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.

**Keywords**
pulmonary fibrosis; fibrosis therapy; drug targets; myofibroblast

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**Abbreviations**
S-LO, 5-lipoxygenase; 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; CCR4, C-X-C motif chemokine receptor-4; DLco, 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, lysophosphatidic acid; LTBR, leukotriene B4; 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; PGE2, prostaglandin E2; PDGF, platelet derived growth factor; PGI2, prostacyclin; PHT, pulmonary hypertension; QoL, quality of life; ROS, reactive oxidative species; TGF-β, transforming growth factor-β; Th, T-helper; TM, tetramethylthiuram disulphide; 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.
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 (Dorschied 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 |                                |                                                  |                                |                                                             | 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) |
| 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 |                                                                      |
|                       |                                |                                                  |                                |                                                             |                                                                      |
|                       |                                |                                                  |                                |                                                             |                                                                      |
| 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: ΔFVC/DLco/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 DLco/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 |
| **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: \( \Delta FVC \) at 60 weeks | Results awaited |
| Anti-fibrotic/Anti-angiogenic antibodies (anti-TGF\( \beta \)(1/2/3)) | 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-\( \alpha_\beta_6 \) 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: $\Delta FVC$ 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 ($n = 173$) | $\Delta FVC$ 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 ($n = 174$) | $\Delta FVC$ 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 |
| 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                                                                                                                                                                                                                                                                                                                                                                                                             |
| 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 point: 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-1a, 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 |
| 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 |
| Tetrathiomolydate | 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 DLco at 12 months Reduction in FVC and DLco 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 |
| | | | 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 DLco and quality of life score |
| 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; DLco; 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; DLco, carbon monoxide transfer factor; pred, predicted; FVC, forced vital capacity; HRCT, high resolution computer tomography; IL-13, interleukin 13; IFN-γ, interferon-gamma; LTB4, leukotriene B4; 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; Barbos-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-β) 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+/CD45R0+ 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

IFNγβ

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 this attenuation by 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; Ziegengagen 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-blind, placebo-controlled trial (Wychet, 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 (Antoniaides 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 PDGFRα 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 (Avi-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 (Glaid 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-blind 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-blind 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-blind placebo-controlled trial designed
to examine the effect of the dual endothelin receptor antagonists, macitentan, 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 ($DLCO$) 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-β1 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; McNulty 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) (Bonniald 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 αvβ6, αvβ5, αvβ3, and an as yet unidentified β1 integrin have been shown to activate latent TGF-β1 independently from any proteolytic activity – they all recognize the RGD sequence of LAP-TGF-β1 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-TGFβ1 result in a conformational change of the latent complex and liberates active TGF-β1 [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 (Stromdix, 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 αvβ6 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-β1 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 profibrotic 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 hypoth-

esis 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 (Kolodsiick 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 CCL2 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 (Gharaei-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, CTN 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, protease-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 (Borenstijn 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 prostaglandin E2 (PGE2). PGE2 is the main prostaglandin produced in the lung and is secreted by several cell types, including fibroblasts and epithelial cells (Maher et al., 2010a). PGE2 exerts major anti-fibrotic effects by suppressing fibroblast responses, including proliferation (Bitterman et al., 1986; Lama et al., 2002), differentiation into myofibroblasts (Kolodwick 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 PGE2 levels are decreased in IPF lung, whereas levels of leukotrienes derived from the 5-lipoxygenase pathway, including LTD4, 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 PGE2 may provide a mechanistic explanation for increased resistance of myofibroblasts to apoptosis, in comparison with increased epithelial cell apoptosis (Maher et al., 2010a). PGE2 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 PGE2 are coming to light with roles identified for both EP2 (Kolodwick 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 LTD4 levels, but secondary end points include performance at lung function testing and progression free survival.

Prostacyclin (PGI2) is another arachidonic acid metabolite derived via the COX-2 pathway. Similar to PGE2, PGI2 possesses a number of anti-fibrotic properties, and COX-2 derived PGI2 has been shown to play an important role in limiting the development of bleomycin-induced lung fibrosis (Lowgren et al., 2006). Recent work has demonstrated that
intrapерitoneal administration of iloprost, a PGI₂ 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 O₂⁻ 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 anastomoses 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 antiangiogenic 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 tissue (Keane et al., 1997; 2001). Moreover, both 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 BIIB 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 anastomoses (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

Lyosphosphatidic acid

Recent work has identified the bioactive phospholipid derivate lyosphosphatidic acid (LPA), acting via stimulation of its multiple G-protein-coupled receptors LPA1-6, 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; 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 LPA1 receptor, findings consistent with previous observations that LPA is capable of mediating cellular contraction in a number of different cell types (Chhrzansowska-Wodnicka and Burridge, 1996). In support of a role for LPA in human disease, BALF LPA levels and LPA 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 LPA1 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 LPA1 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 (Kikkuchi 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 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.

**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.

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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.

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