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Idiopathic pulmonary fibrosis: recent advances on pharmacological therapy

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Idiopathic pulmonary fibrosis: recent advances on pharmacological therapy

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Abstract

Idiopathic pulmonary fibrosis (IPF) is the most common and lethal of the idiopathic interstitial pneumonias with an estimated 5-year survival of approximately 20%. In the last two decades our understanding of disease pathogenesis has substantially evolved and novel compounds have been developed consequent to the increasing knowledge of the mechanisms underlying disease pathobiology. The disease appears to be driven - following chronic injury - by abnormal/dysfunctional alveolar epithelial cells that promote fibroblast recruitment and proliferation, resulting in scarring of the lung and irreversible loss of function. With very few exceptions, clinical trials evaluating novel potential therapies have provided disappointing results. More recently, pirfenidone and nintedanib, two compounds with pleiotropic mechanisms of action, have proven effective in slowing functional decline and disease progression in IPF patients with mild to moderate functional impairment, highlighting the importance of timely diagnosis and administration of treatment in early stages of disease. However, due to the complexity and uncertainties intrinsic to IPF, it is essential that each therapeutic strategy be tailored to the individual patient, after evaluation of potential benefits and risks. This article provides an overview of the most recent clinical trials in IPF and discusses how their results are going to change the clinical and clinical research landscape in IPF. A number of agents with high potential are currently being tested and many more are ready for clinical trials. Their completion is critical for achieving the ultimate goal of curing patients with IPF.

Keywords: clinical trials; idiopathic pulmonary fibrosis; interstitial lung disease; nintedanib; pirfenidone; therapy; treatment.

Abbreviations

AE: acute exacerbation

AEC: alveolar epithelial cell

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ANCOVA: analysis of covariance

DL_{CO}: diffusing capacity of the lung for carbon monoxide

DPLD: diffuse parenchymal lung disease

ECM: extracellular matrix

EMA: European Medicines Agency

FDA: Food and Drug Administration

FEV_1: forced expiratory volume in one second

FGF: fibroblast growth factor

FVC: forced vital capacity

GRADE: Grading of Recommendations Assessment, Development and Evaluation

GSH: glutathione

IIP: idiopathic interstitial pneumonia

IPF: idiopathic pulmonary fibrosis

LOCF: last observation carried forward

NAC: N-acetylcysteine

PDGF: platelet-derived growth factor

PFS: progression-free survival

RCT: randomized controlled trial

SGRQ: St. George's Respiratory Questionnaire

6MWT: 6-minute walk test

TGF-β: transforming growth factor-β

TNF-α: tumour necrosis factor-α

UIP: usual interstitial pneumonia

VC: vital capacity

VEGF: vascular endothelial growth factor

1. Introduction

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Diffuse parenchymal lung diseases (DPLDs) represent a heterogeneous collection of disorders characterized by varying patterns of inflammation and fibrosis but often sharing similar clinical, radiological and physiological features (Travis et al., 2013). While some forms of DPLDs are associated with environmental or occupational exposures (e.g., inhalation of fibrogenic dusts or aerosolized organic antigens), connective tissue diseases (e.g., rheumatoid arthritis, or systemic sclerosis), drug toxicity (e.g., amiodarone, or methotrexate), or radiation, idiopathic interstitial pneumonias (IIPs) are of unknown etiology and are thought to result from a complex interaction between host (genetic) factors and environmental triggers, including cigarette smoke (Spagnolo et al., 2014).

Idiopathic pulmonary fibrosis (IPF), the most common of the IIPs, is a chronic progressive disease, which primarily occurs in older adults (typically current or ex-smoking males over 60 years of age) and manifests with shortness of breath. In the US only, IPF affects between 150,000-200,000 people, and as many as 40,000 people die from IPF each year (Raghu et al., 2014). Similar incidence, prevalence and mortality rates have been reported in Europe (Navaratnam et al., 2011). The diagnosis of IPF requires the exclusion of all known causes of pulmonary fibrosis and the presence of a usual interstitial pneumonia (UIP) pattern, which is defined radiologically by subpleural, basal-predominant reticular abnormalities and honeycombing, with or without traction bronchiectasis (Figure 1), and histologically by patchy involvement of the lung parenchyma by fibrosis and honeycombing in a predominantly subpleural/paraseptal distribution and presence of *fibroblastic foci* (Figure 2) (Raghu et al., 2011). The disease is relentlessly progressive with a median survival time in retrospective longitudinal studies of 3 to 5 years from the time of diagnosis (Bjoraker et al., 1998; Flaherty et al., 2002; Nicholson et al., 2000; King et al., 2001). Although the population outcome for patients diagnosed with IPF is consistent across studies undertaken in different countries and continents, it remains difficult to predict the rate of progression in individual patients.

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Understanding of the pathogenesis of IPF has improved substantially in the last decade. Initially thought of as a predominantly inflammatory process, IPF appears instead to be driven by persistent alveolar epithelial cell (AEC) microinjury followed by an aberrant wound healing response (e.g., expansion of lung fibroblasts and myofibroblasts with secretion of excessive amounts of extracellular matrix (ECM) components), resulting in scarring of the lung, architectural distortion and irreversible loss of function (King et al., 2011). However, the mechanisms by which an initial injury triggers and perpetuates the fibrotic process are still debating. Recent clinical trials have therefore shifted their focus from antiinflammatory and immunosuppressant compounds to molecules targeting components of the wound healing cascade and fibrogenesis. Nonetheless, despite this shift results have been mostly disappointing, probably because of the plethora of mediators and signalling pathways likely to be involved in IPF pathogenesis (Maher, 2012). As such, the only care options which are endorsed by current evidence-based guidelines are oxygen therapy, pulmonary rehabilitation, lung transplantation and enrolment in clinical trials (Raghu et al., 2011).

Since the publication of this guideline document, pirfenidone, which had been on the market in Japan since 2008, has been approved for the treatment of IPF initially in Europe, India and Canada, and recently by the US Food and Drug Administration (FDA) following prior rejection due to what was considered conflicting evidence of efficacy (Noble et al., 2011). On the same day, October 15 2014, the FDA also approved nintedanib to treat IPF. Both drugs reduce functional decline and disease progression in patients with mild to moderate functional impairment, highlighting the importance of timely diagnosis and administration of treatment in early stages of disease.

This article provides an overview of the most recent clinical trials in IPF and discusses how these landmark studies are going to change the therapeutic landscape for this devastating disease.

2. Current approach to pharmacological treatment of IPF

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The management of patients with IPF is largely based on the recommendations of recent evidence-based guidelines (Raghu et al., 2011). These recommendations have been made based on the quality of available data as assessed by the American Thoracic Society GRADE (Grading of Recommendations Assessment, Development and Evaluation) methodology (Schunemann et al., 2006). According to current guidelines, no treatment is recommended for patients with IPF (Table 1). Conversely, the guideline document makes strong recommendations *against* the use of most therapies by virtue of either lack of sufficient-quality data or clear evidence of inefficacy. Some pharmacological agents (combination of N-acetylcysteine [NAC]/prednisone/azathioprine; NAC monotherapy; warfarin; and pirfenidone) received a *weak* recommendation *against* their use (e.g., the majority of patients would not want the intervention, but it could represent a reasonable therapeutic choice in a minority of them), which could be interpreted as an endorsement for the use of that particular treatment, even if only in a minority of patients. However, since the publication of the 2011 guidelines, more robust and better-quality data have become available and some of these recommendations will change in the near future. Specifically, combination of NAC/prednisone/azathioprine (Idiopathic Pulmonary Fibrosis Clinical Research Network, 2012) and warfarin (Noth et al., 2012) have been shown to be unsafe in patients with IPF whereas NAC monotherapy has no effect in preserving forced vital capacity (FVC) (Idiopathic Pulmonary Fibrosis Clinical Research Network, 2014). Similarly, a number of other agents, which were investigational at the time of the guideline document publication, have since proven to be ineffective or even harmful (e.g., ambrisentan; Raghu et al., 2013a) and no longer represent a therapeutic option for IPF patients. Conversely, recent data confirm that pirfenidone slows the deterioration in lung function and reduces disease progression in IPF (King et al., 2014a). Moreover, a phase 2 and two phase 3 clinical trials have, since the release of the guidelines, been published demonstrating the efficacy of the tyrosine kinase inhibitor, nintedanib, as a treatment for IPF (Richeldi et al., 2014).

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3. How we got here and why did it take so long?

In the last decade, a number of high-quality clinical trials have been conducted in IPF but the results have mostly been disappointing (Table 2). Several factors are, at least partially, responsible for such high rate of failure. These include;

Animal models. Over the years, numerous agents have been shown to inhibit experimentally-induced lung fibrosis in mice. Yet, of these >100 compounds showing efficacy in rodent models only two have demonstrated a comparable anti-fibrotic effect in humans. This is mainly because the bleomycin model, the most widely used model of experimentally-induced pulmonary fibrosis, recapitulates only partially the phenotype of progressive pulmonary fibrosis seen in IPF (Moeller et al., 2008). Furthermore, all animal models of fibrosis comprise a defined injury which, after a period of time, results in the development of self-limiting and at least partially resolving fibrosis. In the case of the bleomycin model, the initial insult with bleomycin gives rise to widespread epithelial cell apoptosis and necrosis, which in turn triggers a marked neutrophilic inflammatory response. The extent of epithelial cell death and the subsequent inflammatory response determine the severity of the fibrosis that develops. Prophylactic dosing of animals (i.e., before or within 7 days of bleomycin administration), with compounds which either inhibit apoptosis or attenuate inflammation, results in reduced fibrosis. As a consequence, many compounds have, in the past, erroneously been ascribed anti-fibrotic properties when in reality their effects were either antiinflammatory or anti-apoptotic. The use of therapeutic dosing in animal models of fibrosis will hopefully enhance the value of such *in vivo* experiments in the process of anti-fibrotic drug discovery in the future (Maher and Wells, 2009).

Imperfect knowledge of disease pathogenesis. Concepts of disease pathogenesis have evolved from chronic inflammation to current paradigms of a multifactorial and heterogeneous process in which a combination of genetic, age-related and environmental factors contribute to make the alveolar

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epithelium vulnerable to a variety of insults. Injured type II AECs, in an attempt to restore functional integrity, release cytokines and growth factors, which promote aberrant recruitment and activation of apoptosis-resistant fibroblasts, the key mediators of fibrotic tissue remodeling. The end result is the exaggerated production of ECM components leading to progressive tissue remodelling and scarring (with inevitable loss of function) rather than normal repair (Maher, 2012; Selman and Pardo, 2014). Accordingly, it is only recently that clinical research in IPF has shifted focus from immunomodulatory to anti-fibrotic and anti-proliferative compounds.

Target selection/redundancy of pathway targeting. IPF is characterized by abnormalities in multiple pathways involved in the wound healing process, many of which display considerable redundancy. Therefore, identifying therapeutic targets is extremely challenging. Numerous clinical trials have demonstrated that inhibiting individual mediators or signalling pathways is largely ineffective in slowing the inexorable progression of IPF, whereas more efficacious compounds (e.g., pirfenidone and nintedanib) are pleiotropic in their anti-fibrotic properties.

Development and performance of IPF clinical trials. A number of components are necessary to the design and implementation of a successful clinical trial in IPF, not least of which are precise disease definition and established diagnostic criteria. In both the INPULSIS and the ASCEND trials, central review of the diagnosis of IPF performed by radiologists and pathologists experienced in interstitial lung disease allowed the inclusion of well-defined populations of patients. Indeed, a treatment effect is more likely to be seen in homogeneous IPF population.

Pharmaceutical company interest. Historically, the pharmaceutical industry has been reluctant to invest in research and development of drugs for rare diseases like IPF (Spagnolo et al., 2013). Yet, clinical trials can be pursued, almost exclusively, by industry because of the enormous financial investment and logistic network required for successful drug development. The last decade has witnessed a growing interest in IPF and the lung fibrosis field is more active than ever. Major advances in this disease have

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only been possible thanks to the tremendous concerted effort of dedicated academic researchers/clinicians, patient organizations, health authorities and pharmaceutical companies.

4. What have we learned from negative studies?

Each of the negative clinical trials in IPF has been a source of great disappointment. However, the cumulative knowledge derived from these trials has been instrumental in building the foundation which underpins the recent IPF trial successes. It is important to realize that the first true randomized placebo controlled trial in IPF was only published as recently as 2004 (Raghu et al., 2004). This phase 2b study of interferon-γ-1b used a composite primary endpoint of progression-free survival (PFS); this was defined by either death, a >10% decline in FVC or a >5 mmHg rise in alveolar to arterial (a-A) oxygen gradient. This particular composite end point has never been re-used, in part because the change in a-A gradient proved to have poor reproducibility. Instead, subsequent late-phase IPF trials have used a variety of primary endpoints including survival time, change in 6-minute walk distance, change in lowest 6-minute walk saturation, change in FVC and a range of PFS composites. In recent times studies have increasingly used change in FVC as their primary outcome measure. This evolution in the use of primary endpoints has reflected a growing understanding - all of which has been derived from analysis of clinical trial data - of the variability, reproducibility and relationship to change in disease status of the various clinical measures, which can be applied to individuals with IPF (du Bois et al., 2012). Furthermore, the large datasets generated by multiple trials has enabled estimates to be made of the minimum clinically significant change in measures such as FVC and 6-minute walk distance (du Bois et al., 2011a; du Bois et al., 2014; du Bois et al., 2011b). Lessons have not only been learnt regarding the choice of efficacy endpoints for IPF studies but also in how to best measure those end-points, especially FVC. Most late-phase IPF studies now provide the same standardized spirometry equipment for each centre with central oversight of spirometry training and routine calibration. More recently,

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digital technology has permitted central assessment of the quality of individual subject's trial spirometry through analysis of the flow-volume loop. Such approaches have the effect of reducing measurement variability and thus enhance the power of studies to detect therapy-related change in FVC. The last decade of IPF trials has also seen the development of more robust and sophisticated statistical methods for handling inevitable missing endpoint data. In large phase 3 clinical trials as many as 15-20% of subjects can have missing primary endpoint data (Collard, 2010). This occurs principally because of death but also due to drop outs and because some patients are too unwell to undertake maneuvers such as spirometry. In early studies, such missing primary endpoint events were handled with one of two extremes, either carrying forward the last observation or assigning zero to deaths and, in some cases, also to values missing for reasons other than death. In trials where there are imbalances between arms in missing values these methods of handling missing data can either magnify or disguise the true differences between groups. To address this issue, the TOMORROW and INPULSIS studies moved away from using change from baseline in FVC at 12 months to using change in FVC decline over 12 months (Richeldi et al., 2011; Richeldi et al., 2014). This latter approach permits all FVC values recorded over the course of the study to be utilized in deriving a slope reflecting the rate of change in the FVC. This method, relying as it does on multiple measures and no imputation of missing data, is much less influenced by missing observations. This is borne out by the results of sensitivity analyses, which demonstrate that different methods of imputing missing data points had little influence on the observed rate of change between treatment arms in the two INPULSIS trials (Richeldi et al., 2014).

Cumulatively, international randomized controlled trials (RCTs) have now involved the participation of over 3,000 individuals with IPF. Whilst all of the trials conducted to date have assessed the role of specific drug therapies, an important added benefit of the prospective observation of large numbers of individuals with a well-defined diagnosis has been the insight provided into the natural history of IPF

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(Martinez et al., 2005). Prior to the advent of clinical trials, much of what was known about IPF had been derived from retrospective cohorts usually followed at dedicated tertiary centres. It has only been by following individuals with IPF prospectively that the true morbidity and mortality associated with acute exacerbations (AE) of IPF has been realized (Collard et al., 2007). Furthermore, insights derived from clinical trials have helped inform the current diagnostic approach to IPF, which has now been enshrined in updated international guidelines (Raghu et al., 2011).

5. Recent phase 3 randomized controlled trials

Pirfenidone. Pirfenidone, an orally administered pyridine, was initially identified as having analgesic, anti-inflammatory and anti-pyretic activities in animals. However, the unexpected identification of antifibrotic effects in animals redefined the interest in the compound (Lasky, 2004). Subsequently, pirfenidone has been shown both in *in vitro* experiments and *in vivo* animal models of pulmonary fibrosis to exert anti-fibrotic, antiinflammatory and anti-oxidant properties through down-regulation of profibrotic growth factors including platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β; inhibition of inflammatory cytokine (e.g., tumour necrosis factor [TNF]-α) production and release; and reduction of lipid peroxidation and oxidative stress (Iyer et al., 1999; Maher, 2010; Schaefer, 2011).The first promising effect in IPF patients was observed in a prospective, open-label phase 2 study published in 1999 (Raghu et al., 1999).

The first RCT of pirfenidone in IPF was conducted in Japan by Azuma and co-workers (Azuma et al., 2005). The study did not meet its primary end-point of change in lowest arterial oxygen saturation during a 6-minute walk test (6MWT), although at 9 months the rate of decline in vital capacity (VC) was significantly reduced in the treatment arm. Since the publication of this study, four phase 3 RCTs of pirfenidone in IPF have been conducted and published.

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Taniguchi (Taniguchi et al., 2010). 275 Japanese patients (20-75 years of age) were randomly assigned in a 2:1:2 ratio to high-dose (1800 mg/day) or low-dose (1200 mg/day) pirfenidone, or placebo. The primary endpoint was the change in VC from baseline to week 52. The study met its primary outcome as the loss in VC was significantly higher in the placebo arm (-0.16 L) compared to both the high-dose (-0.09 L; $p = 0.042$) and low-dose pirfenidone arms (0.08 L; $p = 0.039$). High-dose pirfenidone was also associated with a significant improvement in PFS time compared to placebo ($p = 0.028$). Conversely, the incidence of acute exacerbations (either during the study of within 28 days after the termination of the study) did not differ among the high-dose (5.6%), low-dose (5.5%) and placebo (4.8%) groups.

CAPACITY (Noble et al., 2011). The CAPACITY (Clinical Studies Assessing Pirfenidone in Idiopathic Pulmonary Fibrosis: Research of Efficacy and Safety Outcomes) program consisted of two almost identical multinational trials (PIPF-004 and PIPF-006). Eligible patients were aged 40-80 years with a diagnosis of IPF made in the previous 48 months. Inclusion criteria included a predicted FVC ≥50%, predicted diffusing capacity of the lung for carbon monoxide (DL_{CO}) of ≥35%, either predicted FVC or predicted DL_{CO} ≤90% and a 6MWT distance of at least 150 m. In study 004, 435 patients were assigned in a 2:1:2 dosing ratio to pirfenidone 2403 mg/day, pirfenidone 1197 mg/day, or placebo, whereas study 006 ($n = 344$) had only two treatment arms (pirfenidone 2403 mg/day and placebo). The primary endpoint of both trials was change in percentage predicted FVC from baseline to week 72. In study 004, mean FVC change at week 72 was -8.0% in the pirfenidone 2403 mg/day arm compared to -12.4% in the placebo arm ($p = 0.001$). In addition, high-dose pirfenidone reduced the proportion of patients with FVC decline ≥10% compared to placebo (20% vs. 35%, respectively). A loss of ≥10% in FVC is a clinically meaningful event for an IPF patient as it translates into an increased rate of death of up to eight-fold in the following year (du Bois et al., 2011a). Study 006 did not meet its primary end point as mean changes in percent predicted FVC in the pirfenidone and placebo arms were almost

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identical (-9.0 and -9.6%, respectively). Of note, while the magnitude of decline in FVC over time was similar in the pirfenidone arms of 004 and 006 studies, the rate of decline in the placebo groups differed. Specifically, the placebo arm of study 006 experienced an attenuated FVC decline (-9.6%) compared to that observed in placebo arms of previous large RCTs in IPF (Ley et al., 2011). Pirfenidone 2403 mg/day prolonged PFS (time to confirmed ≥10% decline in percentage predicted FVC, ≥15% decline in percentage predicted DL_{CO} or death) in study 004, but not in study 006. Conversely, pirfenidone 2403 mg/day significantly reduced decline in 6MWT distance in study 006, but not in study 004. The study by Taniguchi *et al*. and the two CAPACITY trials were of sufficient methodological quality to be included in a Cochrane systematic review and meta-analysis, which demonstrated that pirfenidone significantly reduces both the rate of lung function deterioration and the risk of disease progression in patients with IPF (Spagnolo et al., 2010). Based on these cumulative data, in 2011, pirfenidone was approved by the European Medicines Agency (EMA) for the treatment of patients with mild to moderate IPF (e.g., FVC >50%).

ASCEND (King et al., 2014a). In ASCEND (Assessment of Pirfenidone to Confirm Efficacy and Safety in Idiopathic Pulmonary Fibrosis) 555 IPF patients were randomly assigned to either pirfenidone 2403 mg/day (n = 278) or placebo (n = 277). Eligible patients were between the ages of 40 and 80 years and had received a centrally confirmed diagnosis of IPF. Additional inclusion criteria included a range of 50% to 90% of the predicted FVC, a range of 30% to 90% of the predicted DL_{CO}, a ratio of the forced expiratory volume in one second (FEV_1) to the FVC of 0.80 or more and a 6MWT distance of at least 150 m. The primary end point was change from baseline to week 52 in the percentage of predicted FVC. The study met its primary endpoint as assessed by a rank analysis of covariance (ANCOVA). Pirfenidone treatment also significantly reduced the proportion of patients who had a decline of 10% or more in the percentage predicted FVC or who died, and significantly increased the proportion of patients with no decline in the percentage of the predicted FVC compared with placebo (16.5% vs.

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31.8% [p<0.001], and 22.7% vs. 9.7% [p<0.001], respectively). In addition, pirfenidone significantly reduced the decline in the 6MWT distance (p = 0.04) and improved PFS (p<0.001). Furthermore, in a pre-specified pooled analysis incorporating data from ASCEND and the two CAPACITY studies, pirfenidone reduced all-cause mortality and IPF-related mortality at 1 year by 48% (p = 0.01) and 68% (p = 0.006), respectively, compared with placebo. Conversely, pirfenidone treatment had no effect on dyspnoea as assessed by the University of California San Diego Shortness of Breath Questionnaire. Consistent with the known safety profile of the drug, most common treatment-related adverse events were gastrointestinal upset and photosensitivity rash, which were generally mild to moderate in severity, reversible, and without clinically significant sequelae (Valeyre et al., 2014).

Nintedanib. Formally known by the development code BIBF 1120, nintedanib is a potent intracellular inhibitor of fibroblast growth factor receptor (FGFR) 1, 2 and 3, platelet-derived growth factor receptor (PDGFR) α and β, and vascular endothelial growth factor receptor (VEGFR) 1, 2 and 3 (Hilberg et al., 2008). It also inhibits the *Src* family tyrosine kinases Lck, Lyn and Flt-3 (Hilberg et al., 2008). Nintedanib was originally developed as an angiostatic factor for cancer treatment, and is approved for lung cancer patients with advanced adenocarcinoma after first-line chemotherapy. In addition, it is currently being investigated in ovarian (phase 3), Fallopian tube (phase 2), thyroid (phase 2), peritoneal (phase 2), prostate (phase 2), liver (phase 2), renal (phase 2) and colorectal (phase 2) cancer as well as myeloma (phase 1). However, nintedanib has also been shown to exert anti-fibrotic activities in bleomycin-induced pulmonary fibrosis in rodents (Chaudhary et al., 2007; Wollin et al., 2014). In primary human lung fibroblasts from patients with IPF, nintedanib inhibits FGF-, PDGF- and VEGF-induced profibrotic effects, reduces TGF-β-induced collagen deposition, and inhibits TGF-β-induced fibroblast to myofibroblast differentiation (Hostettler et al., 2014; Chaudary et al., 2007)

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TOMORROW (Richeldi et al., 2011). In this phase 2b study, the TOMORROW (To Improve Pulmonary Fibrosis With BIBF 1120), 432 patients with IPF were treated with one of four escalating doses of nintedanib (50 mg once a day, and 50 mg, 100 mg, or 150 mg all twice a day) or placebo for 52 weeks. Eligible patients were 40 years of age or older and had received a centrally confirmed diagnosis of IPF less than 5 years before screening. Additional inclusion criteria included a FVC of 50% or more of the predicted value and a DL_{CO} that was 30% to 79% of the predicted value. The primary end point was the annual rate of decline in FVC. While the study did not achieve statistical significance (because of the hierarchical testing procedure adopted with correction for multiplicity), in the group receiving 150 mg twice a day FVC declined by 0.06 liters per year as compared to 0.19 liters per year in the placebo group, corresponding to a 68.4% reduction in the rate of loss. Moreover, the proportion of patients who had a decrease in FVC >10% or >200 ml was smaller in the highest-dose group than in the placebo group (23.8% vs. 44.0%; p = 0.004). Additional beneficial effects of nintedanib 150 mg twice daily compared to placebo included a lower incidence of AE of IPF (2.4 vs. 15.7 per 100 patient-years, respectively) and a significantly improved quality of life as assessed by St. George's Respiratory Questionnaire (SGRQ). Specifically, there were improvements in two domains of the SGRQ - symptoms and activity. Adverse events lead to study discontinuation in 25.9%, 23.3%, 16.3%, 14.0% and 30.6% of patients in the placebo, 50 mg once daily, 50 mg twice daily, 100 mg twice daily and 150 mg twice daily groups, respectively.

INPULSIS (Richeldi et al., 2014). The INPULSIS 1 and INPULSIS 2 were two parallel phase 3 studies that evaluated the efficacy and safety of nintedanib at the dose of 150 mg twice daily compared with placebo in patients with IPF. Patients were eligible if they were 40 years of age or older and had received a centrally confirmed diagnosis of IPF within the previous 5 years. Additional eligibility criteria included a FVC of 50% or more of the predicted value and a DL_{CO} that was 30% to 79% of the predicted value. A total of 1,066 patients were randomly assigned in a 3:2 ratio to receive nintedanib or

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placebo (309 vs. 204 in INPULSIS 1 and 329 vs. 219 in INPULSIS 2, respectively). The primary end point was the annual rate of decline in FVC. Both trials met their primary outcome. In fact, the adjusted annual rate of change in FVC was -115 ml among patients on nintedanib and -240 ml among patients on placebo in INPULSIS 1 (difference: 125 ml; $p<0.001$) and -114 ml and -207 ml in INPULSIS 2 (difference: 93 ml; $p<0.001$), respectively. In addition, in both trials a significantly greater proportion of patients in the placebo arm than in the nintedanib arm had an absolute decline in the percentage of predicted FVC >5% at week 52. As for the two key secondary endpoints, change in quality of life (as assessed by SGRQ) was non-significant in INPULSIS 1 ($p = 0.97$) but significant in INPULSIS 2 ($p = 0.02$), whereas time to the first AE-IPF was significant in INPULSIS 2 ($p = 0.005$) but not in INPULSIS 1 ($p = 0.67$). However, a pre-specified sensitivity analysis of pooled data on the time to the first adjudicated AE (confirmed or suspected) showed that nintedanib had a significant benefit as compared with placebo. Finally, in a pre-specified pooled analysis, no significant difference in death from any cause (5.5% in the nintedanib group vs. 7.8% in the placebo group; $p = 0.14$) or death from a respiratory cause was observed, although these trials were not powered to detect statistically significant differences in mortality. Overall, nintedanib showed an acceptable safety profile. The most frequent drug-related adverse event in both trials was diarrhoea (with rates of 61.5% and 18.6% in the nintedanib and placebo groups, respectively, in INPULSIS 1 and 63.2% and 18.3% in the two groups, respectively, in INPULSIS 2). However, the majority of events were of mild or moderate intensity and most patients continued to receive nintedanib for the duration of the treatment period.

N-acetylcysteine. N-acetylcysteine (NAC) is a small molecule that acts as a scavenger for reactive oxidative species. NAC was first reported to have clinical benefit in the early 1960s, when it was shown to be an effective mucolytic agent in patients with cystic fibrosis (Hurst et al., 1967). Other clinical applications for NAC supplementation include, amongst others, treatment of acetaminophen

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overdose, prevention of chronic obstructive pulmonary disease exacerbation and prevention of contrast-induced kidney damage during imaging procedures (Millea 2009). In addition, NAC is commonly used as a nutritional supplement.

An oxidant-antioxidant imbalance is believed to play a role in the pathogenesis of IPF based on the finding that levels of the endogenous antioxidant glutathione (GSH) are markedly reduced in the lungs of patients (Cantin et al., 1989). The observation that high-dose oral NAC increases lung levels of GSH and improves lung function in patients with fibrosing alveolitis (Behr et al., 1997), paved the way for the IFIGENIA trial (Idiopathic Pulmonary Fibrosis International Group Exploring N-Acetylcysteine), a double-blind, randomized, placebo-controlled multicenter study that assessed the efficacy over one year of high-dose NAC (600 mg three times daily) added to prednisone and azathioprine (which at that time was the *standard therapy*) in patients with IPF (Demedts et al., 2005). As compared with *standard therapy* (the "placebo" arm), *triple therapy* (NAC/prednisone/azathioprine) significantly slowed the decline of both VC and DL_{CO} (the primary end points), as assessed by change between baseline and month 12. Although positive, this study had important drawbacks, mainly related the lack of a survival benefit (though the study was not powered for mortality), the use of the last observation carried forward (LOCF) method of analysis, which inflates type I error (e.g., it may overestimate the treatment effect), and the high rate of patients who did not complete the treatment period (about 30%). The IPFnet-sponsored PANTHER study (Prednisone, Azathioprine, and N-acetylcysteine: A Study That Evaluates Response in IPF) was specifically designed to address some of the issues raised by the IFIGENIA trial and to test the assumption that prednisolone and azathioprine should be considered "standard of care" for patients with IPF (Idiopathic Pulmonary Fibrosis Clinical Research Network, 2012). Patients in the study were randomized in a 1:1:1 ratio to NAC/prednisone/azathioprine (*triple therapy*), NAC alone or placebo. Unexpectedly, a pre-specified efficacy and safety interim analysis planned after approximately 50% of data collection had occurred revealed that *triple therapy*, as

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compared with placebo, was associated with a statistically significant increase in all-cause mortality, all-cause hospitalizations, and treatment-related severe adverse events, thus strongly arguing against the use of this treatment approach in patients with IPF. The combination therapy arm was therefore terminated early and PANTHER continued as a two-group study (i.e., NAC vs. placebo). The completed study did not meet its primary outcome of change in FVC over a 60-week period. In fact, FVC decline over the study period was -0.18 litres in the NAC arm and -0.19 litres in the placebo arm ($p = 0.77$) (Idiopathic Pulmonary Fibrosis Clinical Research Network, 2014). Similarly, NAC was not superior to placebo in reducing the rates of death or AE of IPF. Moreover, a significant increase in serious cardiac events was observed in the NAC arm compared to placebo (6.8% versus 1.5%, respectively).

6. What have we learned from positive studies? Implications on better understanding of disease pathogenesis

Basic IPF research has long suffered a disconnect from IPF clinical practice. Over the last couple of decades, pre-clinical studies, usually conducted *in vitro* in fibroblasts or *in vivo* in the rodent bleomycin model, have uncovered multiple abnormalities in a range of key wound healing cascades all of which appear to be capable of driving the development of fibrosis (Maher et al., 2007). Whilst these insights have been mechanistically interesting, there has been a failure to link individual abnormalities to the development or progression of human fibrotic disease. Redundancy and pleiotropism of signalling pathways undoubtedly means that many receptor or growth factor abnormalities, which appear to promote or inhibit fibrosis in mice are of limited importance in human disease. Furthermore, the major tool used in pre-clinical assessment of candidate therapeutics has tended to be the bleomycin model. As has already been alluded to, unlike IPF, which is a progressive fibrotic disorder with minimal inflammation, bleomycin-induced fibrosis arises as a consequence of acute, widespread alveolar

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epithelial cell apoptosis and accompanying inflammatory cell influx and tends to be self-limiting with partial resolution after about 28 days (Maher and Wells, 2009; Moeller et al., 2008).

So, with these issues in mind does the recent success of pirfenidone and nintedanib in clinical trials teach us anything about IPF pathogenesis and, if so, will these insights help pave the way for more rationale IPF drug discovery in the future? The answer to these questions is not entirely straightforward. Nintedanib's principal action is on the receptors for VEGF, PDGF and FGF (Chaudhary et al., 2007). All of these growth factors have been previously implicated in the development of pulmonary fibrosis and so the efficacy of nintedanib in IPF, to a certain extent, validates previous pre-clinical observations. Pirfenidone has effects on multiple signalling cascades, presumably through kinase inhibition, but it remains unclear whether pirfenidone has a single key target or whether its effects derive from its broad spectrum of action. Relatively little pre-clinical data has been published on the *in vitro* or *in vivo* effects of either nintedanib or pirfenidone in fibrosing disease. Both drugs have, however, been shown to be effective in reducing bleomycin-induced fibrosis in rodents when dosed therapeutically and so provide a degree of validation for this model (Chaudhary et al., 2007; Oku et al., 2008; Kakugawa et al., 2004). Whilst these drugs confirm that fibrosis can be ameliorated through abrogation of wound healing pathways, neither compound really provides a way of better designing or informing *in vitro* fibrosis research. Accordingly, further pre-clinical studies are required before it will be possible to derive major pathogenic insights from the therapeutic effects of either nintedanib or pirfenidone. Both drugs do, however, at least provide a benchmark against which the anti-fibrotic effects of other compounds can be gauged.

7. Implications for clinical practice of recent negative clinical trials

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The results of recently completed clinical trials in IPF have tremendous implications in clinical practice. Warfarin was considered a therapeutic option for a minority of IPF patients based on the results of an open label study conducted in Japan showing that anticoagulant therapy significantly reduces mortality associated with AE (Kubo et al., 2005). Yet, the study had important methodological limitations, including absence of blinding; significant withdrawal rate in the anticoagulant group (26%; e.g., it is possible that patients who left the study were more ill and would have had higher mortality); and unusually high incidence of AE (57% overall and 64% in the placebo group). A double-blind, randomized, placebo-controlled study (the AntiCoagulant Effectiveness in Idiopathic Pulmonary Fibrosis [ACE-IPF]) was therefore specifically designed to evaluate the efficacy of anticoagulation in IPF (Noth et al., 2012). However, the ACE study was terminated early (after 145 of the planned 256 subjects were enrolled) due to excess mortality in the warfarin arm (14 warfarin vs. 3 placebo deaths; adjusted hazard ratio = 4.85). Warfarin treatment was also associated with an increase in all-cause and respiratory-related hospitalizations, and AE of IPF, strongly arguing against the use of warfarin for treating patients with IPF. Accordingly, recommendations on the use of anticoagulants in IPF will soon change. Similarly, the demonstration that previously recommended "standard-of-care" (prednisone/azathioprine/NAC) is actually associated with increased mortality, excess of hospitalizations and increased serious adverse events compared to placebo has already impacted dramatically the approach to treatment of IPF patients (Idiopathic Pulmonary Fibrosis Clinical Research Network, 2012). While formal recommendations have not yet been produced, most physicians no longer initiate immunosuppressive therapy in newly diagnosed patient with IPF and have chosen to withdraw such treatment in patients initiated on immunosuppression prior to announcement of the PANTHER data in October 2011. However, in patients who have demonstrated sustained stability whilst on "triple therapy" and in those without a *definite* diagnosis of IPF (particularly if

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fibrotic nonspecific interstitial pneumonia or chronic hypersensitivity pneumonitis is in the differential diagnosis) the decision regarding the use of immunosuppression is now far from straightforward.

The implications of the PANTHER study, which showed that NAC is ineffective in slowing the rate of FVC decline in patients with IPF, are less predictable (Idiopathic Pulmonary Fibrosis Clinical Research Network, 2014). According to current guidelines, acetylcysteine monotherapy may represent a reasonable therapeutic choice for a minority of patients (Raghu et al., 2011). Yet, in clinical practice NAC is widely used, mainly in patients with "severe" IPF (e.g., FVC <50%), or in those not eligible for enrolment in a clinical trial. However, the recent approval by the FDA of both pirfenidone and nintedanib for the same indication and with no restriction based on disease severity will likely limit further the use of NAC monotherapy (e.g., to patients who are unable to tolerate either drug or for whom they are contraindicated).

8. Open questions

The results of the INPULSIS 1 and INPULSIS 2 and the ASCEND trials represent a major breakthrough for patients with IPF. Based on the results of these studies it is likely that clinicians and patients will soon have genuine choices when it comes to selecting pharmacological therapy for IPF. In addition, there is now indirect evidence demonstrating that reducing the rate of functional decline in IPF (as measured by serial change in FVC) translates into a reduced rate of mortality. In fact, a pre-specified analysis of the pooled population of the ASCEND and CAPACITY studies (n = 1,247 patients) showed that pirfenidone, as compared with placebo, reduces both all-cause and IPF-related mortality (King et al., 2014a). Similarly, nintedanib treatment resulted in a trend toward a reduced mortality mirroring the slowing of functional deterioration (Richeldi et al., 2014). Nevertheless, these data should be viewed as a starting point in the search for better IPF treatments especially as a number of crucial points remain to be addressed.

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Confidence in clinical response and interpretation of results in therapeutic trials of IPF. There are many considerations and challenges involved in the design and completion of a treatment trial in IPF, including heterogeneity in disease progression and uncertainty regarding the optimal outcome measures, which make it difficult to be confident of a robust clinical response. Indeed, IPF patients progress and lose lung function at different rates, whereas approximately two-thirds of them do not show FVC decline over the course of a one-year trial. Accordingly, while categorical change in FVC is a more informative metric of clinical meaningfulness than group mean, efficacy endpoints are driven by only a subgroup of patients (e.g., those who experience progression over the course of the clinical trial), although progression may be continuous yet undetectable using common clinical tests. A cohort enrichment strategy allowing for more homogeneous study populations who experience an increased number of events would reduce substantially the size of the study population and the duration of the trial, thus allowing the timely, successful completion of the study. An alternative approach for enabling the event rate would be the implementation of composite endpoints, which ideally should be constituted by quantifiable measures that reflect a spectrum of pathophysiological consequences of disease progression (Kaul et al., 2010). However, at present, there are no universally accepted composite end-points in IPF. Survival is probably the only indisputable endpoint in a deadly disease like IPF, but a properly powered mortality study in patients with mild to moderate functional impairment is impracticable due to the number of patients and length of trial needed (King et al., 2014b). Mortality studies might be best suited for patients with advanced disease, or rapid progressors, which would offer the theoretical advantage of higher event rates, smaller sample size and shorter trial duration. However, the disease is not well characterized in this population and there are insufficient reliable data to estimate the appropriate sample size and study duration (King et al., 2014b).

Severe IPF. The complexity of the diagnostic process in IPF makes misdiagnosis and delayed diagnosis common (Schoenheit et al., 2011). Therefore, it is not infrequent that IPF patients present with

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advanced disease and severe functional impairment (percentage of the predicted FVC <50%). Yet, ASCEND, CAPACITY and INPULSIS all limited their enrollment to patients with mild-to-moderate functional impairment (e.g., percentage of the predicted FVC >50%). The assumption behind such restricted criteria, which are adopted for most IPF clinical trials, is mainly that advanced disease is less likely to be amenable to any anti-fibrotic therapy, although there are no convincing data in this regard, and that these patients may have different and unpredictable response rates and more frequent and severe adverse events. Therefore, although the FDA has approved both pirfenidone and nintedanib regardless of disease severity (and nintedanib has also been approved by the EMA with the same indication), we cannot know with certainty at the moment whether these drugs are also safe and efficacious in individuals with advanced disease.

Patients without a diagnosis of "definite IPF". According to the 2011 evidence-based guidelines, patients presenting with non-diagnostic chest CT scans require a confirmatory surgical lung biopsy. However, in clinical practice, surgical lung biopsy is performed in only a minority of patients, either because of the risks associated with the procedure - particularly in elderly subjects - or because of the reluctance of most individuals to undergo surgery (Raghu et al., 2011). Moreover, albeit in a minority of cases, even the availability and integration of clinical, radiological, and histologic data may not be sufficient to establish a secure diagnosis of IPF. Such patients (e.g., those with a "probable" or "possible" IPF) are commonly excluded from clinical trials - which require a *definite* diagnosis of IPF - although the INPULSIS trials allowed a proportion of them to enroll (Richeldi et al., 2014). It is therefore uncertain whether the results of clinical trials undertaken in patients with *definite* IPF also apply to those with "probable" or "possible" IPF.

Comorbid Disease. IPF is frequently accompanied (or complicated) by common comorbid conditions, which contribute to its poor prognosis and impaired quality of life (Fell, 2012). In addition, the presence of comorbid conditions such as pulmonary hypertension, and vascular or coronary artery

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disease often preclude IPF patients from participating in clinical trials. In clinical practice, the management of such patients is the most challenging, and solid, prospective data on which to make recommendations for treatment of concomitant conditions in patients with IPF are urgently needed.

A minority of patients are eligible to clinical trials. With very few exceptions, clinical trials in IPF have enrolled selected patients (e.g., those with mild-to-moderate functional impairment), in whom the rates of functional decline and disease progression are both relatively low and variable (King et al., 2014a; Richeldi et al., 2014). Many patients are excluded mainly due to their age, disease severity, or comorbidities, while rapid progressors might have failed inclusion criteria by the time they got access to the trial, thus highlighting the difficulty in accruing adequate numbers of eligible patients within a reasonably short period of time (Nathan et al., 2011). It is important that, in future, clinical trials seek to enrol a broader and more representative population of patients with IPF. Only by doing so will it be possible for clinicians to have confidence in the clinical applicability of data generated in randomised controlled trials of novel IPF therapies.

9. Conclusions

Clinical research on pharmacological treatment of IPF has until very recently witnessed repeated failure and with it a growing sense of frustration. Improved knowledge of disease pathogenesis coupled with the availability of compounds with pleiotropic anti-fibrotic properties is finally changing the landscape of IPF treatment. However, slowing the deterioration of lung function and reducing disease progression in patients with mild-to-moderate IPF, which is undoubtedly a major achievement, should be viewed as a new starting point. In fact, a number of key questions remain unanswered: do the beneficial effect of pirfenidone and nintedanib also apply to patients who fall outside the inclusion criteria of these trials or to patients with other forms of fibrosing lung disease? Do these compounds have a synergistic or additive effect when administered together? How can we identify those patients

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who will progress rapidly or those more likely to respond to a specific treatment? These questions notwithstanding, the IPF community now has a platform on which to build treatments which are, at last, able to alter the natural history of this devastating disease.

This manuscript has not been published and is not under consideration for publication elsewhere.

Conflict of interest statement:

Dr Paolo Spagnolo serves as consultant for Roche. In the last three years PS has served as consultant for Chiesi and has received consulting fees from Boehringer Ingelheim and speaker's fees from InterMune and Boehringer Ingelheim;
Dr Toby M. Maher is in receipt of unrestricted academic industry grants from GSK, UCB and Novartis. In the last three years TM or his institution have received advisory board or consultancy fees from; Boehringer Ingelheim, Bayer, Biogen, ProMedior, GSK, InterMune, Novartis, Lanthio, Roche, Takeda, Sanofi-Aventis and UCB. TM has received speaker's fees from UCB, Boehringer Ingelheim, InterMune and AstraZeneca. TM has participated as an investigator in industry sponsored clinical trials run by Boehringer Ingelheim, Gilead, GSK, InterMune, Novartis, Roche and Celgene;
Prof Luca Richeldi has received grants for research and fees for lectures, advisory board meetings, and steering committee meetings from InterMune, Boehringer Ingelheim, Roche, Takeda, Shionogi, Biogen Idec, Sanofi-Aventis, MedImmune and ImmuneWorks.

Figure legend

Figure 1. Usual interstitial pneumonia (UIP) pattern in a patient with idiopathic pulmonary fibrosis (IPF). High-resolution CT shows a characteristic combination of peripheral, subpleural, and predominantly basilar reticular abnormalities with associated honeycomb change (arrows) and traction bronchiectasis.

Figure 2. Usual interstitial pneumonia (UIP) pattern. UIP is characterized by a patchy geographical distribution (*spatial heterogeneity*) of the fibrotic process alternating relatively preserved lung (arrows) and fibrosis (circles). *Temporal heterogeneity* refers to areas of early, active fibrosis (so-called «fibroblastic foci»; asterisk) abruptly akin to old, established fibrosis (diamond). Extensive lymphoplasmacytic inflammation is generally absent. Hematoxylin-eosin 40X. Courtesy Giulio Rossi MD (University Hospital of Modena, Italy).

Figure 3. Honeycomb change. Areas of honeycomb change are composed of cystic fibrotic air spaces (*), which are frequently lined by bronchiolar epithelium and filled with mucin (#). Smooth muscle hyperplasia is commonly seen in areas of fibrosis and honeycomb change. Hematoxylin-eosin 40X. Courtesy Giulio Rossi MD (University Hospital of Modena, Italy).

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Table 1. Recommendations on pharmacological treatment of IPF according to current guidelines

	Recommendation							
	For				***Against***			
Strength of the recommendation	**Weak**		**Strong**		**Weak**		**Strong**	
Quality of evidence	**L/VL**	**M/H**	**L/VL**	**M/H**	**L/VL**	**M/H**	**L/VL**	**M/H**
Therapeutic agent								
Azathioprine + corticosteroids							**X**	
Azathioprine + corticosteroids + NAC*					**X**			
Bosentan								**X**
Colchicine							**X**	
Corticosteroids alone							**X**	
Cyclosporin A							**X**	
Cyclophosphamide + corticosteroids							**X**	
Etanercept								**X**
Interferon-γ-1b								**X**
NAC alone*					**X**			
Pirfenidone*					**X**			
Warfarin*					**X**			

(Modified from Raghu et al., 2011)

L: low; VL: very low; M: medium; H: high
NAC: N-acetylcysteine;

*Recommendations on these therapeutic options will change in the near future based on the results of recent clinical trials

Note: official recommendations on the use of imatinib, sildenafil, ambrisentan and nintedanib in patients with IPF are not available as the trials investigating these compounds have been published after the publication of the 2011 guidelines.

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Table 2. Overview of negative phase II and phase III randomized controlled trials in IPF

Study agent / treatment	Mechanism of action	Primary endpoint / Number of patients	Outcome / comment	Reference
Azathioprine + prednisone + NAC vs. NAC vs. placebo (PANTHER)	Immunosuppressant + anti-inflammatory + antioxidant	Change in FVC over 60 weeks N = 236	The combination-therapy arm was terminated early due to increased rate of death and hospitalization. No physiological or clinical benefit for combination therapy. No effect of NAC in reducing FVC decline.	(Idiopathic Pulmonary Fibrosis Clinical Research Network, 2012; Idiopathic Pulmonary Fibrosis Clinical Research Network, 2014)
Etanercept	TNF inhibitor	Changes from baseline in FVC % predicted, DL_{CO} % predicted and $P(A\text{-}a)O_2$ at rest over 48 weeks N = 88	No differences in the predefined endpoints	(Raghu et al., 2008)
Everolimus	mTOR inhibitor - proliferation signal inhibitor	Time to disease progression (time to the second of any two of 10% decline in FVC or TLC, 15% decline in DL_{CO}, 4% decline in room air oxygen saturation) N = 89	Everolimus treatment was associated with increased disease progression and higher frequency of adverse events	(Malouf et al., 2011)
Macitentan - MUSIC	Dual endothelin-receptor antagonist	Change in FVC from baseline up to month 12 N = 178	No difference between treatments in pulmonary function tests, or time to disease worsening or death	(Raghu, et al., 2013b)
Interferon gamma-1b	Anti-inflammatory, antifibrotic and pro-Th1	Progression-free survival (defined as the time to disease progression or death) N = 330	No effect on progression-free survival, pulmonary function or quality of life	(Raghu et al., 2004)
Interferon gamma-1b - INSPIRE	Anti-inflammatory, antifibrotic and pro-Th1	Overall survival N = 826	The study was terminated at the second interim analysis due to lack of benefit compared with placebo	(King et al., 2009)
Sildenafil - STEP	Phosphodiesterase 5 inhibitor	Proportion of patients with an increase in the 6-minute walk distance of ≥20% N = 180	The study enrolled patients with advanced IPF (DL_{CO} <35% of the predicted value). No benefit for sildenafil for the primary outcome	(Idiopathic Pulmonary Fibrosis Clinical Research Network, 2010)
Warfarin - ACE	Anticoagulant	Composite endpoint (time to death, hospitalization or ≥10% decline in FVC) N = 145	The study was terminated early due to increased mortality in the warfarin arm.	(Noth et al., 2012)
Bosentan - BUILD-1	Dual endothelin receptor antagonist	Change from baseline up to month 12 in exercise capacity, as measured by 6MWT N = 158	No superiority of bosentan over placebo in the primary endpoint.	(King et al., 2008)
Bosentan - BUILD-3	Dual endothelin receptor antagonist	Time to IPF worsening (decline in FVC ≥10% and decline in DL_{CO} ≥15% or acute exacerbation) or death N = 616	No difference between treatment groups in the primary endpoint analysis.	(King, et al., 2011b)
Ambrisentan - ARTEMIS	Endothelin A receptor antagonist	Time to disease progression (death, decline in FVC ≥10%, decline in DL_{CO} ≥15% or acute exacerbation) N = 492	The study was terminated early due to increased risk for disease progression and hospitalization.	(Raghu, et al., 2013a)
Imatinib	PDGFR-α and -β inhibitor	Time to disease progression (10% decline in FVC from baseline) or death N = 119	No effect of imatinib on survival or lung function.	(Daniels et al., 2010)
Co-trimoxazole	Antibiotic	Change in FVC over 1 year N = 181	Only 89% of patients had definite/probable IPF. Co-trimoxazole was added to *standard treatment*. No effect on lung function	(Shulgina et al., 2013)

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DL_{CO}: diffusing capacity of the lung for carbon monoxide; FVC: forced vital capacity; mTOR: mammalian target of rapamycin; NAC: N-acetylcysteine; $P(A\text{-}a)O_2$: alveolar to arterial oxygen pressure difference; PDGFR: platelet-derived growth factor receptors; 6MWT: six-minute walk test; TLC: total lung capacity; TNF: tumour necrosis factor.

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Fig. 1

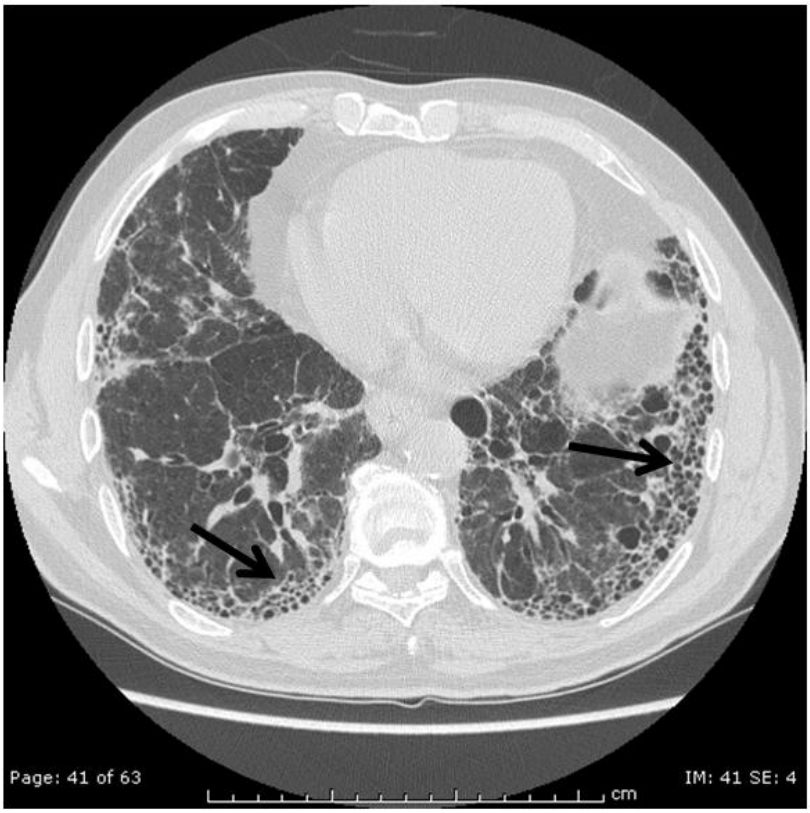

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Fig. 2

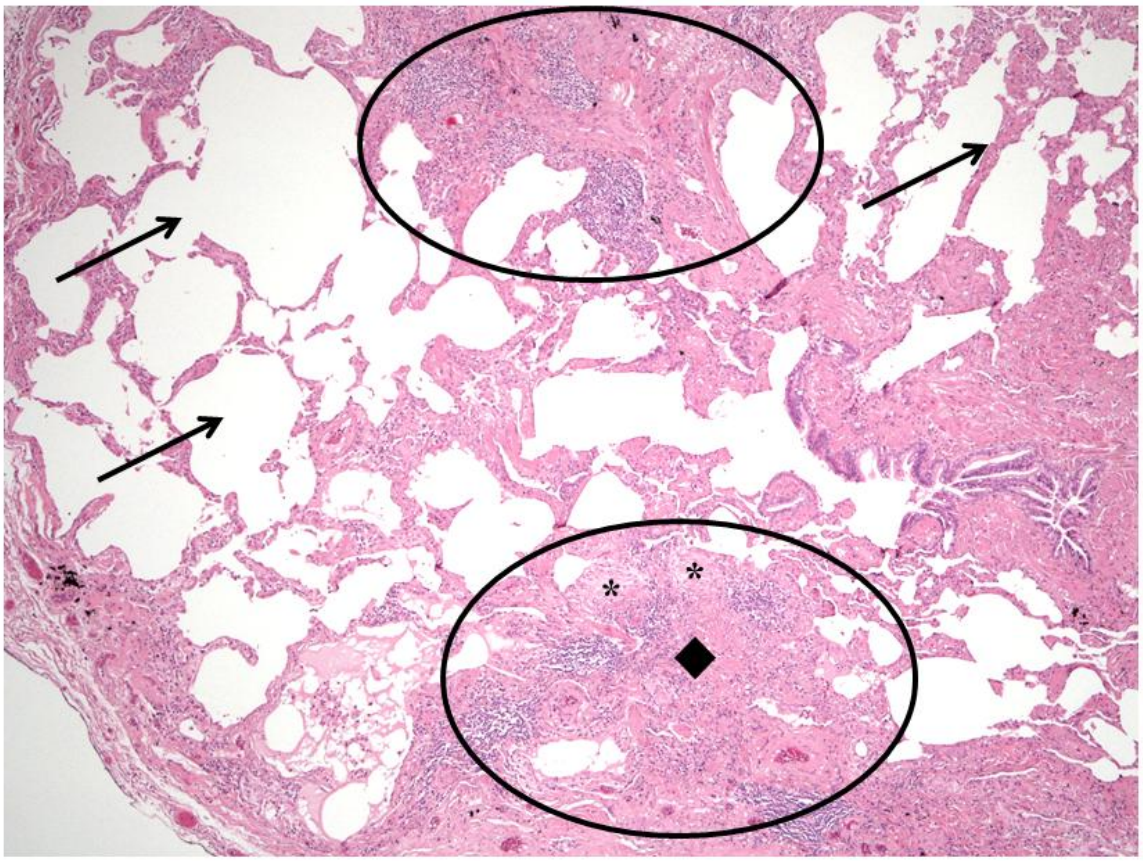

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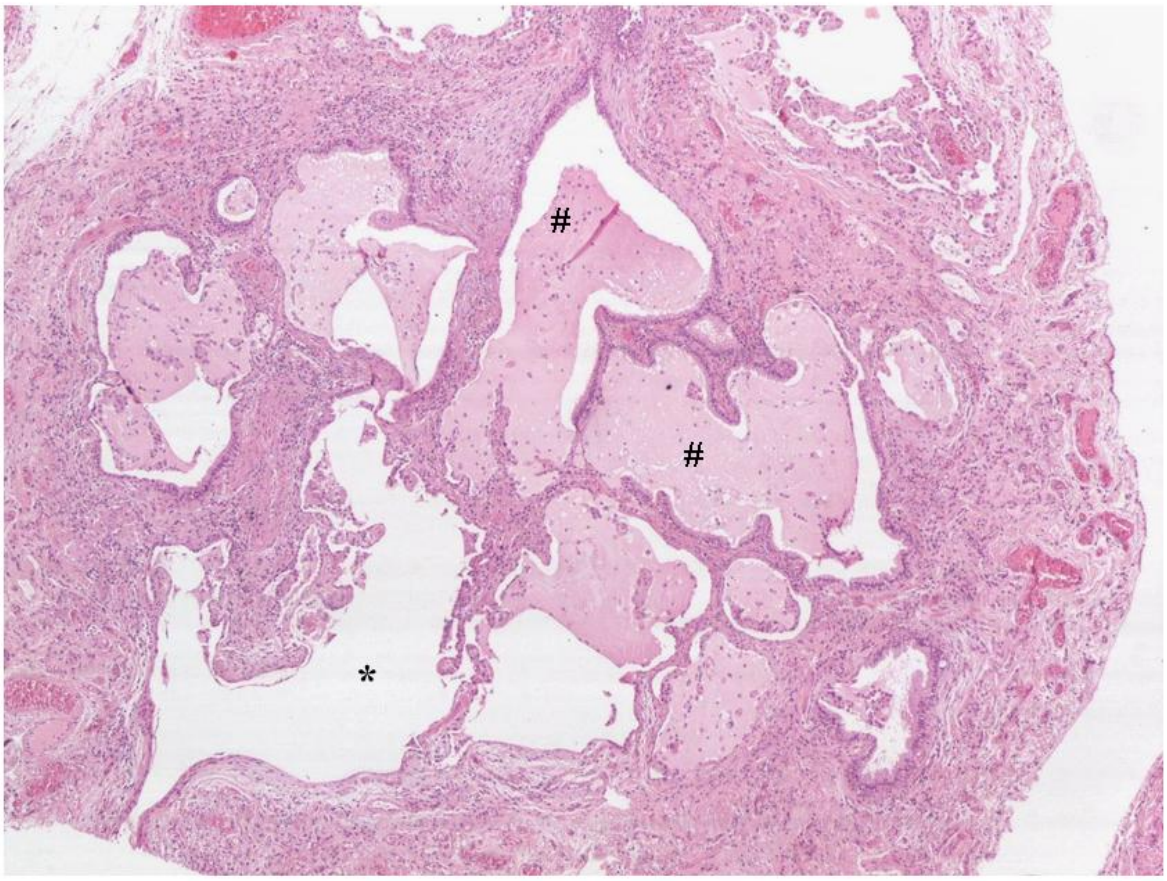
Fig. 3



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