur J Pharmacol 599: 44–53.

Demedts M, Behr J, Buhl R, Costabel U, Dekhuijzen R, Jansen HM *et al*. (2005). High-dose acetylcysteine in idiopathic pulmonary fibrosis. N Engl J Med 353: 2229–2242.

Deterding RR, Jacoby CR, Shannon JM (1996). Acidic fibroblast growth factor and keratinocyte growth factor stimulate fetal rat pulmonary epithelial growth. Am J Physiol 271: L495–L505.

Deterding RR, Havill AM, Yano T, Middleton SC, Jacoby CR, Shannon JM *et al*. (1997). Prevention of bleomycin-induced lung injury in rats by keratinocyte growth factor. Proc Assoc Am Physicians 109: 254–268.

Di Sario A, Bendia E, Svegliati Baroni G, Ridolfi F, Casini A, Ceni E *et al*. (2002). Effect of pirfenidone on rat hepatic stellate cell proliferation and collagen production. J Hepatol 37: 584–591.

Ding LW, Wu Z, Liu T, Ullenbruch M, Liu J, Phan S (2010). Activation of stem cell factor/c-kit signaling pathway in pulmonary fibrosis. Am J Respir Crit Care Med 181 (Suppl.): A3536.

Dohi M, Hasegawa T, Yamamoto K, Marshall BC (2000). Hepatocyte growth factor attenuates collagen accumulation in a murine model of pulmonary fibrosis. Am J Respir Crit Care Med 162: 2302–2307.

Dorscheid DR, Wojcik KR, Sun S, Marroquin B, White SR (2001). Apoptosis of airway epithelial cells induced by corticosteroids. Am J Respir Crit Care Med 164: 1939–1947.

Douglas WW, Ryu JH, Bjoraker JA, Schroeder DR, Myers JL, Tazelaar HD *et al*. (1997). Colchicine versus prednisone as treatment of usual interstitial pneumonia. Mayo Clin Proc 72: 201–209.

Douglas WW, Ryu JH, Schroeder DR (2000). Idiopathic pulmonary fibrosis: impact of oxygen and colchicine, prednisone, or no therapy on survival. Am J Respir Crit Care Med 161: 1172–1178.

Elias JA, Jimenez SA, Freundlich B (1987). Recombinant gamma, alpha, and beta interferon regulation of human lung fibroblast proliferation. Am Rev Respir Dis 135: 62–65.

Elias JA, Freundlich B, Adams S, Rosenbloom J (1990). Regulation of human lung fibroblast collagen production by recombinant interleukin-1, tumor necrosis factor, and interferon-gamma. Ann N Y Acad Sci 580: 233–244.

Erler JT, Bennewith KL, Nicolau M, Dornhofer N, Kong C, Le QT *et al*. (2006). Lysyl oxidase is essential for hypoxia-induced metastasis. Nature 440: 1222–1226.

Ezquerro IJ, Lasarte JJ, Dotor J, Castilla-Cortazar I, Bustos M, Penuelas I *et al*. (2003). A synthetic peptide from transforming growth factor beta type III receptor inhibits liver fibrogenesis in rats with carbon tetrachloride liver injury. Cytokine 22: 12–20.

Feghali-Bostwick CA, Tsai CG, Valentine VG, Kantrow S, Stoner MW, Pilewski JM *et al*. (2007). Cellular and humoral autoreactivity in idiopathic pulmonary fibrosis. J Immunol 179: 2592–2599.

Fichtner-Feigl S, Strober W, Kawakami K, Puri RK, Kitani A (2006). IL-13 signaling through the IL-13alpha2 receptor is involved in induction of TGF-beta1 production and fibrosis. Nat Med 12: 99–106.

Finch PW, Rubin JS, Miki T, Ron D, Aaronson SA (1989). Human KGF is FGF-related with properties of a paracrine effector of epithelial cell growth. Science 245: 752–755.

Flaherty KR, Toews GB, Lynch JP 3rd, Kazerooni EA, Gross BH, Strawderman RL *et al*. (2001). Steroids in idiopathic pulmonary fibrosis: a prospective assessment of adverse reactions, response to therapy, and survival. Am J Med 110: 278–282.

Flaherty KR, Mumford JA, Murray S, Kazerooni EA, Gross BH, Colby TV *et al*. (2003). Prognostic implications of physiologic and radiographic changes in idiopathic interstitial pneumonia. Am J Respir Crit Care Med 168: 543–548.

Gasse P, Riteau N, Charron S, Girre S, Fick L, Petrilli V *et al*. (2009). Uric acid is a danger signal activating NALP3 inflammasome in lung injury inflammation and fibrosis. Am J Respir Crit Care Med 179: 903–913.

Gharaee-Kermani M, Denholm EM, Phan SH (1996). Costimulation of fibroblast collagen and transforming growth factor beta1 gene expression by monocyte chemoattractant protein-1 via specific receptors. J Biol Chem 271: 17779–17784.

Giaid A, Michel RP, Stewart DJ, Sheppard M, Corrin B, Hamid Q (1993). Expression of endothelin-1 in lungs of patients with cryptogenic fibrosing alveolitis. Lancet 341: 1550–1554.

Giri SN, Leonard S, Shi X, Margolin SB, Vallyathan V (1999). Effects of pirfenidone on the generation of reactive oxygen species in vitro. J Environ Pathol Toxicol Oncol 18: 169–177.

Giri SN, Biring I, Nguyen T, Wang Q, Hyde DM (2002). Abrogation of bleomycin-induced lung fibrosis by nitric oxide synthase inhibitor, aminoguanidine in mice. Nitric Oxide 7: 109–118.

Goldstein RH, Polgar P (1982). The effect and interaction of bradykinin and prostaglandins on protein and collagen production by lung fibroblasts. J Biol Chem 257: 8630–8633.

Gressner AM, Weiskirchen R (2006). Modern pathogenetic concepts of liver fibrosis suggest stellate cells and TGF-beta as major players and therapeutic targets. J Cell Mol Med 10: 76–99.

Gribbin J, Hubbard RB, Le Jeune I, Smith CJ, West J, Tata LJ (2006). Incidence and mortality of idiopathic pulmonary fibrosis and sarcoidosis in the UK. Thorax 61: 980–985.

Grunert S, Jechlinger M, Beug H (2003). Diverse cellular and molecular mechanisms contribute to epithelial plasticity and metastasis. Nat Rev Mol Cell Biol 4: 657–665.

Gu L, Tseng S, Horner RM, Tam C, Loda M, Rollins BJ (2000). Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. Nature 404: 407–411.

Gunther A, Lubke N, Ermert M, Schermuly RT, Weissmann N, Breithecker A *et al.* (2003). Prevention of bleomycin-induced lung fibrosis by aerosolization of heparin or urokinase in rabbits. Am J Respir Crit Care Med 168: 1358–1365.

Gurujeyalakshmi G, Giri SN (1995). Molecular mechanisms of antifibrotic effect of interferon gamma in bleomycin-mouse model of lung fibrosis: downregulation of TGF-beta and procollagen I and III gene expression. Exp Lung Res 21: 791–808.

Gurujeyalakshmi G, Hollinger MA, Giri SN (1999). Pirfenidone inhibits PDGF isoforms in bleomycin hamster model of lung fibrosis at the translational level. Am J Physiol 276: L311–L318.

Hale ML, Margolin SB, Krakauer T, Roy CJ, Stiles BG (2002). Pirfenidone blocks the in vitro and in vivo effects of staphylococcal enterotoxin B. Infect Immun 70: 2989–2994.

Hamada N, Kuwano K, Yamada M, Hagimoto N, Hiasa K, Egashira K *et al.* (2005). Anti-vascular endothelial growth factor gene therapy attenuates lung injury and fibrosis in mice. J Immunol 175: 1224–1231.

Hancock A, Armstrong L, Gama R, Millar A (1998). Production of interleukin 13 by alveolar macrophages from normal and fibrotic lung. Am J Respir Cell Mol Biol 18: 60–65.

Hashimoto N, Phan SH, Imaizumi K, Matsuo M, Nakashima H, Kawabe T *et al.* (2010). Endothelial-mesenchymal transition in bleomycin-induced pulmonary fibrosis. Am J Respir Cell Mol Biol 43: 161–172.

Haslett PA, Corral LG, Albert M, Kaplan G (1998). Thalidomide costimulates primary human T lymphocytes, preferentially inducing proliferation, cytokine production, and cytotoxic responses in the CD8+ subset. J Exp Med 187: 1885–1892.

Hayakawa Y, Tazawa S, Ishikawa T, Niiya K, Sakuragawa N (1995). Transcriptional regulation of tissue- and urokinase-type plasminogen activator genes by thrombin in human fetal lung fibroblasts. Thromb Haemost 74: 704–710.

Hecker L, Vittal R, Jones T, Jagirdar R, Luckhardt TR, Horowitz JC *et al.* (2009). NADPH oxidase-4 mediates myofibroblast activation and fibrogenic responses to lung injury. Nat Med 15: 1077–1081.

Henderson WR Jr, Chi EY, Ye X, Nguyen C, Tien YT, Zhou B *et al.* (2010). Inhibition of Wnt/beta-catenin/CREB binding protein (CBP) signaling reverses pulmonary fibrosis. Proc Natl Acad Sci USA 107: 14309–14314.

Hernandez-Rodriguez NA, Cambrey AD, Harrison NK, Chambers RC, Gray AJ, Southcott AM *et al.* (1995). Role of thrombin in pulmonary fibrosis. Lancet 346: 1071–1073.

Hewitson TD, Kelynack KJ, Tait MG, Martic M, Jones CL, Margolin SB *et al.* (2001). Pirfenidone reduces in vitro rat renal fibroblast activation and mitogenesis. J Nephrol 14: 453–460.

Higashiyama H, Yoshimoto D, Kaise T, Matsubara S, Fujiwara M, Kikkawa H *et al.* (2007). Inhibition of activin receptor-like kinase 5 attenuates bleomycin-induced pulmonary fibrosis. Exp Mol Pathol 83: 39–46.

Hogaboam CM, Lukacs NW, Chensue SW, Strieter RM, Kunkel SL (1998). Monocyte chemoattractant protein-1 synthesis by murine lung fibroblasts modulates CD4+ T cell activation. J Immunol 160: 4606–4614.

Horan GS, Wood S, Ona V, Li DJ, Lukashev ME, Weinreb PH *et al.* (2008). Partial inhibition of integrin alpha(v)beta6 prevents pulmonary fibrosis without exacerbating inflammation. Am J Respir Crit Care Med 177: 56–65.

Howell DCJ, Goldsack NR, Marshall RP, McAnulty RJ, Starke R, Purdy G *et al.* (2001). Direct thrombin inhibition reduces lung collagen, accumulation, and connective tissue growth factor mrna levels in bleomycin-induced pulmonary fibrosis. Am J Pathol 159: 1383–1395.

Howell DCJ, Johns RH, Lasky JA, Shan B, Scotton CJ, Laurent GJ *et al.* (2005). Absence of proteinase-activated receptor-1 signaling affords protection from bleomycin-induced lung inflammation and fibrosis. Am J Pathol 166: 1353–1365.

Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV *et al.* (2010). Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. Am J Pathol 176: 85–97.

Imokawa S, Sato A, Hayakawa H, Kotani M, Urano T, Takada A (1997). Tissue factor expression and fibrin deposition in the lungs of patients with idiopathic pulmonary fibrosis and systemic sclerosis. Am J Respir Crit Care Med 156: 631–636.

Iyer SN, Wild JS, Schiedt MJ, Hyde DM, Margolin SB, Giri SN (1995). Dietary intake of pirfenidone ameliorates bleomycin-induced lung fibrosis in hamsters. J Lab Clin Med 125: 779–785.

Iyer SN, Gurujeyalakshmi G, Giri SN (1999). Effects of pirfenidone on transforming growth factor-beta gene expression at the transcriptional level in bleomycin hamster model of lung fibrosis. J Pharmacol Exp Ther 291: 367–373.

Iyer SN, Hyde DM, Giri SN (2000). Anti-inflammatory effect of pirfenidone in the bleomycin-hamster model of lung inflammation. Inflammation 24: 477–491.

Jack CI, Jackson MJ, Johnston ID, Hind CR (1996). Serum indicators of free radical activity in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 153: 1918–1923.

Jakubzick C, Choi ES, Joshi BH, Keane MP, Kunkel SL, Puri RK *et al.* (2003). Therapeutic attenuation of pulmonary fibrosis via targeting of IL-4- and IL-13-responsive cells. J Immunol 171: 2684–2693.

Jenkins RG, Su X, Su G, Scotton CJ, Camerer E, Laurent GJ *et al.* (2006). Ligation of protease-activated receptor 1 enhances alpha(v)beta6 integrin-dependent TGF-beta activation and promotes acute lung injury. J Clin Invest 116: 1606–1614.

Kagan HM, Li W (2003). Lysyl oxidase: properties, specificity, and biological roles inside and outside of the cell. J Cell Biochem 88: 660–672.

Kakugawa T, Mukae H, Hayashi T, Ishii H, Abe K, Fujii T *et al.* (2004). Pirfenidone attenuates expression of HSP47 in murine bleomycin-induced pulmonary fibrosis. Eur Respir J 24: 57–65.

Kaminski N, Rosas IO (2006). Gene expression profiling as a window into idiopathic pulmonary fibrosis pathogenesis: can we identify the right target genes? Proc Am Thorac Soc 3: 339–344.

Karpus WJ, Lukacs NW, Kennedy KJ, Smith WS, Hurst SD, Barrett TA (1997). Differential CC chemokine-induced enhancement of T helper cell cytokine production. J Immunol 158: 4129–4136.

Kasper M, Haroske G (1996). Alterations in the alveolar epithelium after injury leading to pulmonary fibrosis. Histol Histopathol 11: 463–483.

Katzenstein AL (1985). Pathogenesis of 'fibrosis' in interstitial pneumonia: an electron microscopic study. Hum Pathol 16: 1015–1024.

Katzenstein AL, Myers JL (1998). Idiopathic pulmonary fibrosis: clinical relevance of pathologic classification. Am J Respir Crit Care Med 157: 1301–1315.

Kawanami O, Ferrans VJ, Crystal RG (1982). Structure of alveolar epithelial cells in patients with fibrotic lung disorders. Lab Invest 46: 39–53.

Keane MP, Arenberg DA, Lynch JP 3rd, Whyte RI, Iannettoni MD, Burdick MD *et al.* (1997). The CXC chemokines, IL-8 and IP-10, regulate angiogenic activity in idiopathic pulmonary fibrosis. J Immunol 159: 1437–1443.

Keane MP, Belperio JA, Arenberg DA, Burdick MD, Xu ZJ, Xue YY *et al.* (1999). IFN-gamma-inducible protein-10 attenuates bleomycin-induced pulmonary fibrosis via inhibition of angiogenesis. J Immunol 163: 5686–5692.

Keane MP, Belperio JA, Burdick MD, Lynch JP, Fishbein MC, Strieter RM (2001). ENA-78 is an important angiogenic factor in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 164: 2239–2242.

Keerthisingam CB, Jenkins RG, Harrison NK, Hernandez-Rodriguez NA, Booth H, Laurent GJ *et al.* (2001). Cyclooxygenase-2 deficiency results in a loss of the anti-proliferative response to transforming growth factor-beta in human fibrotic lung fibroblasts and promotes bleomycin-induced pulmonary fibrosis in mice. Am J Pathol 158: 1411–1422.

Kelly BG, Lok SS, Hasleton PS, Egan JJ, Stewart JP (2002). A rearranged form of Epstein-Barr virus DNA is associated with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 166: 510–513.

Khalil N, O'Connor RN, Flanders KC, Unruh H (1996). TGF-beta 1, but not TGF-beta 2 or TGF-beta 3, is differentially present in epithelial cells of advanced pulmonary fibrosis: an immunohistochemical study. Am J Respir Cell Mol Biol 14: 131–138.

Kikuchi A, Yamamoto H, Kishida S (2007). Multiplicity of the interactions of Wnt proteins and their receptors. Cell Signal 19: 659–671.

Kim KK, Kugler MC, Wolters PJ, Robillard L, Galvez MG, Brumwell AN *et al.* (2006). Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. Proc Natl Acad Sci USA 103: 13180–13185.

Kim KK, Wei Y, Szekeres C, Kugler MC, Wolters PJ, Hill ML *et al.* (2009). Epithelial cell alpha3beta1 integrin links beta-catenin and Smad signaling to promote myofibroblast formation and pulmonary fibrosis. J Clin Invest 119: 213–224.

King TE Jr, Tooze JA, Schwarz MI, Brown KR, Cherniack RM (2001). Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. Am J Respir Crit Care Med 164: 1171–1181.

King TE Jr, Behr J, Brown KK, du Bois RM, Lancaster L, de Andrade JA *et al.* (2008). BUILD-1: a randomized placebo-controlled trial of bosentan in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 177: 75–81.

King TE Jr, Albera C, Bradford WZ, Costabel U, Hormel P, Lancaster L *et al.* (2009). Effect of interferon gamma-1b on survival in patients with idiopathic pulmonary fibrosis (INSPIRE): a multicentre, randomised, placebo-controlled trial. Lancet 374: 222–228.

Koch HP (1985). Thalidomide and congeners as anti-inflammatory agents. Prog Med Chem 22: 165–242.

Kolb M, Kirschner J, Riedel W, Wirtz H, Schmidt M (1998). Cyclophosphamide pulse therapy in idiopathic pulmonary fibrosis. Eur Respir J 12: 1409–1414.

Kollias G, Douni E, Kassiotis G, Kontoyiannis D (1999). On the role of tumor necrosis factor and receptors in models of multiorgan failure, rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease. Immunol Rev 169: 175–194.

Kolodsick JE, Peters-Golden M, Larios J, Toews GB, Thannickal VJ, Moore BB (2003). Prostaglandin E2 inhibits fibroblast to myofibroblast transition via E. prostanoid receptor 2 signaling and cyclic adenosine monophosphate elevation. Am J Respir Cell Mol Biol 29: 537–544.

Kolodsick JE, Toews GB, Jakubzick C, Hogaboam C, Moore TA, McKenzie A *et al.* (2004). Protection from fluorescein isothiocyanate-induced fibrosis in IL-13-deficient, but not IL-4-deficient, mice results from impaired collagen synthesis by fibroblasts. J Immunol 172: 4068–4076.

Komuro I, Kurihara H, Sugiyama T, Yoshizumi M, Takaku F, Yazaki Y (1988). Endothelin stimulates c-fos and c-myc expression and proliferation of vascular smooth muscle cells. FEBS Lett 238: 249–252.

Kondoh Y, Taniguchi H, Yokoi T, Nishiyama O, Ohishi T, Kato T *et al.* (2005). Cyclophosphamide and low-dose prednisolone in idiopathic pulmonary fibrosis and fibrosing nonspecific interstitial pneumonia. Eur Respir J 25: 528–533.

Konigshoff M, Kramer M, Balsara N, Wilhelm J, Amarie OV, Jahn A *et al.* (2009). WNT1-inducible signaling protein-1 mediates pulmonary fibrosis in mice and is upregulated in humans with idiopathic pulmonary fibrosis. J Clin Invest 119: 772–787.

Korfei M, Ruppert C, Mahavadi P, Henneke I, Markart P, Koch M *et al.* (2008). Epithelial endoplasmic reticulum stress and apoptosis in sporadic idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 178: 838–846.

Krowka MJ, Ahmad S, de Andrade JA, Frost A, Glassberg M, Lancaster L *et al.* (2007). A ranomized, double-blind trial, placebo-controlled study to evaluate the safety and efficacy of iloprost inhalation in adults with abnormal pulmonary arterial pressure and exercise limitation associated with idiopathic pulmonary fibrosis. Chest (Suppl) 132: 633a.

Kubo H, Nakayama K, Yanai M, Suzuki T, Yamaya M, Watanabe M *et al.* (2005). Anticoagulant therapy for idiopathic pulmonary fibrosis. Chest 128: 1475–1482.

Kuhn C, McDonald JA (1991). The roles of the myofibroblast in idiopathic pulmonary fibrosis. Ultrastructural and immunohistochemical features of sites of active extracellular matrix synthesis. Am J Pathol 138: 1257–1265.

Kuwano K, Kunitake R, Kawasaki M, Nomoto Y, Hagimoto N, Nakanishi Y *et al*. (1996). P21Waf1/Cip1/Sdi1 and p53 expression in association with DNA strand breaks in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 154: 477–483.

Lama V, Moore BB, Christensen P, Toews GB, Peters-Golden M (2002). Prostaglandin E2 synthesis and suppression of fibroblast proliferation by alveolar epithelial cells is cyclooxygenase-2-dependent. Am J Respir Cell Mol Biol 27: 752–758.

Lama VN, Phan SH (2006). The extrapulmonary origin of fibroblasts: stem/progenitor cells and beyond. Proc Am Thorac Soc 3: 373–376.

Latsi PI, du Bois RM, Nicholson AG, Colby TV, Bisirtzoglou D, Nikolakopoulou A *et al*. (2003). Fibrotic idiopathic interstitial pneumonia: the prognostic value of longitudinal functional trends. Am J Respir Crit Care Med 168: 531–537.

Lawson WE, Grant SW, Ambrosini V, Womble KE, Dawson EP, Lane KB *et al*. (2004). Genetic mutations in surfactant protein C are a rare cause of sporadic cases of IPF. Thorax 59: 977–980.

Lawson WE, Crossno PF, Polosukhin VV, Roldan J, Cheng DS, Lane KB *et al*. (2008). Endoplasmic reticulum stress in alveolar epithelial cells is prominent in IPF: association with altered surfactant protein processing and herpesvirus infection. Am J Physiol Lung Cell Mol Physiol 294: L1119–L1126.

Leask A (2009). Signaling in fibrosis: targeting the TGF beta, endothelin-1 and CCN2 axis in scleroderma. Front Biosci (Elite Ed) 1: 115–122.

Leask A, Abraham DJ (2003). The role of connective tissue growth factor, a multifunctional matricellular protein, in fibroblast biology. Biochem Cell Biol 81: 355–363.

Lee CG, Cho SJ, Kang MJ, Chapoval SP, Lee PJ, Noble PW *et al*. (2004). Early growth response gene 1-mediated apoptosis is essential for transforming growth factor beta1-induced pulmonary fibrosis. J Exp Med 200: 377–389.

Lepisto J, Peltonen J, Vaha-Kreula M, Niinikoski J, Laato M (1995). Platelet-derived growth factor isoforms PDGF-AA, -AB and -BB exert specific effects on collagen gene expression and mitotic activity of cultured human wound fibroblasts. Biochem Biophys Res Commun 209: 393–399.

Li X, Rayford H, Uhal BD (2003a). Essential roles for angiotensin receptor AT1a in bleomycin-induced apoptosis and lung fibrosis in mice. Am J Pathol 163: 2523–2530.

Li X, Zhang H, Soledad-Conrad V, Zhuang J, Uhal BD (2003b). Bleomycin-induced apoptosis of alveolar epithelial cells requires angiotensin synthesis de novo. Am J Physiol Lung Cell Mol Physiol 284: L501–L507.

Li X, Molina-Molina M, Abdul-Hafez A, Ramirez J, Serrano-Mollar A, Xaubet A *et al*. (2006). Extravascular sources of lung angiotensin peptide synthesis in idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 291: L887–L895.

Liu T, Jin H, Ullenbruch M, Hu B, Hashimoto N, Moore B *et al*. (2004). Regulation of found in inflammatory zone 1 expression in bleomycin-induced lung fibrosis: role of IL-4/IL-13 and mediation via STAT-6. J Immunol 173: 3425–3431.

Liu G, Friggeri A, Yang Y, Milosevic J, Ding Q, Thannickal VJ *et al*. (2010a). miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. J Exp Med 207: 1589–1597.

Liu S, Taghavi R, Leask A (2010b). Connective tissue growth factor is induced in bleomycin-induced skin scleroderma. J Cell Commun Signal 4: 25–30.

Lovgren AK, Jania LA, Hartney JM, Parsons KK, Audoly LP, Fitzgerald GA *et al*. (2006). COX-2-derived prostacyclin protects against bleomycin-induced pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 291: L144–L156.

McAnulty RJ, Campa JS, Cambrey AD, Laurent GJ (1991). The effect of transforming growth factor beta on rates of procollagen synthesis and degradation in vitro. Biochim Biophys Acta 1091: 231–235.

MacNee W, Rahman I (1995). Oxidants/antioxidants in idiopathic pulmonary fibrosis. Thorax 50 (Suppl. 1): S53–S58.

Maeyama T, Kuwano K, Kawasaki M, Kunitake R, Hagimoto N, Matsuba T *et al*. (2001). Upregulation of Fas-signalling molecules in lung epithelial cells from patients with idiopathic pulmonary fibrosis. Eur Respir J 17: 180–189.

Maher TM (2010). Pirfenidone in idiopathic pulmonary fibrosis. Drugs Today (Barc) 46: 473–482.

Maher TM, Evans IC, Bottoms SE, Mercer PF, Thorley AJ, Nicholson AG *et al*. (2010a). Diminished prostaglandin E2 contributes to the apoptosis paradox in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 182: 73–82.

Maher TM, Evans IC, Laurent GJ, McAnulty RJ (2010b). PGE2 paradoxically increases fibroblast apoptosis but reduces airway epithelial cell apoptosis in response to FasL via activation of the EP4 receptor. Am J Respir Crit Care Med 181 (Suppl.): A4182.

Marchal-Somme J, Uzunhan Y, Marchand-Adam S, Kambouchner M, Valeyre D, Crestani B *et al*. (2007). Dendritic cells accumulate in human fibrotic interstitial lung disease. Am J Respir Crit Care Med 176: 1007–1014.

Markart P, Luboeinski T, Korfei M, Schmidt R, Wygrecka M, Mahavadi P *et al*. (2009). Alveolar oxidative stress is associated with elevated levels of nonenzymatic low-molecular-weight antioxidants in patients with different forms of chronic fibrosing interstitial lung diseases. Antioxid Redox Signal 11: 227–240.

Markart P, Nass R, Ruppert C, Hundack L, Wygrecka M, Korfei M *et al*. (2010). Safety and tolerability of inhaled heparin in idiopathic pulmonary fibrosis. J Aerosol Med Pulm Drug Deliv 23: 161–172.

Marshall RP, Webb S, Bellingan GJ, Montgomery HE, Chaudhari B, McAnulty RJ *et al*. (2002). Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome. Am J Respir Crit Care Med 166: 646–650.

Marshall RP, Gohlke P, Chambers RC, Howell DC, Bottoms SE, Unger T *et al*. (2004). Angiotensin II and the fibroproliferative response to acute lung injury. Am J Physiol Lung Cell Mol Physiol 286: L156–L164.

Mehrad B, Burdick MD, Strieter RM (2009). Fibrocyte CXCR4 regulation as a therapeutic target in pulmonary fibrosis. Int J Biochem Cell Biol 41: 1708–1718.

Miyazaki Y, Araki K, Vesin C, Garcia I, Kapanci Y, Whitsett JA *et al*. (1995). Expression of a tumor necrosis factor-alpha transgene in murine lung causes lymphocytic and fibrosing alveolitis. A mouse model of progressive pulmonary fibrosis. J Clin Invest 96: 250–259.

Mizuno S, Matsumoto K, Li MY, Nakamura T (2005). HGF reduces advancing lung fibrosis in mice: a potential role for MMP-dependent myofibroblast apoptosis. FASEB J 19: 580–582.

Moeller A, Ask K, Warburton D, Gauldie J, Kolb M (2008). The bleomycin animal model: a useful tool to investigate treatment options for idiopathic pulmonary fibrosis? Int J Biochem Cell Biol 40: 362–382.

Moeller A, Gilpin SE, Ask K, Cox G, Cook D, Gauldie J *et al.* (2009). Circulating fibrocytes are an indicator of poor prognosis in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 179: 588–594.

Montaldo C, Cannas E, Ledda M, Rosetti L, Congiu L, Atzori L (2002). Bronchoalveolar glutathione and nitrite/nitrate in idiopathic pulmonary fibrosis and sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 19: 54–58.

Montuschi P, Ciabattoni G, Paredi P, Pantelidis P, du Bois RM, Kharitonov SA *et al.* (1998). 8-Isoprostane as a biomarker of oxidative stress in interstitial lung diseases. Am J Respir Crit Care Med 158: 1524–1527.

Moodley YP, Scaffidi AK, Misso NL, Keerthisingam C, McAnulty RJ, Laurent GJ *et al.* (2003). Fibroblasts isolated from normal lungs and those with idiopathic pulmonary fibrosis differ in interleukin-6/gp130-mediated cell signaling and proliferation. Am J Pathol 163: 345–354.

Moodley YP, Caterina P, Scaffidi AK, Misso NL, Papadimitriou JM, McAnulty RJ *et al.* (2004). Comparison of the morphological and biochemical changes in normal human lung fibroblasts and fibroblasts derived from lungs of patients with idiopathic pulmonary fibrosis during FasL-induced apoptosis. J Pathol 202: 486–495.

Moore BB, Kolodsick JE, Thannickal VJ, Cooke K, Moore TA, Hogaboam C *et al.* (2005). CCR2-mediated recruitment of fibrocytes to the alveolar space after fibrotic injury. Am J Pathol 166: 675–684.

Moore BB, Murray L, Das A, Wilke CA, Herrygers AB, Toews GB (2006). The role of CCL12 in the recruitment of fibrocytes and lung fibrosis. Am J Respir Cell Mol Biol 35: 175–181.

Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J *et al.* (1999). The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. Cell 96: 319–328.

Murphy KM, Ouyang W, Farrar JD, Yang J, Ranganath S, Asnagli H *et al.* (2000). Signaling and transcription in T helper development. Annu Rev Immunol 18: 451–494.

Murphy-Ullrich JE, Poczatek M (2000). Activation of latent TGF-beta by thrombospondin-1: mechanisms and physiology. Cytokine Growth Factor Rev 11: 59–69.

Mutsaers SE, Marshall RP, Goldsack NR, Laurent GJ, McAnulty RJ (1998). Effect of endothelin receptor antagonists (BQ-485, Ro 47-0203) on collagen deposition during the development of bleomycin-induced pulmonary fibrosis in rats. Pulm Pharmacol Ther 11: 221–225.

Nakazato H, Oku H, Yamane S, Tsuruta Y, Suzuki R (2002). A novel anti-fibrotic agent pirfenidone suppresses tumor necrosis factor-alpha at the translational level. Eur J Pharmacol 446: 177–185.

Nash JR, McLaughlin PJ, Butcher D, Corrin B (1993). Expression of tumour necrosis factor-alpha in cryptogenic fibrosing alveolitis. Histopathology 22: 343–347.

Nyhan KC, Faherty N, Murray G, Cooey LB, Godson C, Crean JK *et al.* (2010). Jagged/Notch signalling is required for a subset of TGFbeta1 responses in human kidney epithelial cells. Biochim Biophys Acta 1803: 1386–1395.

Oikonomou N, Harokopos V, Zalevsky J, Valavanis C, Kotanidou A, Szymkowski DE *et al.* (2006). Soluble TNF mediates the transition from pulmonary inflammation to fibrosis. PloS ONE 1: e108.

Oku H, Nakazato H, Horikawa T, Tsuruta Y, Suzuki R (2002). Pirfenidone suppresses tumor necrosis factor-alpha, enhances interleukin-10 and protects mice from endotoxic shock. Eur J Pharmacol 446: 167–176.

Oriente A, Fedarko NS, Pacocha SE, Huang SK, Lichtenstein LM, Essayan DM (2000). Interleukin-13 modulates collagen homeostasis in human skin and keloid fibroblasts. J Pharmacol Exp Ther 292: 988–994.

Ortiz LA, Lasky J, Hamilton RF Jr, Holian A, Hoyle GW, Banks W *et al.* (1998). Expression of TNF and the necessity of TNF receptors in bleomycin-induced lung injury in mice. Exp Lung Res 24: 721–743.

Otsuka M, Takahashi H, Shiratori M, Chiba H, Abe S (2004). Reduction of bleomycin induced lung fibrosis by candesartan cilexetil, an angiotensin II type 1 receptor antagonist. Thorax 59: 31–38.

Overall CM, Wrana JL, Sodek J (1989). Transforming growth factor-beta regulation of collagenase, 72 kDa-progelatinase, TIMP and PAI-1 expression in rat bone cell populations and human fibroblasts. Connect Tissue Res 20: 289–294.

Ozawa Y, Suda T, Naito T, Enomoto N, Hashimoto D, Fujisawa T *et al.* (2009). Cumulative incidence of and predictive factors for lung cancer in IPF. Respirology 14: 723–728.

Pan Q, Kleer CG, van Golen KL, Irani J, Bottema KM, Bias C *et al.* (2002). Copper deficiency induced by tetrathiomolybdate suppresses tumor growth and angiogenesis. Cancer Res 62: 4854–4859.

Pandit KV, Corcoran D, Yousef H, Yarlagadda M, Tzouvelekis A, Gibson KF *et al.* (2010). Inhibition and role of let-7d in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 182: 220–229.

Park SH, Saleh D, Giaid A, Michel RP (1997). Increased endothelin-1 in bleomycin-induced pulmonary fibrosis and the effect of an endothelin receptor antagonist. Am J Respir Crit Care Med 156: 600–608.

Peacock AJ, Dawes KE, Shock A, Gray AJ, Reeves JT, Laurent GJ (1992). Endothelin-1 and endothelin-3 induce chemotaxis and replication of pulmonary artery fibroblasts. Am J Respir Cell Mol Biol 7: 492–499.

Peao MN, Aguas AP, de Sa CM, Grande NR (1994). Neoformation of blood vessels in association with rat lung fibrosis induced by bleomycin. Anat Rec 238: 57–67.

Pedram A, Razandi M, Hu RM, Levin ER (1997). Vasoactive peptides modulate vascular endothelial cell growth factor production and endothelial cell proliferation and invasion. J Biol Chem 272: 17097–17103.

Peters-Golden M, Bailie M, Marshall T, Wilke C, Phan SH, Toews GB *et al.* (2002). Protection from pulmonary fibrosis in leukotriene-deficient mice. Am J Respir Crit Care Med 165: 229–235.

Phan SH (2002). The myofibroblast in pulmonary fibrosis. Chest 122: 286S–289S.

Phelps DT, Deneke SM, Daley DL, Fanburg BL (1992). Elevation of glutathione levels in bovine pulmonary artery endothelial cells by N-acetylcysteine. Am J Respir Cell Mol Biol 7: 293–299.

Phillips RJ, Burdick MD, Hong K, Lutz MA, Murray LA, Xue YY *et al.* (2004). Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. J Clin Invest 114: 438–446.

Piguet PF, Collart MA, Grau GE, Kapanci Y, Vassalli P (1989). Tumor necrosis factor/cachectin plays a key role in bleomycin-induced pneumopathy and fibrosis. J Exp Med 170: 655–663.

Piguet PF, Ribaux C, Karpuz V, Grau GE, Kapanci Y (1993). Expression and localization of tumor necrosis factor-alpha and its mRNA in idiopathic pulmonary fibrosis. Am J Pathol 143: 651–655.

Plataki M, Koutsopoulos AV, Darivianaki K, Delides G, Siafakas NM, Bouros D (2005). Expression of apoptotic and antiapoptotic markers in epithelial cells in idiopathic pulmonary fibrosis. Chest 127: 266–274.

Raghow R, Postlethwaite AE, Keski-Oja J, Moses HL, Kang AH (1987). Transforming growth factor-beta increases steady state levels of type I procollagen and fibronectin messenger RNAs posttranscriptionally in cultured human dermal fibroblasts. J Clin Invest 79: 1285–1288.

Raghu G, Depaso WJ, Cain K, Hammar SP, Wetzel CE, Dreis DF *et al.* (1991). Azathioprine combined with prednisone in the treatment of idiopathic pulmonary fibrosis: a prospective double-blind, randomized, placebo-controlled clinical trial. Am Rev Respir Dis 144: 291–296.

Raghu G, Brown KK, Bradford WZ, Starko K, Noble PW, Schwartz DA *et al.* (2004). A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis. N Engl J Med 350: 125–133.

Raghu G, Brown KK, Costabel U, Cottin V, du Bois RM, Lasky JA *et al.* (2008). Treatment of idiopathic pulmonary fibrosis with etanercept: an exploratory, placebo-controlled trial. Am J Respir Crit Care Med 178: 948–955.

Rahman I, Skwarska E, Henry M, Davis M, O'Connor CM, FitzGerald MX *et al.* (1999). Systemic and pulmonary oxidative stress in idiopathic pulmonary fibrosis. Free Radic Biol Med 27: 60–68.

Ramos C, Montano M, Garcia-Alvarez J, Ruiz V, Uhal BD, Selman M *et al.* (2001). Fibroblasts from idiopathic pulmonary fibrosis and normal lungs differ in growth rate, apoptosis, and tissue inhibitor of metalloproteinases expression. Am J Respir Cell Mol Biol 24: 591–598.

Reddy NM, Kleeberger SR, Cho HY, Yamamoto M, Kensler TW, Biswal S *et al.* (2007). Deficiency in Nrf2-GSH signaling impairs type II cell growth and enhances sensitivity to oxidants. Am J Respir Cell Mol Biol 37: 3–8.

Renzoni EA (2004). Neovascularization in idiopathic pulmonary fibrosis: too much or too little? Am J Respir Crit Care Med 169: 1179–1180.

Renzoni EA, Walsh DA, Salmon M, Wells AU, Sestini P, Nicholson AG *et al.* (2003). Interstitial vascularity in fibrosing alveolitis. Am J Respir Crit Care Med 167: 438–443.

Rose CE Jr, Sung SS, Fu SM (2003). Significant involvement of CCL2 (MCP-1) in inflammatory disorders of the lung. Microcirculation 10: 273–288.

Rosenbloom J, Feldman G, Freundlich B, Jimenez SA (1986). Inhibition of excessive scleroderma fibroblast collagen production by recombinant gamma-interferon. Association with a coordinate decrease in types I and III procollagen messenger RNA levels. Arthritis Rheum 29: 851–856.

Rubin JS, Osada H, Finch PW, Taylor WG, Rudikoff S, Aaronson SA (1989). Purification and characterization of a newly identified growth factor specific for epithelial cells. Proc Natl Acad Sci USA 86: 802–806.

Saito A, Okazaki H, Sugawara I, Yamamoto K, Takizawa H (2003). Potential action of IL-4 and IL-13 as fibrogenic factors on lung fibroblasts in vitro. Int Arch Allergy Immunol 132: 168–176.

Saleh D, Barnes PJ, Giaid A (1997a). Increased production of the potent oxidant peroxynitrite in the lungs of patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 155: 1763–1769.

Saleh D, Furukawa K, Tsao MS, Maghazachi A, Corrin B, Yanagisawa M *et al.* (1997b). Elevated expression of endothelin-1 and endothelin-converting enzyme-1 in idiopathic pulmonary fibrosis: possible involvement of proinflammatory cytokines. Am J Respir Cell Mol Biol 16: 187–193.

Schlingensiepen KH, Fischer-Blass B, Schmaus S, Ludwig S (2008). Antisense therapeutics for tumor treatment: the TGF-beta2 inhibitor AP 12009 in clinical development against malignant tumors. Recent Results Cancer Res 177: 137–150.

Scotton CJ, Chambers RC (2007). Molecular targets in pulmonary fibrosis: the myofibroblast in focus. Chest 132: 1311–1321.

Scotton CJ, Chambers RC (2010). Bleomycin revisited: towards a more representative model of IPF? Am J Physiol Lung Cell Mol Physiol 299: L439–L441.

Scotton CJ, Krupiczojc MA, Konigshoff M, Mercer PF, Lee YC, Kaminski N *et al.* (2009). Increased local expression of coagulation factor X contributes to the fibrotic response in human and murine lung injury. J Clin Invest 119: 2550–2563.

Selman M, Pardo A (2002). Idiopathic pulmonary fibrosis: an epithelial/fibroblastic cross-talk disorder. Respir Res 3: 3.

Selman M, Carrillo G, Salas J, Padilla RP, Perez-Chavira R, Sansores R *et al.* (1998). Colchicine, d-penicillamine, and prednisone in the treatment of idiopathic pulmonary fibrosis: a controlled clinical trial. Chest 114: 507–512.

Shah M, Foreman DM, Ferguson MW (1995). Neutralisation of TGF-beta 1 and TGF-beta 2 or exogenous addition of TGF-beta 3 to cutaneous rat wounds reduces scarring. J Cell Sci 108: 985–1002.

Shahar I, Fireman E, Topilsky M, Grief J, Schwarz Y, Kivity S *et al.* (1999). Effect of endothelin-1 on alpha-smooth muscle actin expression and on alveolar fibroblasts proliferation in interstitial lung diseases. Int J Immunopharmacol 21: 759–775.

Shahzeidi S, Sarnstrand B, Jeffery PK, McAnulty RJ, Laurent GJ (1991). Oral N-acetylcysteine reduces bleomycin-induced collagen deposition in the lungs of mice. Eur Respir J 4: 845–852.

Shi-Wen X, Chen Y, Denton CP, Eastwood M, Renzoni EA, Bou-Gharios G *et al.* (2004). Endothelin-1 promotes myofibroblast induction through the ETA receptor via a rac/phosphoinositide 3-kinase/Akt-dependent pathway and is essential for the enhanced contractile phenotype of fibrotic fibroblasts. Mol Biol Cell 15: 2707–2719.

Sime PJ, Xing Z, Graham FL, Csaky KG, Gauldie J (1997). Adenovector-mediated gene transfer of active transforming growth factor-beta1 induces prolonged severe fibrosis in rat lung. J Clin Invest 100: 768–776.

Sime PJ, Marr RA, Gauldie D, Xing Z, Hewlett BR, Graham FL *et al.* (1998). Transfer of tumor necrosis factor-alpha to rat lung induces severe pulmonary inflammation and patchy interstitial fibrogenesis

with induction of transforming growth factor-beta1 and myofibroblasts. Am J Pathol 153: 825–832.

Simler NR, Howell DC, Marshall RP, Goldsack NR, Hasleton PS, Laurent GJ *et al.* (2002). The rapamycin analogue SDZ RAD attenuates bleomycin-induced pulmonary fibrosis in rats. Eur Respir J 19: 1124–1127.

Sonnylal S, Shi-Wen X, Leoni P, Naff K, Van Pelt CS, Nakamura H *et al.* (2010). Selective expression of connective tissue growth factor in fibroblasts in vivo promotes systemic tissue fibrosis. Arthritis Rheum 62: 1523–1532.

Stefani G, Slack FJ (2008). Small non-coding RNAs in animal development. Nat Rev Mol Cell Biol 9: 219–230.

Strieter RM (2005). Pathogenesis and natural history of usual interstitial pneumonia: the whole story or the last chapter of a long novel. Chest 128: 526S–532S.

Subramanian SV, Polikandriotis JA, Kelm RJ Jr, David JJ, Orosz CG, Strauch AR (2004). Induction of vascular smooth muscle alpha-actin gene transcription in transforming growth factor beta1-activated myofibroblasts mediated by dynamic interplay between the Pur repressor proteins and Sp1/Smad coactivators. Mol Biol Cell 15: 4532–4543.

Suga M, Iyonaga K, Ichiyasu H, Saita N, Yamasaki H, Ando M (1999). Clinical significance of MCP-1 levels in BALF and serum in patients with interstitial lung diseases. Eur Respir J 14: 376–382.

Suganuma H, Sato A, Tamura R, Chida K (1995). Enhanced migration of fibroblasts derived from lungs with fibrotic lesions. Thorax 50: 984–989.

Sutinen S, Rainio P, Sutinen S, Huhti E, Pokela R (1980). Ultrastructure of terminal respiratory epithelium and prognosis in chronic interstitial pneumonia. Eur J Respir Dis 61: 325–336.

Tabata C, Tabata R, Kadokawa Y, Hisamori S, Takahashi M, Mishima M *et al.* (2007). Thalidomide prevents bleomycin-induced pulmonary fibrosis in mice. J Immunol 179: 708–714.

Tager AM, LaCamera P, Shea BS, Campanella GS, Selman M, Zhao Z *et al.* (2008). The lysophosphatidic acid receptor LPA1 links pulmonary fibrosis to lung injury by mediating fibroblast recruitment and vascular leak. Nat Med 14: 45–54.

Tamargo RJ, Bok RA, Brem H (1991). Angiogenesis inhibition by minocycline. Cancer Res 51: 672–675.

Tang YW, Johnson JE, Browning PJ, Cruz-Gervis RA, Davis A, Graham BS *et al.* (2003). Herpesvirus DNA is consistently detected in lungs of patients with idiopathic pulmonary fibrosis. J Clin Microbiol 41: 2633–2640.

Taniguchi H, Ebina M, Kondoh Y, Ogura T, Azuma A, Suga M *et al.* (2010). Pirfenidone in idiopathic pulmonary fibrosis. Eur Respir J 35: 821–829.

Turner-Warwick M (1963). Precapillary systemic-pulmonary anastomoses. Thorax 18: 225–237.

Turner-Warwick M, Burrows B, Johnson A (1980). Cryptogenic fibrosing alveolitis: response to corticosteroid treatment and its effect on survival. Thorax 35: 593–599.

Uhal BD, Joshi I, Hughes WF, Ramos C, Pardo A, Selman M (1998). Alveolar epithelial cell death adjacent to underlying myofibroblasts in advanced fibrotic human lung. Am J Physiol 275: L1192–L1199.

Uhal BD, Kim JK, Li X, Molina-Molina M (2007). Angiotensin-TGF-beta 1 crosstalk in human idiopathic pulmonary fibrosis: autocrine mechanisms in myofibroblasts and macrophages. Curr Pharm Des 13: 1247–1256.

Ulloa L, Doody J, Massague J (1999). Inhibition of transforming growth factor-beta/SMAD signalling by the interferon-gamma/STAT pathway. Nature 397: 710–713.

Waghray M, Cui Z, Horowitz JC, Subramanian IM, Martinez FJ, Toews GB *et al.* (2005). Hydrogen peroxide is a diffusible paracrine signal for the induction of epithelial cell death by activated myofibroblasts. FASEB J 19: 854–856.

Walters DM, Cho HY, Kleeberger SR (2008). Oxidative stress and antioxidants in the pathogenesis of pulmonary fibrosis: a potential role for Nrf2. Antioxid Redox Signal 10: 321–332.

Wang R, Zagariya A, Ibarra-Sunga O, Gidea C, Ang E, Deshmukh S *et al.* (1999). Angiotensin II induces apoptosis in human and rat alveolar epithelial cells. Am J Physiol 276: L885–L889.

Wang R, Ibarra-Sunga O, Verlinski L, Pick R, Uhal BD (2000a). Abrogation of bleomycin-induced epithelial apoptosis and lung fibrosis by captopril or by a caspase inhibitor. Am J Physiol Lung Cell Mol Physiol 279: L143–L151.

Wang WL, Healy ME, Sattler M, Verma S, Lin J, Maulik G *et al.* (2000b). Growth inhibition and modulation of kinase pathways of small cell lung cancer cell lines by the novel tyrosine kinase inhibitor STI 571. Oncogene 19: 3521–3528.

Watterson KR, Lanning DA, Diegelmann RF, Spiegel S (2007). Regulation of fibroblast functions by lysophospholipid mediators: potential roles in wound healing. Wound Repair Regen 15: 607–616.

Whyte M, Hubbard R, Meliconi R, Whidborne M, Eaton V, Bingle C *et al.* (2000). Increased risk of fibrosing alveolitis associated with interleukin-1 receptor antagonist and tumor necrosis factor-alpha gene polymorphisms. Am J Respir Crit Care Med 162: 755–758.

Wilborn J, Crofford LJ, Burdick MD, Kunkel SL, Strieter RM, Peters-Golden M (1995). Cultured lung fibroblasts isolated from patients with idiopathic pulmonary fibrosis have a diminished capacity to synthesize prostaglandin E2 and to express cyclooxygenase-2. J Clin Invest 95: 1861–1868.

Wilborn J, Bailie M, Coffey M, Burdick M, Strieter R, Peters-Golden M (1996). Constitutive activation of 5-lipoxygenase in the lungs of patients with idiopathic pulmonary fibrosis. J Clin Invest 97: 1827–1836.

Willis BC, Liebler JM, Luby-Phelps K, Nicholson AG, Crandall ED, du Bois RM *et al.* (2005). Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor-beta1: potential role in idiopathic pulmonary fibrosis. Am J Pathol 166: 1321–1332.

Wipff PJ, Hinz B (2008). Integrins and the activation of latent transforming growth factor beta1 – an intimate relationship. Eur J Cell Biol 87: 601–615.

Wipff PJ, Rifkin DB, Meister JJ, Hinz B (2007). Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. J Cell Biol 179: 1311–1323.

Wynn TA (2004). Fibrotic disease and the T(H)1/T(H)2 paradigm. Nat Rev Immunol 4: 583–594.

Xaubet A, Marin-Arguedas A, Lario S, Ancochea J, Morell F, Ruiz-Manzano J *et al.* (2003). Transforming growth factor-beta1 gene polymorphisms are associated with disease progression in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 168: 431–435.

Xaubet A, Roca-Ferrer J, Pujols L, Ramirez J, Mullol J, Marin-Arguedas A *et al.* (2004). Cyclooxygenase-2 is up-regulated in lung parenchyma of chronic obstructive pulmonary disease and down-regulated in idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis 21: 35–42.

Xu S, Denton CP, Holmes A, Dashwood MR, Abraham DJ, Black CM (1998). Endothelins: effect on matrix biosynthesis and proliferation in normal and scleroderma fibroblasts. J Cardiovasc Pharmacol 31 (Suppl. 1): S360–S363.

Xu YD, Hua J, Mui A, O'Connor R, Grotendorst G, Khalil N (2003). Release of biologically active TGF-beta1 by alveolar epithelial cells results in pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 285: L527–L539.

Xu MY, Porte J, Knox AJ, Weinreb PH, Maher TM, Violette SM *et al.* (2009). Lysophosphatidic acid induces alphavbeta6 integrin-mediated TGF-beta activation via the LPA2 receptor and the small G protein G alpha(q). Am J Pathol 174: 1264–1279.

Yamada M, Kuwano K, Maeyama T, Hamada N, Yoshimi M, Nakanishi Y *et al.* (2008). Dual-immunohistochemistry provides little evidence for epithelial-mesenchymal transition in pulmonary fibrosis. Histochem Cell Biol 129: 453–462.

Yanagisawa K, Osada H, Masuda A, Kondo M, Saito T, Yatabe Y *et al.* (1998). Induction of apoptosis by Smad3 and down-regulation of Smad3 expression in response to TGF-beta in human normal lung epithelial cells. Oncogene 17: 1743–1747.

Zappala CJ, Latsi PI, Nicholson AG, Colby TV, Cramer D, Renzoni EA *et al.* (2010). Marginal decline in forced vital capacity is associated with a poor outcome in idiopathic pulmonary fibrosis. Eur Respir J 35: 830–836.

Zavadil J, Cermak L, Soto-Nieves N, Bottinger EP (2004). Integration of TGF-beta/Smad and Jagged1/Notch signalling in epithelial-to-mesenchymal transition. EMBO J 23: 1155–1165.

Zhang K, Gharaee-Kermani M, McGarry B, Phan SH (1994a). In situ hybridization analysis of rat lung alpha 1(I) and alpha 2(I) collagen gene expression in pulmonary fibrosis induced by endotracheal bleomycin injection. Lab Invest 70: 192–202.

Zhang K, Rekhter MD, Gordon D, Phan SH (1994b). Myofibroblasts and their role in lung collagen gene expression during pulmonary fibrosis. A combined immunohistochemical and in situ hybridization study. Am J Pathol 145: 114–125.

Zhang K, Gharaee-Kermani M, McGarry B, Remick D, Phan SH (1997). TNF-alpha-mediated lung cytokine networking and eosinophil recruitment in pulmonary fibrosis. J Immunol 158: 954–959.

Zhang F, Nielsen LD, Lucas JJ, Mason RJ (2004). Transforming growth factor-beta antagonizes alveolar type II cell proliferation induced by keratinocyte growth factor. Am J Respir Cell Mol Biol 31: 679–686.

Zhang Y, Liu P, Gao X, Qian W, Xu K (2010). rAAV2-TGF-beta(3) decreases collagen synthesis and deposition in the liver of experimental hepatic fibrosis rat. Dig Dis Sci 55: 2821–2830.

Zhu Y, Liu Y, Zhou W, Xiang R, Jiang L, Huang K *et al.* (2010). A prostacyclin analogue, iloprost, protects from bleomycin-induced pulmonary fibrosis in mice. Respir Res 11: 34.

Ziegenhagen MW, Schrum S, Zissel G, Zipfel PF, Schlaak M, Muller-Quernheim J (1998). Increased expression of proinflammatory chemokines in bronchoalveolar lavage cells of patients with progressing idiopathic pulmonary fibrosis and sarcoidosis. J Investig Med 46: 223–231.

Ziesche R, Hofbauer E, Wittmann K, Petkov V, Block LH (1999). A preliminary study of long-term treatment with interferon gamma-1b and low-dose prednisolone in patients with idiopathic pulmonary fibrosis. N Engl J Med 341: 1264–1269.

Zisman DA, Lynch JP 3rd, Toews GB, Kazerooni EA, Flint A, Martinez FJ (2000). Cyclophosphamide in the treatment of idiopathic pulmonary fibrosis: a prospective study in patients who failed to respond to corticosteroids. Chest 117: 1619–1626.

Zisman DA, Schwarz M, Anstrom KJ, Collard HR, Flaherty KR, Hunninghake GW (2010). A controlled trial of sildenafil in advanced idiopathic pulmonary fibrosis. N Engl J Med 363: 620–628.

Zuo F, Kaminski N, Eugui E, Allard J, Yakhini Z, Ben-Dor A *et al.* (2002). Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. Proc Natl Acad Sci USA 99: 6292–6297.