Idiopathic Pulmonary Fibrosis and Antifibrotic Treatments

Focus on Experimental Studies

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- **Context.**—Idiopathic pulmonary fibrosis (IPF) is a progressive fatal disease that up to now has been associated with a poor outcome. Some advances have been made in understanding the multiple interrelated pathogenic pathways underlying IPF. The disease is now believed to result from complex interactions among genetic, epigenetic, transcriptional, posttranscriptional, metabolic, and environmental factors. The discovery and validation of theranostic biomarkers are necessary to enable a more precise and earlier diagnosis of IPF and to improve the prediction of future disease behavior. Two drugs recently approved by the US Food and Drug Administration, pirfenidone and nintedanib, have shown the ability to reduce the progression of the disease, although survival benefits are only minimal and neither drug prevents or reverses the disease.

**Objective.**—To provide a critical overview of the main experimental studies carried out for testing the principal effects of pirfenidone and nintedanib on IPF.

**Data Sources.**—Experimental (animal and in vitro) studies concerning both drugs were used.

**Conclusions.**—Pirfenidone has a longer history of preclinical experimental studies than nintedanib. Many studies have been reported more recently (after 2014) and some of them evaluated the association of both drugs, thus suggesting their combination in future therapeutic approaches. Future investigations focusing on targets at molecular, cellular, and tissue levels are necessary to have a better in-depth knowledge of the properties of these drugs and to explore the potential efficacy of both or other drug combinations.


Idiopathic pulmonary fibrosis (IPF) is one of the most common idiopathic interstitial lung diseases characterized by progressive lung scarring and a very poor overall prognosis with a median survival of 2 to 3 years. The disease occurs primarily in older adults (age 55 years or older) who present with generic symptoms such as cough, dyspnea, or fatigue frequently causing insensitive detection and late diagnosis. The diagnosis is based on clinical, radiologic, and histopathologic evaluation. Within the past 15 years, the scientific community has made significant progress toward standardized diagnostic and prognostic algorithms for IPF that led to the generation of the 2011 American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Latin American Thoracic Association guidelines, and, more recently, updated diagnostic criteria by the Fleischner Society. Usual interstitial pneumonia (UIP) is the histologic pattern of the disease and is characterized by a temporally and geographically heterogeneous mixture of fibrosis, scarring, and honeycombing along with less affected parenchyma as a consequence of repeated injuries to various sites of the lung tissue, each occurring at a different stage of development. UIP can also be seen in other clinicopathologic settings such as connective tissue diseases (CTD), hypersensitivity pneumonitis (HP), and pneumocidiosis. However, the presence of other histologic lesions such as follicular aggregates, plasma cell/lymphocyte ratio, and bridging fibrosis are now reported as important details to take into consideration for a more confident pathologic diagnosis of non–IPF-UIP, particularly for UIP pattern in CTD and chronic HP. Despite extensive research efforts, the etiopathogenesis and pathophysiology of IPF remain poorly understood and consequently only slight improvement has been made for appropriate management and effective therapies.

Initially, IPF was considered an inflammatory disease, therefore treatment with corticosteroids, azathioprine, and cyclophosphamide were extensively used in clinics. On the contrary, recent clinical trials and evidence-based guidelines suggest that anti-inflammatory agents are not helpful in the treatment of IPF but increase mortality. In this context, based on a deeper knowledge of the pathogenetic mechanisms of IPF, other therapeutic approaches are ongoing, targeting different pathways such as alveolar epithelial cell damage and activation (eg, fresolimus-mab), telomere attrition (eg, nandrolone), cellular senescence (eg, desatinib), epigenetic drift (eg, 5′-azacytidine), mesen-
chymal stem cell (MSC) exhaustion (eg, placenta-derived or bone marrow–derived MSC), loss of proteostasis (eg, everolimus), and mitochondrial dysfunction (eg, MitoQ). Two recently approved and currently in use antifibrotic treatments, pirfenidone and nintedanib, have been shown to be beneficial by slowing disease progression.

Pirfenidone, an oral pyridine derivative bioavailable synthetic compound was approved for the treatment of mild to moderate IPF in 2011 in the European Union (EU) and in 2014 in the United States. To date, randomized placebo–controlled phase III studies have demonstrated that pirfenidone significantly slows disease progression.10–13

Nintedanib is a multiple tyrosine kinase inhibitor that was approved for therapeutic use both in the EU and in the United States in 2014. Nintedanib was studied in a phase II (TOMORROW) trial for IPF and encouraging results from this trial formed the basis for larger phase III (INPULSIS) trials.14,15 These drugs have shown therapeutic benefits overall in early IPF stages, thus early diagnosis and treatment is crucial for reducing functional decline, slowing disease progression, and improving quality of life. Although their effectiveness and safety in clinical management have been tested, there are currently some open questions, mainly addressing the optimal time of treatment and a more precise knowledge of their therapeutic/adverse effects. To date, the exact mechanism and pharmacodynamics of both drugs remain not well defined and there is a greatly felt need to better understand the principal pathways, in order to identify predictive markers of responsiveness, resistance, toxicity, and potential increased beneficial effects in both or other drug combinations.

Some in vivo and in vitro studies have demonstrated anti-inflammatory, antioxidant, and antifibrotic effects of pirfenidone and a principal inhibitory action of nintedanib on vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) receptors.16

This review article is a critical overview of the main experimental studies carried out to clarify the mechanisms of action of the 2 drugs. The principal molecular pathways investigated in the experimental studies, including animal and in vitro models, will be reported for both drugs in 2 sections. The 2 sections, named “Pirfenidone and Nintedanib: Preclinical Research Studies” and “Pirfenidone and Nintedanib: Postclinical Research Studies,” figuratively represent the 2 eras, before and after the clinical use of the 2 drugs (Figure 1; Table).

PIRFENIDONE AND NINTEDANIB: PRECLINICAL RESEARCH STUDIES

Preclinical studies were crucial to understanding the effect of these drugs on inflammation and overall fibrosis. Fibrotic remodeling represents the principal aspect in IPF development and progression.

A limited number of experimental studies were carried out in the preclinical era. They focused on drug efficacy and toxicity, although with some flaws, mainly related to the choice of inappropriate animal models and limited careful lung tissue evaluation (both clinical and experimental).

Pirfenidone

The first in vivo evidence of drug efficacy was demonstrated in several experimental studies by Iyer and colleagues17–20 by using bleomycin-induced lung fibrosis in a hamster model fed with a diet containing pirfenidone. The authors showed a dose-dependent protective role of pirfenidone in reducing bleomycin-induced lung toxicity and retarding lung fibrosis. A down-regulation of lung procollagen gene expression (procollagen I and III) related both to attenuation of the inflammatory events and to transforming growth factor (TGF)–β transcriptional suppression was partially evident in hamsters treated with the pirfenidone diet.

The antifibrotic role of pirfenidone was also studied in a murine experimental model of cyclophosphamide–induced lung fibrosis.21 In particular, a lower total lung hydroxyproline content was demonstrated after treatment, even if no difference was found in the histologic lung fibrosis scores.21

The protective effect of pirfenidone, using intratracheal instillation of amiodarone, showed a significant prevention in septal thickening, fibrosis, and amiodarone-induced TGF-β expression in hamster lungs. On the contrary, bronchoalveolar lavage (BAL) fluid analysis showed that pirfenidone had no effect on inflammatory parameters induced by amiodarone treatment (lactate dehydrogenase, protein content and activity, number of inflammatory cells), except for the influx of eosinophils.22

The first study of the antifibrotic efficacy of pirfenidone, using an experimental mouse model of bleomycin-induced lung fibrosis, was reported in 2004.23 The authors demonstrated that the treatment reduced collagen deposition (also evaluated by lung hydroxyproline content) and the number of macrophages, myofibroblasts, heat shock protein-47 (HSP-47)–positive type II pneumocytes, and interstitial spindle–shaped cells in the lung tissue.23 The role of HSP-47 in the pathogenesis of IPF and the inhibitory effect of pirfenidone on HSP-47 expression were confirmed by the same research group in subsequent studies conducted on normal human lung fibroblasts and in the human type II alveolar epithelial cell line A549 stimulated with TGF-β.24,25

The authors concluded that both studies highlight the importance of an antifibrotic effect of the drug by their direct action on HSP-47 expression on fibroblasts with a subsequent reduction of collagen synthesis.

Liu et al26 confirmed a significant antifibrotic, but no immunosuppressive, effect of pirfenidone in rat lung transplants and isolated fibroblast cell lines focusing on the arginine-arginase pathway, a key mechanism in collagen synthesis. In particular, pirfenidone treatment was shown to down-regulate arginase expression and activity, as well as TGF-β levels, leading to an inhibition of collagen deposition and improvement of pulmonary function.

An additional study using a murine experimental model of bleomycin-induced pulmonary fibrosis investigated comparatively pirfenidone and prednisolone activity both for inflammatory background and fibrotic aspects.27 The study revealed that both drugs had anti-inflammatory effects by attenuating the increase in interleukin (IL)–1β, IL-6, IL-12p40, and monocyte chemoattractant protein expression induced by bleomycin. However, only pirfenidone treatment was related to decreased levels of profibrotic factors such as FGF, TGF-β1, and the stroma cell–derived factors 1α and IL-18. The authors underlined for the first time the importance of further studies focusing on the identification of different forms (active and latent) of TGF-β and on a better understanding of the role of FGF in the pathogenesis of fibrosis.27 In 2012 the antifibrotic activity of pirfenidone was tested for the first time in paraquat-induced lung fibrosis by...
using a rat experimental model. A semiquantitative determination of lung fibrosis on the lung sections showed that the treatment significantly decreased pulmonary fibrosis extension.\textsuperscript{28} Finally, pirfenidone was also tested in combination with edaravone and erythropoietin to evaluate both the expansion of the fibrosis and the degree of inflammation in rabbits with bleomycin-induced lung fibrosis. The administration of pirfenidone alone slowed the progression of bleomycin-induced lung injury, but the triple-drug combination was reported to be more effective in reducing the expansion of the fibrosis.\textsuperscript{29}

Nintedanib

In the preclinical era the antifibrotic effect of nintedanib on lung fibrosis was investigated in only 1 study that used both in vitro and in vivo models.\textsuperscript{30} Nintedanib oral administration in bleomycin-treated rats was demonstrated to reduce fibrosis on isolated lung tissue by influencing collagen deposition and inhibiting the expression of

![Figure 1. Timeline of experimental studies in preclinical and postclinical eras. Insets reporting research studies are marked in yellow for nintedanib, in blue for pirfenidone, and in green for combination/comparison of pirfenidone and nintedanib.](image-url)
profibrotic genes. Furthermore, in bronchial fibroblast cultures from patients with fibrotic lung diseases the activity of nintedanib was compared to 2 other antifibrotic compounds: imatinib mesylate (combined c-abl/c-kit/PDGF receptor kinase inhibitor) and SB-431542 (TGFB-β receptor I kinase inhibitor). Nintedanib administration resulted in a reduction of collagen deposition, inhibition of profibrotic gene expression, and TGFB-β-mediated differentiation of fibroblasts into myofibroblasts in a dose-dependent manner. This last activity was shared by SB-431542, whereas imatinib mesylate was inactive.30 In this study the compound used was “BIBF 1000,” a prototypical kinase inhibitor. Subsequently the compound was replaced by “BIBF 1200,” as this supposedly had a more sustained inhibition on vascular endothelial growth factor receptor (VEGFR) or an additional inhibitory effect on Src-type kinases.31

PIRFENIDONE AND NINTEDANIB: POSTCLINICAL RESEARCH STUDIES

The number of experimental studies, both in vitro and in vivo, has increased since the acceptance of the drugs for clinical use. Several studies have more consistently used the murine bleomycin-induced lung fibrosis model and overall were designed to address the molecular basis of the disease with specific focus on target genes.

Pirfenidone

Conte et al32 investigated the effects of pirfenidone on fibroblast proliferation, myofibroblastic differentiation, and fibrogenic activity by using human lung fibroblasts (derived from histologically normal areas). The research study demonstrated that the treatment reduced the proliferation of fibroblasts and the expression of TGFB-β-induced smooth muscle actin (SMA) and procollagen-I, both at mRNA and protein levels, thus influencing all of the cited processes. Interestingly, pirfenidone also inhibited TGFB-β-induced phosphorylation of Smad3, p38, and Akt, key factors in the long-term effects of TGFB-β. Moreover, the authors demonstrated that pirfenidone did not have any significant cytotoxic effect on human fibroblasts by measuring the lactate dehydrogenase release.32 The cytotoxic in vitro effect of pirfenidone was evaluated more in depth in the same year by Walter et al.33 The authors found a dose-dependent role of the drug in increasing the apoptosis rates and in reducing cell viability to a minimum.

The crucial role of the antifibrotic effects of pirfenidone was also investigated in a murine bleomycin-induced pulmonary fibrosis model focusing for the first time on fibrocyte accumulation related to CC-chemokine ligand (CCL)-2 production.34 Pirfenidone significantly ameliorated bleomycin-induced lung fibrosis (assessed by quantitative histology and collagen measurement) by reducing the increase in the number of fibrocytes in the lung tissue. Moreover, an in vitro study on murine fibrocytes isolated from the lungs demonstrated that the treatment inhibited fibrocyte migration toward CCL-2 expression through the attenuation of CCR2 mRNA transcription in fibrocytes.34 An important research study by Bauer et al35 started from a systematic genomic comparison between bleomycin-treated rats and patients with IPF and resulted in the identification of a common set of 12 disease-relevant translational gene markers. Interestingly, this panel was able to separate almost all patients with IPF from control subjects in 3 independent cohorts and stratified IPF patients according to disease severity. Based on molecular data, the authors highlighted the optimal use of the bleomycin model and suggested this gene set in IPF clinical practice for diagnostic and prognostic purposes.35

Subsequently, several studies focused on the potential antioxidative effects of pirfenidone. Neri et al36 published a study focusing on the role of pirfenidone on p38 phosphorylation. In particular, the authors demonstrated that pirfenidone inhibited the p38-mediated generation of procoagulant microparticles by using A549 lung epithelial cell lines after oxidative stress induction. The authors concluded their work by highlighting this unrecognized useful action of the compound for exploring different target pathways for potentially more effective combination therapies. One year later, the effect of pirfenidone on oxidative-related mechanisms was also investigated by Liu et al,37 focusing on stress-related factors Nrf2/Bach1 and their downstream antioxidant factors heme oxygenase 1 and glutathione peroxidase 1. The authors found that pirfenidone treatment inhibited Bach1 and several related factors’ gene expressions both in vitro (lung fibroblasts induced by TGF-β) and in vivo (mouse lung tissue after bleomycin injury). In the murine lung tissue a decrease in inflammatory infiltration and pulmonary fibrosis was seen after pirfenidone administration. The study provided a new insight into the essential role of oxidative stress on IPF and therefore into a novel approach for therapeutic strategies.37

The antifibrotic property of pirfenidone, prednisone, and acetylsalicylate was compared in a rat bleomycin-induced lung fibrosis model by Yu et al.39 All the treatments were shown to be more effective and associated with lower inflammation, expansion of fibrosis, and expression of TGF-β, TNF-α, and PDGF than the placebo group. A significant difference in effectiveness, mainly on fibrosis, was instead shown between pirfenidone and acetylsalicylate. On the contrary, the authors found a significant increase of Caveolin-1, a protein involved in fibroblast extracellular matrix (ECM) deposition.39 The pirfenidone action on a profibrotic hedgehog (Hh) signaling pathway was carefully studied in both in vitro (lung fibroblast cultures) and in clinical samples (tissue samples from lung transplant) by Didiasova et al.40 The Hh pathway may be activated via a canonical or noncanonical mode of action: in canonical Hh signaling, binding of the Hh ligands to their receptor leads to the derepression of smoothened (SMO), resulting in the activation of glioma-associated oncogene homolog (GLI)1, GLI2, and GLI3 transcription factors; on the other side, in noncanonical Hh signaling, GLI activity is regulated independently of SMO by other signaling pathways, including TGF-β. The authors showed that pirfenidone treatment interfered with the Hh pathway at different levels, such as a down-regulated expression of the Hh pathway components and Hh target genes, including protein GLI2. The authors concluded that pirfenidone interfered with the activity of the GLI2 transcription factor, impairing not only
the Hh pathway but also other signaling systems (eg, TGF-β) regulated by this protein.40

Kurita et al41 investigated for the first time the antifibrotic effect of pirfenidone in autophagy/mitophagy activation in lung fibroblasts, focusing on myofibroblast differentiation during insufficient mitophagy. This mechanism is considered crucial in IPF pathogenesis, enhancing apoptosis and cellular senescence of epithelial cells and fibroblast-myofibroblast differentiation. The study was carried out on PARK2 knockout mice with bleomycin-induced lung fibrosis. PARK2 is indeed one of the best characterized pathways in mitophagy activation. The authors demonstrated that pirfenidone increased PARK2-mediated mitophagy and attenuated lung fibrosis and oxidative modifications in a bleomycin PARK2 knockout model. The antioxidative action of pirfenidone was highlighted in the study as an interesting aspect to investigate more in depth, overall to optimize the efficacy of the drug.41

As already investigated in the “pretrial era,” some authors focused on the role of pirfenidone in mouse paraquat-induced lung fibrosis. In a first experimental study by Pourgholamhossein et al,42 pirfenidone was shown to significantly decrease lung fibrosis, edema, inflammatory cell infiltration, TGF-β1 concentration, and the amount of hydroxyproline in the lung tissue. The authors demonstrated these effects through inhibition of inflammation and oxidative stress and down-regulation of genes encoding for profibrotic cytokines and enzymatic systems for reactive oxygen species (ROS) production.42 More recently, the same research group used a combination therapy of pirfenidone-prednisolone versus only pirfenidone, concluding that the combined therapy could represent a more powerful therapeutic approach in reducing paraquat-induced lung fibrosis.43

Nintedanib

The first study that investigated the antifibrotic and anti-inflammatory activity of nintedanib used both in vitro (lung fibroblast cell lines isolated from patients with lung fibrosis) and in vivo models (bleomycin- and silica-induced lung fibrosis in mice).44 The treatment showed a significant effect on fibrosis (reduced lung collagen content) and inflammation, detected in BAL and in tissue.

In the same year, Hostettler et al45 also performed a study focusing on the antifibrotic effects of nintedanib in fibroblasts derived from lung biopsies of 4 patients with IPF and 4 patients without IPF. The IPF fibroblasts showed higher expression of platelet-derived growth factor receptor (PDGFR) and fibroblast growth factor receptor (FGFR) than controls, and nintedanib inhibited the proliferation of fibroblasts induced by PDGF, FGF, and VEGF. Moreover, this treatment showed an antifibrotic action also enhancing the expression of prrometalloproteinase-2, inhibiting the expression of TIMP-2, and reducing TGF-β-mediated collagen secretion.45

Surfactant protein (SP)-D and SP-A are secreted into the distal airways and pulmonary alveoli by Clara cells and type II pneumocytes, and their expression by these cells increases following many forms of pulmonary injury including IPF. SP-D has effects on different pathologic conditions including in vivo apoptosis reduction. In 2015, Kamio et al46 aimed to investigate the activity of nintedanib on SP-D expression by using A549 human lung epithelial cells. The authors found an upregulation of SP-D mRNA expression after nintedanib administration even if without impact on cell proliferation. The drug action was not influenced by the blocking of FGFR, PDGFR, and VEGF receptor, thus demonstrating that the 2 mechanisms are uncoupled.46

Ackermann et al47 focused on the vascular effect of nintedanib, using a bleomycin-induced lung fibrosis model. The authors demonstrated that nintedanib significantly influenced the reduction of lung fibrosis and vascular proliferation with normalization of the distorted microvascular architecture and an improvement in lung function.47

The mechanism of autophagy was also tested with this drug. Rangarajan et al48 reported for the first time that nintedanib induces noncanonical autophagy, using fibroblasts isolated from lungs of IPF patients. Nintedanib was demonstrated to down-regulate ECM protein expression and to inhibit TGF-β-induced myofibroblast differentiation. This action was demonstrated to be an uncoupled function independent from the antifibrotic effect of the drug.48

As for pirfenidone, the inhibitory effect of nintedanib treatment on fibrocyte accumulation was also investigated. Nintedanib was shown to inhibit migration and differentiation of human fibrocytes induced by growth factors in vitro, as well as to reduce the number of fibrocytes in a murine bleomycin-induced lung fibrosis model.49

To date, nintedanib has been reported to be a multi-targeted tyrosine kinase inhibitor that directly blocks the Src pathway, a crucial process in mediating TGF-β-induced epithelial-mesenchymal transition (EMT). Very recently, bleomycin and/or mechanical ventilation (MV) has been used to induce fibrosis in a murine experimental model of fibroproliferative response in clinical acute respiratory distress syndrome (wild type and Src knockout). The authors found that EMT increased with MV and nintedanib reduced MV-related EMT and pulmonary fibrosis through inhibition of the Src signalling pathway and TGF-β production.50

Pirfenidone and Nintedanib

Antifibrotic activity of the 2 drugs was compared by studying αvβ6 integrin-mediated TGF-β activity in embryonic mouse fibroblasts. The authors observed that both drugs were moderate inhibitors of TGF-β–induced gene expression but they were not involved in αvβ6-mediated TGF-β activity, thus the hypothetic use of these treatments in combination with αvβ6-neutralizing agents could be beneficial for patients with IPF.51

A recent work by a German group52 assessed the expression and function of the FK506-binding protein 10 (FKBP10) as a new potential drug target for the treatment of IPF, since it is involved in processing complex molecules such as collagen. FKBP10 expression was upregulated in a bleomycin-induced fibrosis lung model and lung samples from patients with IPF. The knockdown FKBP10 form obtained by the RNA interference method attenuated the synthesis of collagen I, collagen V, and α-SMA and collagen secretion. This knockdown FKBP10 form showed a similar efficiency of nintedanib in inhibiting collagen secretion, whereas pirfenidone had no effect on this process. The authors, given the important influence of this protein on collagen secretion and remodeling, strongly suggested that in the future a specific anti-FKBP10 treatment should be planned for association with pirfenidone treatment.52

Lehtonen et al53 for the first time examined the effects of pirfenidone and nintedanib on fibroblast and myofibroblast structure and function by using only primary stromal cell lines obtained from 7 patients with IPF (BAL or lung biopsy...
specimens) and 4 controls (“normal” tissue outside the lung tumor). Both pirfenidone and nintedanib were shown to reduce in vitro proliferation of fibroblastic cells in a dose-dependent manner and when both drugs were used in combination, further reduction of proliferation was observed. Both pirfenidone and nintedanib were able to reduce the amount of α-SMA expression and the myofibroblastic appearance although the level of reduction was cell-line dependent.53

In an in vitro study, Knüppel and colleagues54 very recently investigated a novel antifibrotic mechanism. The aim of the study was to comprehensively assess the effects of pirfenidone and nintedanib on the different steps of collagen synthesis and maturation in primary human lung fibroblasts from 7 patients with IPF and 3 healthy donors. For both drugs, electron microscopy of IPF fibroblast cultures revealed fewer and thinner collagen fibrils than in untreated controls. The authors concluded that both drugs influenced important regulatory levels in collagen synthesis and processing, but nintedanib was more effective in down-regulating profibrotic gene expression and collagen secretion.54

**CONCLUSIONS**

The year 2014 marked a new era in the history of care for the patient with IPF with the approval of nintedanib and pirfenidone by the US Food and Drug Administration. However, several questions remain unclear regarding their clinical use, such as the optimal timing, the optimal patient with IPF, and the optimal administration (alone or in combination). Only little is known about the precise molecular targets and their pharmacodynamics. Several research studies about these issues continue to develop, even after their approved clinical use.

In molecular drug target investigations, biomarker discovery and validation represent a crucial part, and researchers should always take into consideration several aspects; the most important one concerns the type of disease. Clinicopathologic heterogeneous fibrotic diseases, such as IPF, represent a more difficult field that should
require a multistep biomarker discovery/validation road map (Figure 2). As reported by Marshall et al. in an interesting overview on strategy for biomarker discovery in fibrotic disease, "markers that are more proximal to disease mechanisms (i.e. derived from the key disease cells/ proteins) are likely to be more robust, hence access to tissues is important in identifying and validating new markers." As occurs in the oncologic field, the application of more specific tissue analysis using high-throughput genomic technologies and subsequent computational analysis might be a more sensitive and specific approach. Newly discovered biomarkers in lung tissue can subsequently be assessed in other, more accessible compartments as provided by less invasive tools (blood, BAL, sputum). Potentially usable clinical biomarkers of drug efficacy require preclinical studies or basic research to test their efficacy and toxicity (Figure 2).

From a careful review of the literature using the keywords “pirfenidone,” “nintedanib,” and “lung” in PubMed (restricted to research only in the English language and experimental studies), we can draw out some important relevant points: (1) pirfenidone has had a longer history of preclinical investigations, whereas the period of experimental studies before clinical use of nintedanib has been markedly shorter; (2) several experimental studies, some with both drugs in comparison and/or in association with each other or with new drugs, have been more recently reported, after 2014, suggesting their combination in future therapeutic approaches; (3) preclinical investigations of both drugs indicate a similar antifibrotic mechanism of action, namely, decreased amounts of histologic fibrosis, decreased tissue levels of collagen and/or hydroxyprolene, and decreased levels of TGF-β and α-SMA; (4) in addition to the experiments on animal models, nintedanib and pirfenidone have been investigated in human lung cell lines, revealing that both drugs are able to reduce fibroblast proliferation; (5) several animal and in vitro studies have demonstrated a significant anti-inflammatory and antioxidant action of pirfenidone.

There are, however, weaknesses in many experimental studies, particularly the oldest ones, such as the frequent use of human cell lines instead of cell lines derived from IPF-affected lungs and the choice of different animal models both in terms of species (hamster, rat, mouse) and inducers of fibrosis (paraquat, silica, bleomycin, cyclophosphamide, amiodarone). Even if all the previously mentioned animal models used for lung fibrosis do not properly mimic the physiologic and histopathologic pattern of UIP, they are reported to be useful in understanding the principal mechanisms of fibrogenesis.

Expert panelists have recently published an American Thoracic Society (ATS) workshop report formalizing recommendations for the correct use of animal models in the preclinical assessment of potential therapies for pulmonary fibrosis. The panelists focused on 3 major themes: choice of animal, practical considerations of fibrosis modeling, and fibrotic endpoints for evolution. It is desirable that future studies include appropriate animal models in the biomarker discovery pipeline as suggested by the ATS report.

Further research studies, including both lung tissue from patients with IPF and appropriate preclinical models, are necessary to have a better in-depth knowledge of the antifibrotic properties of these drugs. We have come a long way in recent years; however, we are still too far from an adequate understanding of the molecular targets of these drugs and we are surely far from personalized medicine.

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