University of Siena – Department of Medical Biotechnologies

Doctorate in Genetics, Oncology and Clinical Medicine (GenOMeC)

XXXIV cycle (2018-2021)

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The impact of antifibrotic therapy in the management of idiopathic pulmonary fibrosis and progressive fibrosing interstitial lung diseases: a real-world comparative study of efficacy between pirfenidone and nintedanib

Scientific disciplinary sector: MED/10 – Respiratory Diseases

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Academic Year 2020/2021

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ABSTRACT

Background: Idiopathic pulmonary fibrosis (IPF) is the most common and lethal among diffuse fibrosing interstitial lung diseases (ILD). Beyond lung transplantion, the therapeutic approach relies on antifibrotic treatment: pirfenidone and nintedanib are the only pharmaceutical drugs approved for IPF, since they have demonstrated to significantly reduce disease progression rate. Still, no solid data has been published to compare these two drugs as well as few studies have investigated their potential efficacy on familial pulmonary fibrosis (FPF) and progressive fibrosing ILD (PF-ILD) in a real-life setting.

Methods: we collected clinical, functional and radiological data from all patients affected with IPF and PF-ILDs that have been treated with pirfenidone and nintedanib at Referral Centre of Siena from 2011 to 2020. The aim of the research was to compare effectiveness of the two drugs in terms of mortality and disease progression in our population.

Results: no significant differences in mortality and progression-free survival were observed between pirfenidone and nintedanib subgroup. Both drugs significantly reduce FVC and DLCO decline rate in respect with pretreatment period. Similar data was observed in the PF-ILD subgroup, while FPF patients showed no significant benefit from antifibrotic treatment in terms of disease progression. Pirfenidone was more effective than nintedanib in preserving FVC in FPF subgroup.

Conclusions: our research study, conducted in a large cohort through a almost decennial time of observation, confirmed the reliable and substantially similar efficacy of pirfenidone and nintedanib in improving life expectancy and progression-free survival of IPF patients. FPF appeared to be less responsive to antifibrotics, but pirfenidone showed a better performance than nintedanib on this field. PF-ILD patients showed a analogue clinical course of IPF subjects in our study: the effectiveness of pirfenidone and nintedanib was reliable and similar, supporting their future use in clinical practice.

ABBREVIATIONS

CA: cancer antigen

CCL: = C-C motif chemokine ligand

CPFE: combined pulmonary fibrosis and emphysema

CTD: connective tissue disease

CXCL = C-X-C motif chemokine

CX3CL: fractalkine

DLCO: diffusion lung capacity for carbon monoxide

FEV1: forced expiratory volume in the 1 second

FGF: fibroblast growth factor

FPF: familial pulmonary fibrosis

FVC: forced vital capacity

HP: hypersensitivity pneumonitis

HRCT: high resolution computed tomography

ICAM-1: intercellular adhesion molecule 1

IFN: interferon

IL: interleukin

ILD: interstitial lung disease

IPF: idiopathic pulmonary fibrosis

KL-6: Krebs von den Lungen-6

LOXL2: lysyl oxidase-like 2

MMP: matrix metalloproteinases

NSIP: non-specific interstitial pneumonia

PDGF: platelet-derived growth factor

PF-ILD: progressive fibrosing interstitial lung disease

RA: rheumatoid arthritis

S100A9: S-100 calcium binding protein

SP: surfactant protein

SSC: systemic sclerosis

TERC: telomerase RNA component

TERT: Telomerase reverse transcriptase

TGF: transforming growth factor

TNF: tumor necrosis factor

TOLLIP: Toll Interacting Protein

UIP: usual interstitial pneumonia

VCAM1: vascular cell adhesion molecule 1

VEGF: vascular endothelial growth factor

YKL-40: chitinase-3-like protein 1.

INTRODUCTION

Interstitial lung diseases (ILD) include more than 200 parenchymal pulmonary disorders, characterized by an involvement of lung interstitial district, whose extension and localization may be very heterogeneous. The majority of ILDs are recognized as rare disease: this aspect makes differential diagnosis and, consequently, prognostic estimation really challenging. Moreover, ILD development may also be associated to non-pulmonary systemic diseases (such as connective tissue diseases (CTD)) or be determined by specific exposure of organic or non-organic molecules. Therefore, although pulmonologists are commonly the referral physician for ILD patients, all these issues make a multidisciplinary approach absolutely necessary for the clinical management, as recently officially endorsed by international guidelines for the diagnosis of idiopathic pulmonary fibrosis (IPF), sarcoidosis and chronic hypersensitivity pneumonitis (HP) (1–3).

However, despite being considered a crucial issue for the clinical management of these patients, early diagnosis of ILD remains nowadays an unresolved issue, since symptomatic onset is commonly insidious, non-specific and slowly progressive. The most common symptoms reported by ILD patients are dry cough and exertional dyspnea: it is estimated a mean diagnostic delay of 2-3 years from symptoms onset, that significantly impacts in life expectancy of these patients (4).

Many ILDs typically show a inexorably progressive course, leading to chronic respiratory failure and death. Notably, IPF, the most common and well-recognized fibrotic ILD, is burdened by a worse prognosis than the majority of malignant diseases (5). However, also other ILDs could present a progressive fibrosing phenotype that may be indistinguishable from IPF, leading to a progressive deterioration in lung function, respiratory symptoms and quality of life (6). These findings, combined with the evidence that steroids and/or immunosuppressants may not be effective or even detrimental in these patients, led the way to the definition of progressive fibrosing ILD (PF-ILD), including many different disease entities that can mimic IPF clinical course and prognosis (Figure 1).

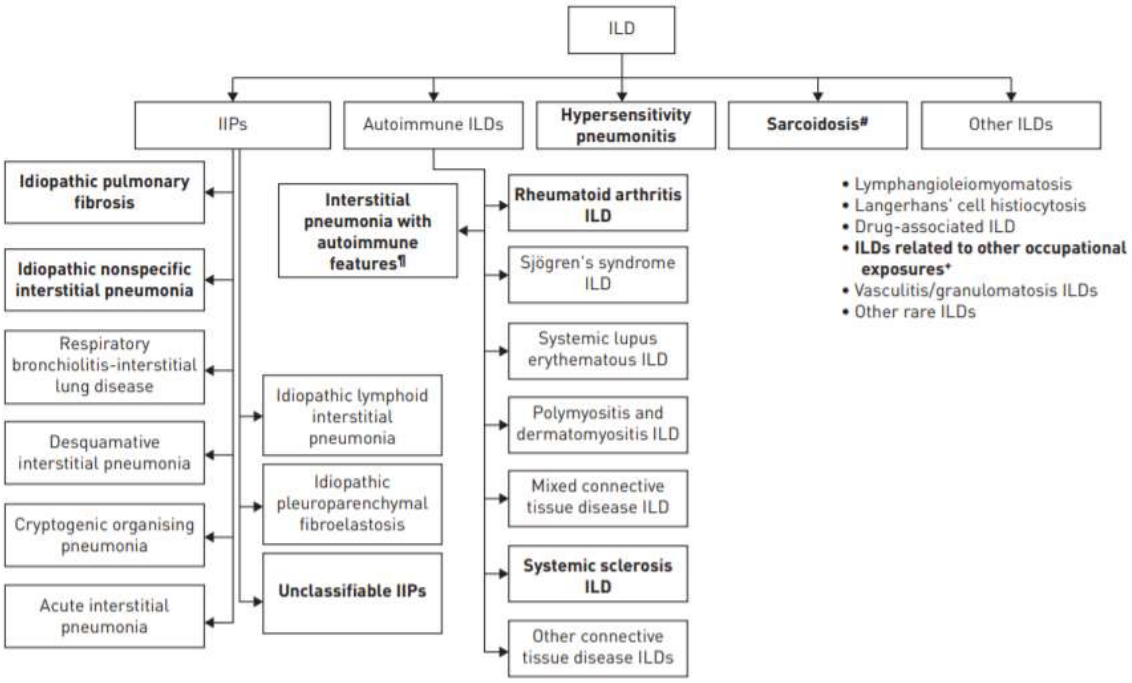

Figure 1. Classification model for ILDs. Potential PF-ILD are reported in bold. From Cottin et al. (6)

IDIOPATHIC PULMONARY FIBROSIS

Definition and epidemiology

IPF is a chronic and irreversible diffuse ILD of unknown origin, characterized by a progressive worsening of respiratory symptoms (exertional dyspnea and typically dry chronic cough), leading to respiratory failure and death in few years, if untreated.

The prevalence of IPF is increasing and appears to be quite homogeneous worldwide. The highest incidence is reported in North America and Europe (3-9 cases per 100,000 person-years), but it is still not clear if this discrepancy is due to a real increase of disease incidence or may be related to a better diagnostic performance and/or adherence to international guidelines (7). Nevertheless, in the last decade the incidence of hospital admissions and death rates related to IPF appeared to be increased as well, suggesting a growing social burden and health impact of disease, maybe due to ageing of Western population (7–9).

From a epidemiological point of view, many factors have been associated to the risk for developing IPF: genetic features (including having close relatives affected by IPF) (10–12), demography (male sex, age > 65 years) (13,14), environmental exposure (especially current or previous smoking (15,16), but also viral colonization or infection (17) and air pollution (18)), and medical comorbidities (diabetes mellitus, obstructive sleep apnea and gastroesophageal reflux disease) (19,20).

Pathogenesis

Thanks to the growing research interest on this field, the comprehension of IPF pathogenesis has been steadily improving in the last decades: starting to be considered a chronic inflammatory disease, subsequently leading to extensive tissue fibrosis (21), IPF is nowadays considered a multifactorial disease, in which genetic, epigenetic, immunological and environmental factors actively contribute to the development and influence the progression of disease (1,22) (Figure 2).

The most widely accepted pathogenetic model of IPF relies on chronic and recurrent micro-injuries (viral infection, organic exposure, mechanic stress) to alveolar epithelium, progressively leading to aberrant ageing and apoptosis processes and subsequent activation and overexpression of pro-fibrotic and pro-coagulant cytokines and chemokines (23). Many biomolecular pathways, including oxidative and endoplasmic reticulum stress, associated to mitochondrial alteration, contribute to the progressive exhaustion of alveolar epithelial cells, causing a impaired alveolar re-epithelialization and a over-secretion of profibrotic mediators (24,25). These processes are also facilitated by genetic susceptibility, that are estimated to contribute for up to a third of all cases: many genetic variants have been linked to a higher risk to develop IPF and, interestingly, they are involved in different biological pathways, underlining the complexity of this disease. Among these, the most common genetic alterations are involved in the production and quality of surfactant (SFTPA2, SFTPC) (26,27), telomere maintenance (TERT and TERC) (28) and regulation of immunological host defence processes (MUC5B and TOLLIP) (12,29,30). In clinical terms, the effective contribution of these genetic variants in the development and progression of IPF is still not fully elucidated, considering

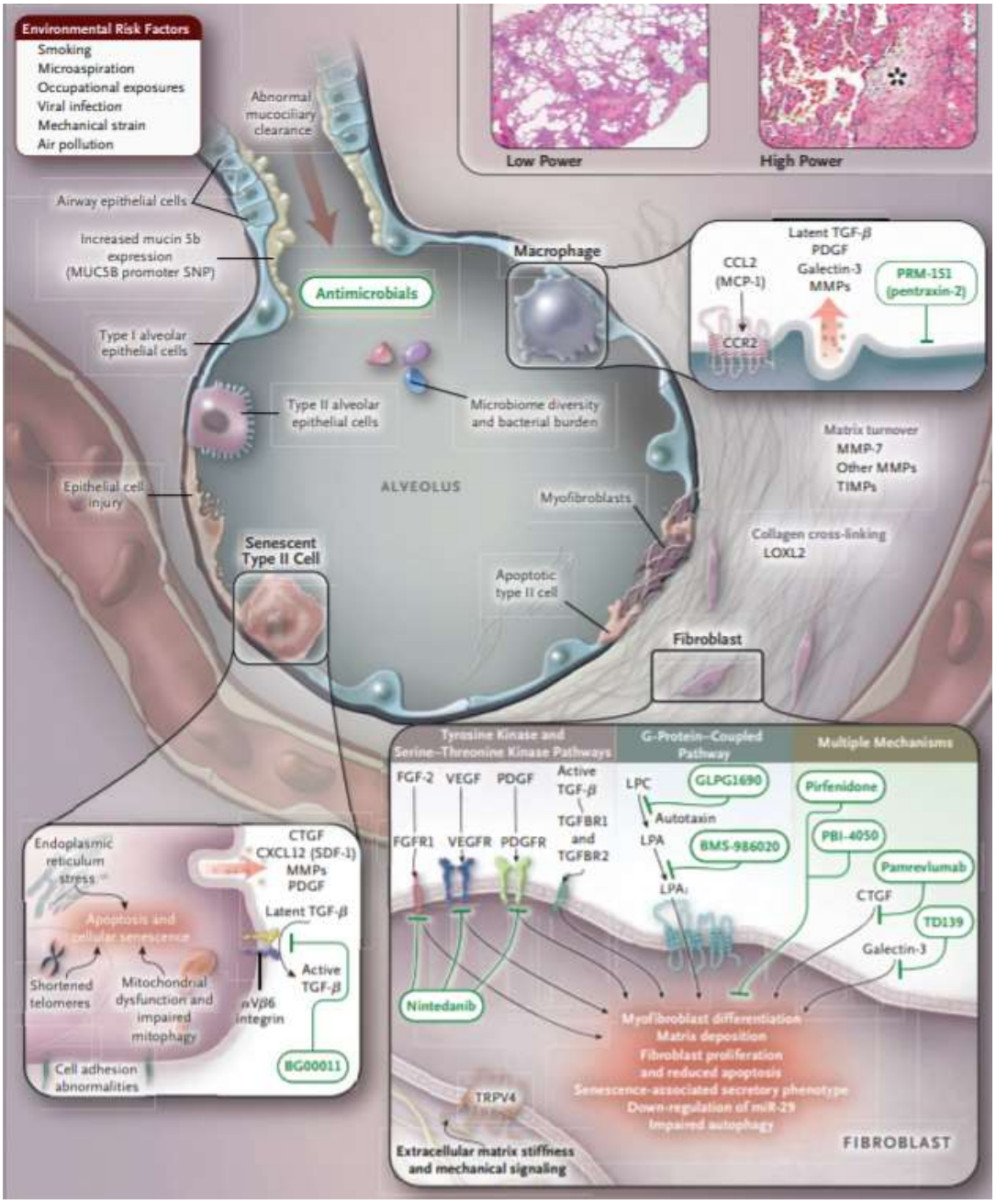

Figure 2. Pathobiological pathways of IPF. Adapted from Lederer and Martinez (31)

also that the most common genetic alteration in IPF patients (MUC5B gain of function) has been also reported to a better survival outcome (12).

The imbalance between profibrotic and antifibrotic factors promotes a dysregulated recruitment of fibroblasts and myofibroblast in the alveolar space, involving not only the resident mesenchymal cells but also blood fibrocytes (32,33). Moreover, epithelial and endothelial-mesenchymal transition

processes have been demonstrated to further foster the myofibroblast proliferation in the airway and their persistent activation (34). These myofibroblasts sustain the maladaptive process repairs of alveolar epithelium injuries through a over-production of extracellular matrix, that induces a progressive architectural distortion leading to the impairment of alveolar-capillary membrane. Moreover, extracellular matrix produced by over-activated myofibroblast is characterized by a altered cellular composition and stiffness, that, triggering a positive vicious circle, further stimulates the activation and secretion activity of myofibroblasts through a dysregulated integrins signaling interaction (35,36). In parallel with these pathways, alveolar epithelial cells are progressively replaced by bronchiolar-like epithelium (the so-called "bronchiolisation of alveolar spaces"): this process is driven by a dysregulated and excessive apoptosis of alveolar pneumocytes, that induces, as repair mechanism, an aberrant activation of respiratory stem cells ("basal cells"), physiologically resident in the bronchioles (37,38). The lung remodeling induced by bronchiolisation contributes as well to the progression of disease, as bronchiolar cells may release pro-fibrotic cytokines and chemokines (such as TGF-β) and also be involved in epithelial-mesenchymal transition, giving a significant contribution to fibrogenesis of lung tissue.

PF-ILD: similarities and differences

A chronic and irreversible disease progression, even if with a substantial heterogeneity, is a hallmark of IPF. The retrospective evaluation of clinical data and respiratory functional parameters may be useful for the diagnosis of IPF, since the invariably progressive course of disease can be considered a defining characteristic of this disease. However, albeit in a smaller percentage of patients than IPF, many other diffuse ILDs have been associated with a clinical behavior and mortality similar to IPF, characterized also by a lack of response to conventional treatment, such as oral steroids and/or immunosuppressant. The evidence of this subset of patients across different ILDs led the way to the definition of "progressive fibrosing-ILD", among which the most common are idiopathic nonspecific

interstitial pneumonia, unclassifiable idiopathic interstitial pneumonia, CTD-ILDs, fibrotic sarcoidosis, fibrotic hypersensitivity pneumonitis (HP) and ILD associated to occupational exposures (e.g. asbestosis) (6,39) (Figure 3).

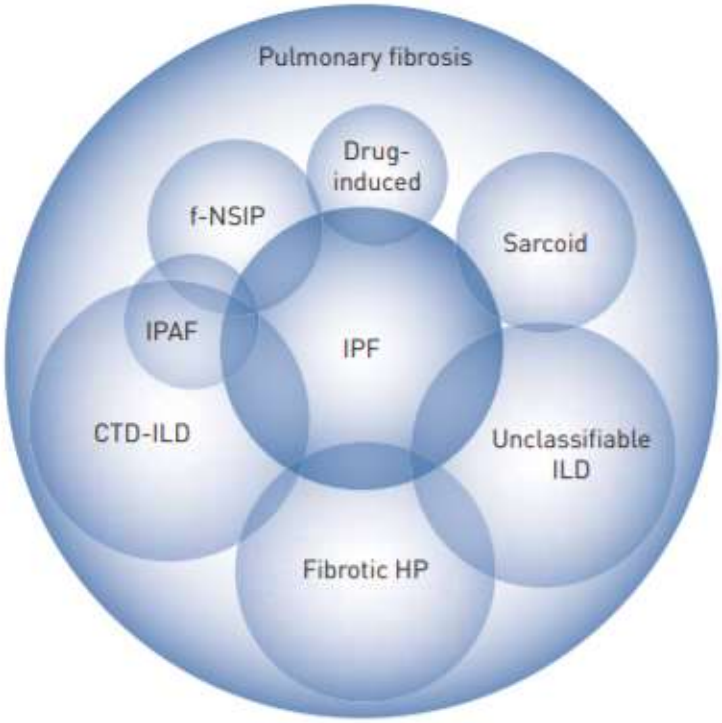

Figure 3. Overlap between IPF and non-IPF ILDs with a progressive clinical course (from Wells et al. (39))

To date, there is still no consensus in the definition of PF-ILD in terms of clinical data: the most recent RCTs investigating the efficacy of antifibrotic treatment on this field defined a progressive disease through a reduction > 10% of FVC in the previous 24 months and/or an increase of fibrotic areas evident on a CT scan and/or a significant worsening of clinical status reported by the patients (40,41). This approach is supported by the evidence that short term disease progression, identified

through the analysis of lung function test trends or serial CT scans (42–45), was a good predictor of worse outcome in many non-IPF diffuse ILDs, including NSIP, fHP and CTD-ILD: in all these studies, a disease progression was invariably associated to a higher mortality rate, supporting the clinical indication to follow-up these patients in order to early detect a progressive phenotype in these populations. Other malignant prognostic determinants for IPF as well for PF-ILD include: a UIP pattern on histologic sampling and/or chest HRCT (46–48), a decline in 6-minute walking test distance (49,50), a clinically significant worsening of patient reported outcome through specific questionnaires (51,52) and serum biomarkers, such as Krebs von den Lungen-6 (KL-6) and surfactant protein A and D (SF-A and SF-D) (53,54).

In terms of biomolecular aspects, many reports described pathogenetic mechanisms common to IPF and other PF-ILDs, that may explain the similar clinical course between the two subgroups. In particular, it may be assumed that, regardless the causing agent of alveolar injury (autoimmune inflammation, inhalation of organic particles or viral infection), the following phases of lung fibrogenesis and dysregulated repair mechanisms might represent the pathologic overexpression of conserved biopathways, involving the aberrant activation of myofibroblasts and secretion of profibrotic cytokines and growth factors. Many papers have explored and demonstrated shared pathogenic mechanisms between IPF and non-IPF progressive ILDs, further supporting the hypothesis that an inexorably progressive phenotype may be the expression of the same biomolecular pathways. Progressive SSC-ILD showed very similar patterns of alveolar cell exhaustion and senescence, immunological alterations and mitochondrial dysfunction if compared to IPF (55,56), while shorter telomere lengths and impaired telomerase activity has been reported also in fHP as well as other idiopathic fibrotic lung diseases (57). Interestingly, patients with lung fibrosis associated to rheumatoid arthritis (RA) showed the same genetic and epigenetic alterations of IPF patients, suggesting a common risk profile between the two diseases in terms of genetic susceptibility (58).

However, although these similarities offer intriguing insights in the pathogenesis of non-IPF ILDs, the trigger mechanisms responsible for disease progression and its perpetuation remain unclear.

Clinical aspects and diagnostic pathway of IPF

From a clinical perspective, diagnosis of IPF is challenging. The most common symptoms associated to IPF is exertional dyspnea, that may be associated or not to chronic dry cough. The onset of symptoms is mainly insidious and is usually ascribed to other medical conditions, such as chronic heart disease, chronic obstructive pulmonary disease, asthma and gastroesophageal reflux disease, physical deconditioning, ageing or environmental exposure (cigarette smoking or atopy) (59). Moreover, IPF is commonly associated to one or more comorbidities that may cause dyspnea or dry cough, further complicating the early recognition of this disease. The physical examination may represent a valid help for the clinical suspicion of interstitial lung disease: bibasal, velcro-like inspiratory crackles are present in the majority of cases at thoracic auscultation, while digital clubbing, despite a low sensitivity and specificity for IPF, may be useful to detect a latent respiratory failure and lead to a prompt in-depth diagnostic evaluation (60).

Since IPF is a rare disease and the sensitivity of medical examination and first-line diagnostic exams (e.g. chest X-ray) is poor, especially in the early stage of disease, it is unsurprising the estimation of a mean diagnostic delay of at least two years associated to this disease. This assumption is not to be underestimated, because an early diagnosis of IPF is crucial to optimize the clinical management of these patients. Indeed, antifibrotic treatment appeared to be more effective in reducing disease progression and to improve life expectancy in patients with more preserved lung function (61,62). Moreover, in patients younger than 65 years old, a early diagnosis of IPF may help respiratory physician to detect a incipient worsening of disease and promptly address the patient to a specific evaluation for lung transplantation.

In 2018, American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines for diagnosis of IPF were published (1). This document was designed to replace the previous ATS/ERS

guidelines of 2011 and, beyond updating the radiological and histopathological criteria for the diagnosis (Figure), it underlined the central role of the multidisciplinary discussion in the diagnostic pathway of IPF.

The diagnostic criteria for IPF were the following:

- Exclusion of known causes of ILD, such as environmental exposure, CTD or other systemic disease (e.g. sarcoidosis) and either:
    - A Usual interstitial pneumonia (UIP) pattern at chest high resolution computed tomography (HRCT)
    - Specific combination of HRCT and histopatological features (Figure) in case of lung tissue sampling.

Recommended Scanning Protocol

1. Noncontrast examination

2. Volumetric acquisition with selection of:
   - Sub-millimetric collimation
   - Shortest rotation time
   - Highest pitch
   - Tube potential and tube current appropriate to patient size:
     - Typically 120 kVp and ⩽240 mAs
     - Lower tube potentials (e.g., 100 kVp) with adjustment of tube current encouraged for thin patients
   - Use of techniques available to avoid unnecessary radiation exposure (e.g., tube current modulation)

3. Reconstruction of thin-section CT images (⩽1.5 mm):
   - Contiguous or overlapping
   - Using a high-spatial-frequency algorithm
   - Iterative reconstruction algorithm if validated on the CT unit (if not, filtered back projection)

4. Number of acquisitions:
   - Supine: inspiratory (volumetric)
   - Supine: expiratory (can be volumetric or sequential)
   - Prone: only inspiratory scans (can be sequential or volumetric); optional (*see text*)
   - Inspiratory scans obtained at full inspiration

5. Recommended radiation dose for the inspiratory volumetric acquisition:
   - 1–3 mSv (i.e., "reduced" dose)
   - Strong recommendation to avoid "ultralow-dose CT" (<1 mSv)

Figure 4. Chest HRCT scanning parameters for diagnosis of IPF. Adapted from Raghu et al. (1)

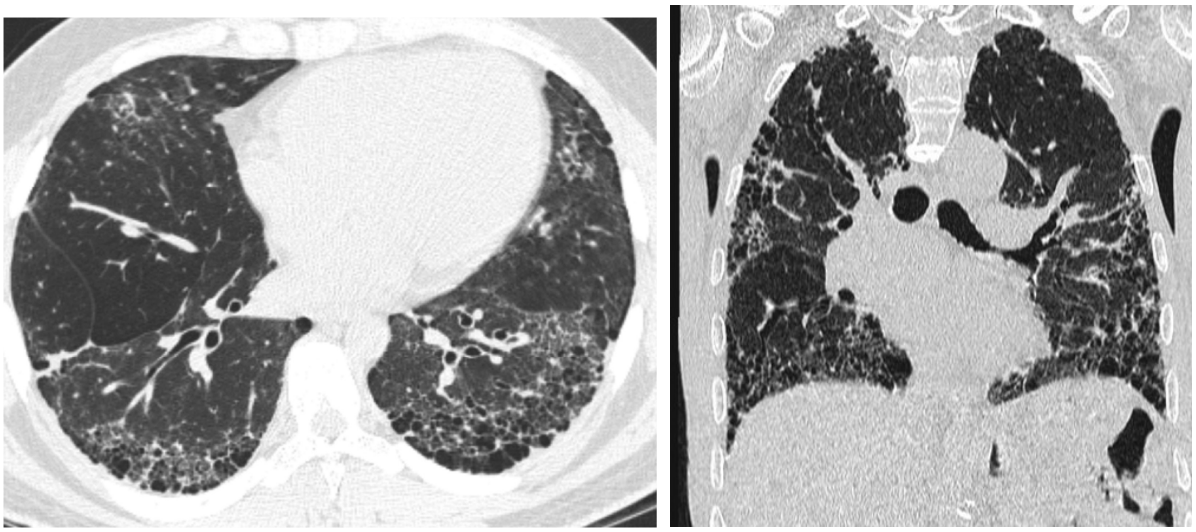

Figure 5. HRCT images of a definite UIP pattern

UIP pattern	Probable UIP	Indeterminate	Alternative diagnosis
- Honeycombing with or without traction bronchiectasis - Predominant subpleural and basal distribution	- Predominant subpleural and basal distribution - Interstitial reticular pattern with or without traction bronchiectasis - Mild ground glass opacities	- Predominant subpleural and basal distribution - Subtle reticulation and/or mild ground glass opacities - CT features or distribution not specific for any disease	- Findings suggestive of alternative diagnosis

Table 1. HRCT patterns of UIP

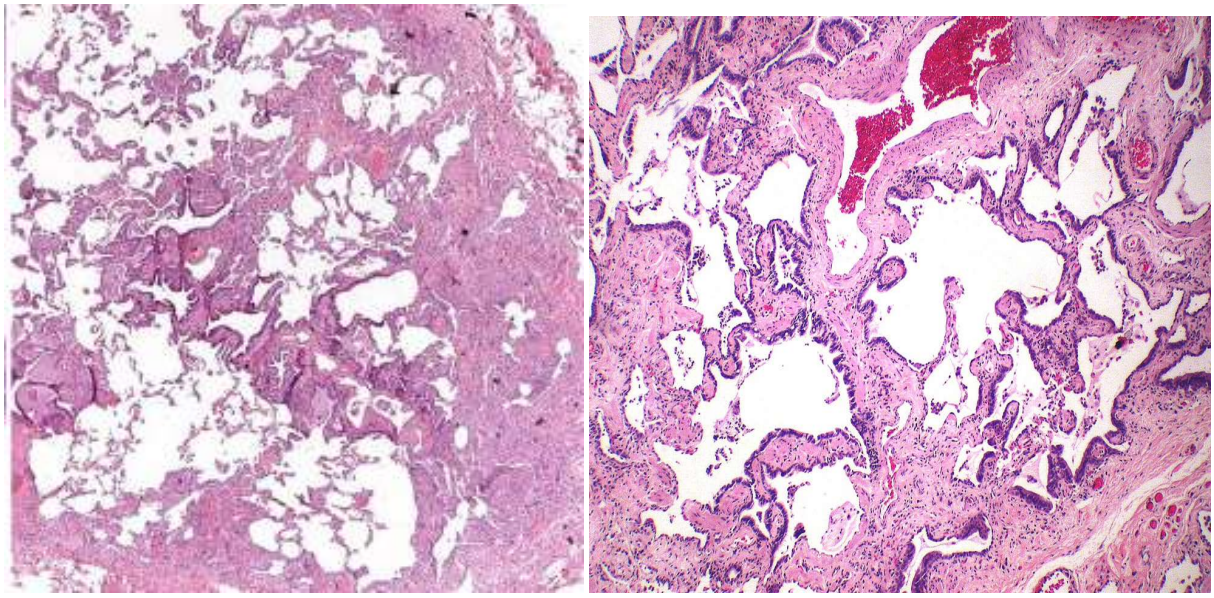

Figure 6. Histopatologic images of lung tissue sampling, showing a UIP pattern.

UIP pattern	Probable UIP	Indeterminate	Alternative diagnosis
- Fibrotic architectural distortion (honeycombing and/ or destructive scarring) - Subpleural and heterogeneous distribution of fibrosis - Evidence of fibroblast foci - Absence of features suggestive for alternative diagnosis	- Similar to UIP but not enough for "definite" UIP (extension, distribution) - Absence of features suggestive for alternative diagnosis - Isolated honeycombing	- Fibrotic architectural distortion, with features suggesting other diagnosis than UIP or systemic disease associated with UIP - Features suggestive for alternative diagnosis	- Features indicative for other diagnosis

Table 2. Histopathology characteristics of different pattern of UIP.

IPF suspected*		Histopathology pattern			
		UIP	Probable UIP	Indeterminate for UIP	Alternative diagnosis
HRCT pattern	UIP	IPF	IPF	IPF	Non-IPF dx
	Probable UIP	IPF	IPF	IPF (Likely)**	Non-IPF dx
	Indeterminate for UIP	IPF	IPF (Likely)**	Indeterminate for IPF***	Non-IPF dx
	Alternative diagnosis	IPF (Likely)** /non-IPF dx	Non-IPF dx	Non-IPF dx	Non-IPF dx

Figure 7. Combination of radiologic and histopathologic pattern for diagnosis of IPF. From Raghu et al (1).

No specific recommendations were stated regarding the composition of the multidisciplinary group, except for a reliable expertise in the management of ILD. Respiratory physicians are required to know and evaluate any potential sign during the medical examination and in the medical history that may be associated or causative of ILD. In particular, occupational and environmental exposure as well as clinical symptoms or signs suggestive for a concomitant CTD need to be evaluated in-depth. In addition, a complete serological testing for serum autoantibodies is strongly recommended in all patients suspected to have IPF: this statement was made as ILD may sometimes anticipate CTD symptoms or even represent the unique localization of disease (63,64). Moreover, many ILD patients might have been treated with oral steroids or immunosuppressants by primary care physicians in the early phase of diseases, inducing a remission of autoimmune clinical features.

Accordingly, the involvement of radiologists specifically experienced in thoracic imaging is essential for a acceptable diagnostic performance of the multidisciplinary discussion: CTDs may be asymptomatic at symptoms onset, while exposure-related ILD (e.g. chronic hypersensitivity pneumonitis) may not be associated to a specific agent in a relevant percentage of cases. Therefore, the detection and identification of specific radiological signs or features that may be associated to

systemic diseases is essential for a correct classification of CT patterns and to define the following diagnostic pathway (Figure 8).

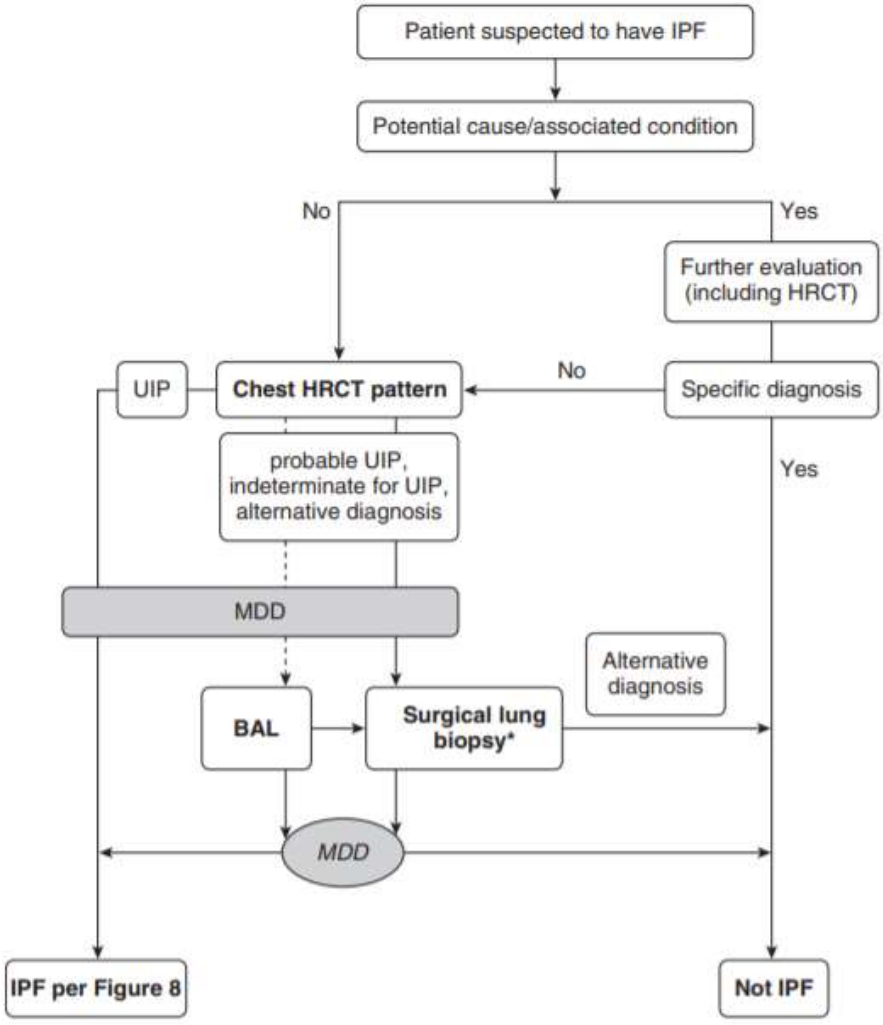

Figure 8. Diagnostic algorithm for IPF. From Raghu et al. (1)

Prognosis

The prognosis of IPF is poor: if not treated, life expectancy at diagnosis is estimated in a range from 3 to 5 years, that is significantly worse of many malignant disease (5). Moreover, clinical course of IPF is unpredictable: the majority of patients experiences a slow and progressive worsening of respiratory symptoms, associated to the consensual impairment of respiratory functional parameters, eventually causing chronic respiratory failure and death. On the other hand, a more aggressive disease progression may be observed in a percentage of patients, characterized by a inexorable and irreversible clinical and respiratory functional deterioration, leading to exitus in few years. Moreover, a minority of patients may experience a sudden and severe worsening of respiratory symptoms that are typically associated to acute respiratory failure and bilateral ground-glass opacities or parenchymal consolidation at CT scan, that are not explained by heart failure, pleural disease or pulmonary embolism. These devastating events are defined as "acute exacerbation of disease" and are associated with a mortality of > 90% within 6 months after discharge.(65) (Figure 9).

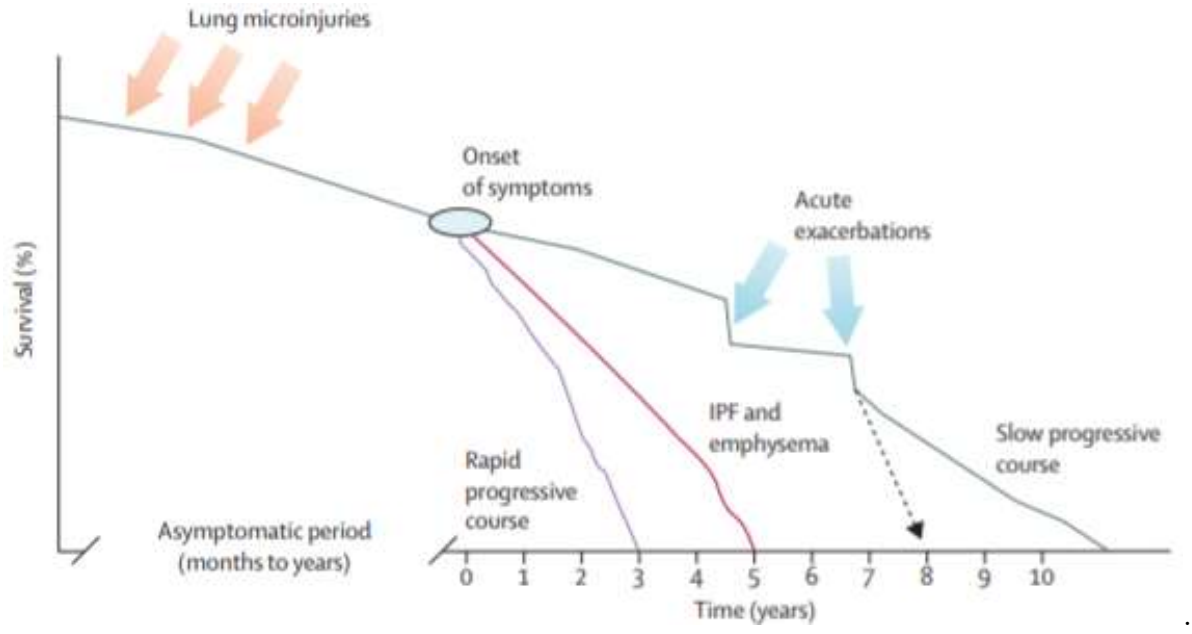

Figure 9. A proposed model to describe different clinical phenotypes of IPF. From King et al. (65)

Acute exacerbations may occur in all diffuse ILDs, but are significantly more common in IPF patients: although they may be triggered by specific events (such as an infection, drug toxicity or aspiration), they remain idiopathic in the majority of cases(66).

So far, no specific therapeutic indication has been proposed for the management of acute exacerbation of IPF, except the treatment of a known cause and respiratory support with high-flows oxygen therapy and/or non-invasive mechanical ventilation (67). In suitable patients, extracorporeal membrane oxygenation may represent an option as a bridge to lung transplantation therapy.

Concerning prognostic estimation, many clinical, demographic, radiological and immunological features have been proposed for the risk stratification of IPF progression and early mortality. Male sex, age > 70 years and smoking status are invariably reported as negative prognostic factor, as well as a more severe impairment of lung volumes or gas exchange (68).

Both respiratory and non-respiratory comorbidities are associated with a significant impairment of life expectancy and quality of life in these patients (69): moreover, IPF represents *per se* a risk factor for lung cancer and pulmonary hypertension. Combined pulmonary fibrosis and emphysema (CPFE) is a well-recognized disease entity, characterized by the evidence of fibrotic areas typical for UIP at lower lobes associated to emphysematous alveolar destruction at upper lobes. CPFE is associated with a more accelerated disease progression and a higher risk of mortality: paradoxically, patients with CPFE may show only mild or normal lung volumes at spirometry, associated to a severe or very severe impairment of pulmonary diffusion capacity (70). Concerning radiological features, a definite UIP pattern at CT scan and the extension or macrocystic phenotype of honeycombing areas are associated with a more aggressive disease progression (47).

Many biomarkers have been proposed to be used in routinary clinical practice for prognostic estimation and evaluation of disease severity and, eventually, response to antifibrotic treatment. Considering the complexity and multidimensionality of IPF pathogenesis, it is not surprising that serum and BAL biomarkers proposed in the literature are quite numerous and may reflect different

biomolecular mechanisms involved in IPF pathobiology. However, to date, due to the heterogeneity of study design and methodological issues, there is still no international consensus for the implementation of any biomarker in the clinical management of ILD patients. Interestingly, many molecules have demonstrated an interesting potential in predicting clinical outcome both in IPF and non-IPF patients, suggesting the existence of shared pathogenic mechanisms (especially related to epithelial dysfunction and ECM remodeling) among progressive fibrosing ILD (Table 3).

Notably, it is widely accepted that no biomarker has demonstrated so far a reliable accuracy in discriminating IPF from non-IPF ILDs: consequently, the last ATS/ERS guidelines recommend against the use of biomarker during the diagnostic pathway of IPF (1).

Disease	*Pathogenic mechanism*	*Biomarkers*
IPF	• Epithelial cell dysfunction	- KL-6 (53,71)
		- TERT; TERC [11,28]
		- CA 19.9; CA 125 (72)
		- YKL-40 (73)
		- SP-A; SP-D (74)
		- MUC5b [12]
		- TOLLIP [30]
	• ECM remodeling	- MMP-7 (74)
		- ICAM-1; VCAM-1 (75)
		- Integrins (35)
		- tenascin C (76)
	• Immune dysregulation	- MUC5b [29]
		- CCL-18 (77)
		- S100A family (78)
		- LOXL2 (79)
PF-ILD	• Epithelial cell dysfunction	- KL-6 (80,81)
		- SP-A; SP-D (82)
		- CA 19.9; CA 125 (83)
	• ECM remodeling	- MMP-7; MMP-12 (84)
		- VCAM1 (84)
	• Immune dysregulation	- CCL18 (85)
		- IL-6, IL-2 (86)
		- CXCL4, CXCL10, CX3CL1 (87)
		- Chitotriosidase (88)

Table 3. Principal proposed biomarkers for prognostic estimation of IPF and PF-ILD. CA: cancer antigen; CCL: = C-C motif chemokine ligand; CXCL = C-X-C motif chemokine; CX3CL: fractalkine; ICAM-1: intercellular adhesion molecule 1; IL: interleukin; LOXL2: lysyl oxidase-like 2; KL-6: Krebs Von den Lungen-6; MMP: matrix metalloproteinases; SP: surfactant protein; S100A9: S-100 calcium binding protein; TERC: telomerase RNA component; TERT: Telomerase reverse transcriptase; TOLLIP: Toll Interacting Protein; VCAM1: vascular cell adhesion molecule 1; YKL-40: chitinase-3-like protein 1.

Treatment

No pharmacological drugs have been specifically approved for IPF treatment until the start of antifibrotic era, marked by the approval of pirfenidone for clinical use in 2008 in Japan. Although antifibrotic treatment cannot arrest disease progression or least of all reverse fibrotic destruction of lung parenchyma, it remains the only pharmacological approach currently approved for the treatment of IPF. Another milestone for the therapeutic landscape of IPF was the publication of the PANTHER trial in 2012 (89): the three-drugs regimen composed by prednisone, azathioprine and N-acetyl-cysteine was associated with a significant increased rate of death and hospitalization against placebo, leading to the premature stop of the trial after 32 weeks of treatment. This trial, in addition to the growing evidence supporting the limited role of inflammation in the pathogenesis of IPF, marked the end of the antinflammatory and immunosuppressant approach for IPF. Figure 10 showed the recommendations reported in the last ATS/ERS clinical guidelines for the treatment of IPF.

Agent	2015 Guideline
New and revised recommendations	
Anticoagulation (warfarin)	Strong recommendation against use*
Combination prednisone + azathioprine + *N*-acetylcysteine	Strong recommendation against use†
Selective endothelin receptor antagonist (ambrisentan)	Strong recommendation against use†
Imatinib, a tyrosine kinase inhibitor with one target	Strong recommendation against use*
Nintedanib, a tyrosine kinase inhibitor with multiple targets	Conditional recommendation for use*
Pirfenidone	Conditional recommendation for use*
Dual endothelin receptor antagonists (macitentan, bosentan)	Conditional recommendation against use†
Phosphodiesterase-5 inhibitor (Sildenafil)	Conditional recommendation against use*
Unchanged recommendations	
Antiacid therapy	Conditional recommendation for use‡
N-acetylcysteine monotherapy	Conditional recommendation against use†
Antipulmonary hypertension therapy for idiopathic pulmonary fibrosis-associated pulmonary hypertension	Reassessment of the previous recommendation was deferred
Lung transplantation: single vs. bilateral lung transplantation	Formulation of a recommendation for single vs. bilateral lung transplantation was deferred

Figure 10. 2015 ATS/ERS guidelines for treatment of IPF (adapted from Raghu et al. (90))

PIRFENIDONE

Overview

Pirfenidone is a 5-methyl-1-phenyl-2-(1H)-pyridone and was originally conceived as a anti-pyretic and analgesic drug (91). However, researches conducted by Iyer et al. focused on the potential anti-inflammatory effects of this compound in a hamster model of bleomycin-induced lung damage: the researchers observed that pirfenidone was able to arrest lung fibrosis development, showing an important reduction of bleomycin-induced lung-toxicity (92,93).

These preliminary results led the way to the development of an orally available compound that was eventually approved for the treatment of IPF patients in Japan in 2008, making pirfenidone the first drug specifically indicated for this disease. Pirfenidone was approved for clinical use for IPF in European Union in 2011 and in the US in 2014. Although pirfenidone can be considered the first drug conceived as a antifibrotic, there are still many concerns regarding its mechanism of action and how it can be effective in reducing disease progression in IPF patients. Since early 2000s, many researches have been conducted to investigate this issue, revealing that pirfenidone effects are probably multidimensional and may interfere with multiple pathogenic pathways recognized in IPF pathogenesis (94).

First of all, pirfenidone have demonstrated anti-inflammatory properties through the inhibition of a wide range of intra and extracellular pro-inflammatory cytokines. The most consistent evidences on this field come from murine or mouse models: the administration of pirfenidone caused a significant reduction of production of many pro-inflammatory mediators, including TNF-α, TGF-β, IFNγ and IL-6 (95), and, on the counter part, to enhance the release of anti-inflammatory cytokines, such as IL-10 (96) . It also appeared to prevent or delay the onset of endotoxic shock and pulmonary inflammation in mouse models after the injection of lipopolysaccharide (LPS) endotoxin (96,97). Moreover, pirfenidone showed to inhibit the secretion of TGF- β, IL-1β, IL-12 and monocyte chemoattractant protein (MCP-1) in murine models of bleomycin-induced lung fibrosis,

demonstrating a wider anti-inflammatory activity than prednisolone (98). In the same model, the analysis of bronchoalveolar lavage fluid revealed that pirfenidone attenuated the alveolar recruitment and activation of macrophages, lymphocytes and neutrophils, further underlining the potential of this drug in reducing the inflammatory-mediated lung damage and, maybe, preventing lung fibrosis development (99).

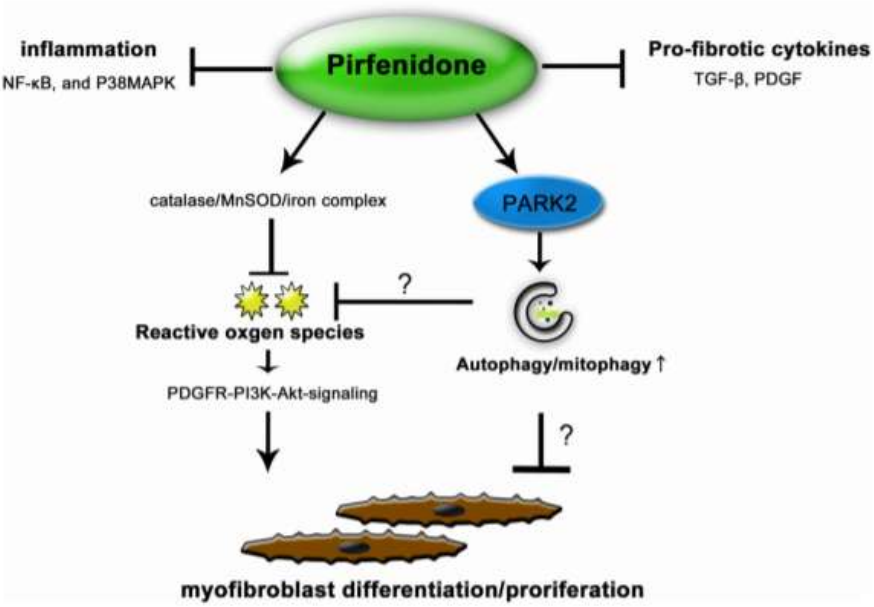

Figure 11. Proposed mechanisms of action of pirfenidone, including antinflammatory, antifibrotic and antioxidative properties of the drug. Adapted from Kurita et al. (100)

Focusing specifically on anti-fibrotic properties, pirfenidone can interfere with multiple pathways of pulmonary fibrogenesis. The inhibition of TGF-β1, that plays a crucial role in the pathogenesis of pulmonary fibrosis, is probably the most important activity of pirfenidone. Through the suppression of this signaling pathway, pirfenidone led to a significant decrease of fibronectin, heat shock protein and type I-III collagen secretion, inhibiting also the proliferation of fibroblasts and preventing their transformation in myofibroblasts (101–105). All these mechanisms caused a reduction in the

deposition of extracellular matrix in the lungs, contributing to slow down the progression of disease. Pirfenidone may also facilitate anti-oxidant pathways, through its activity of scavenger of reactive oxygen species (106,107). Interestingly, the anti-fibrotic properties of pirfenidone appears to be not limited to the lungs, since the drug have demonstrated a reliable efficacy in reducing fibrosis progression also in other tissues, like retina, liver and kidney (108–110), or modulating fibrotic remodeling of myocardium through the inhibition of angiotensin II pathway (111).

Effectiveness of pirfenidone in IPF: randomized clinical trials and real-world evidences

The first clinical evidence supporting the potential effectiveness of pirfenidone in IPF came from an phase II open-label study, which showed for the first time a reduction of functional disease progression through a pharmacological approach. Moreover, despite the limited sample size (54 patients, it was suggested that pirfenidone could also improve survival, since treated patients showed a mortality of 22% at 1 year and of 37% at 2 years, that were apparently better than historical cohorts of untreated patients (112).

In 2005, Azuma et al. published the first randomized clinical trial (RCTs) of pirfenidone in the management of patients with IPF. Despite not reaching the primary outcome (change of nadir oxygen saturation during 6-minute walking test, the Authors observed a significant deceleration of FVC decline after nine months of treatment, associated to a decrease of acute exacerbation incidence. These promising results led to the approval of pirfenidone for treatment of IPF in 2008 in Japan (113).

The effectiveness of this drug in IPF was further confirmed by international, multicenter and placebo-controlled CAPACITY trial, which enclosed two parallel RCTs (004 and 006): the aims of CAPACITY were to evaluate the efficacy of pirfenidone in reducing FVC decline (primary outcome) and to identify the optimal therapeutic dose of the drug. To be included in the study, patients were required to have a mild-to-moderate lung volumes impairment and a DLCO >35%. The pooled results from CAPACITY 004 and 006 confirmed the efficacy of pirfenidone in reducing FVC progression rate and clearly showed a dose-dependent effect of the drug, since the daily dosage of 2403 mg

appeared to achieve the best benefits. However, CAPACITY 006 failed to reach the primary outcome, since the placebo population showed an unexpected slow decline of FVC (9% vs 12.5% of CAPACITY 004 placebo population) (Figure 12). Accordingly, pirfenidone showed to improve progression-free survival (expressed in terms of time to decline of FVC > 10% or DLCO > 15%) and mortality rate in CAPACITY 004 but not in 006; nevertheless, 6-minute walking test distance was significantly improved only in 006 trial (114).

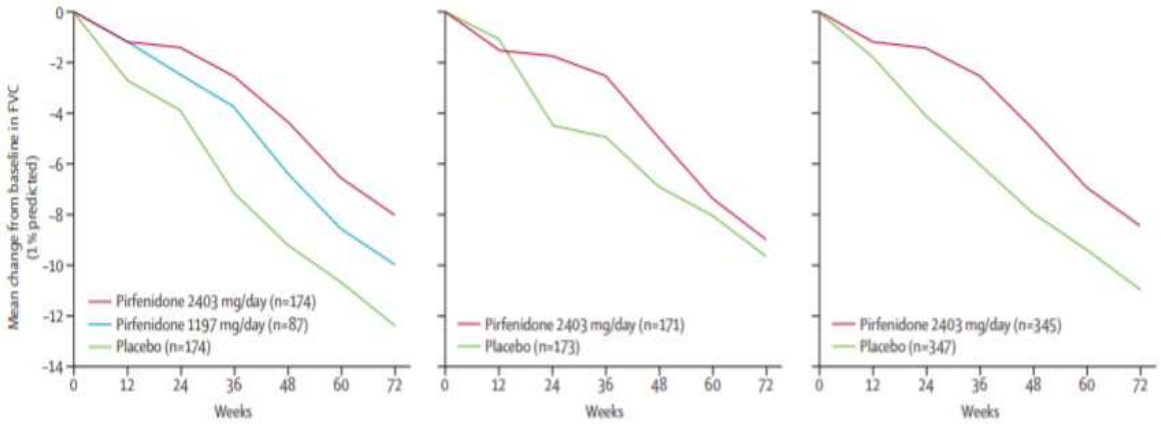

Figure 12. Mean change of FVC from baseline. Adapted from Noble et al (114)

Despite partially controversial, the results of CAPACITY trials led to the approval of pirfenidone use in IPF patients in European Union, that granted the license for the treatment in 2011, but not in the United States, in which regulatory authorities required a further confirmatory trial to better explore the clinical effectiveness of pirfenidone. The double-blind, placebo-controlled phase III RCT named ASCEND was designed for this aim and enrolled patients with IPF with similar inclusion criteria with respect to CAPACITY. The results showed a significant reduction of FVC rate of decline after 52 weeks of treatment with pirfenidone 2403 mg/die (primary outcome) and confirmed the improvement in terms of progression-free survival reported by CAPACITY 004 (115). Overall, ASCEND findings

led to the approval for clinical use of pirfenidone in US in 2014, that became the first anti-fibrotic drug worldwide approved for the treatment of IPF (Figure 13).

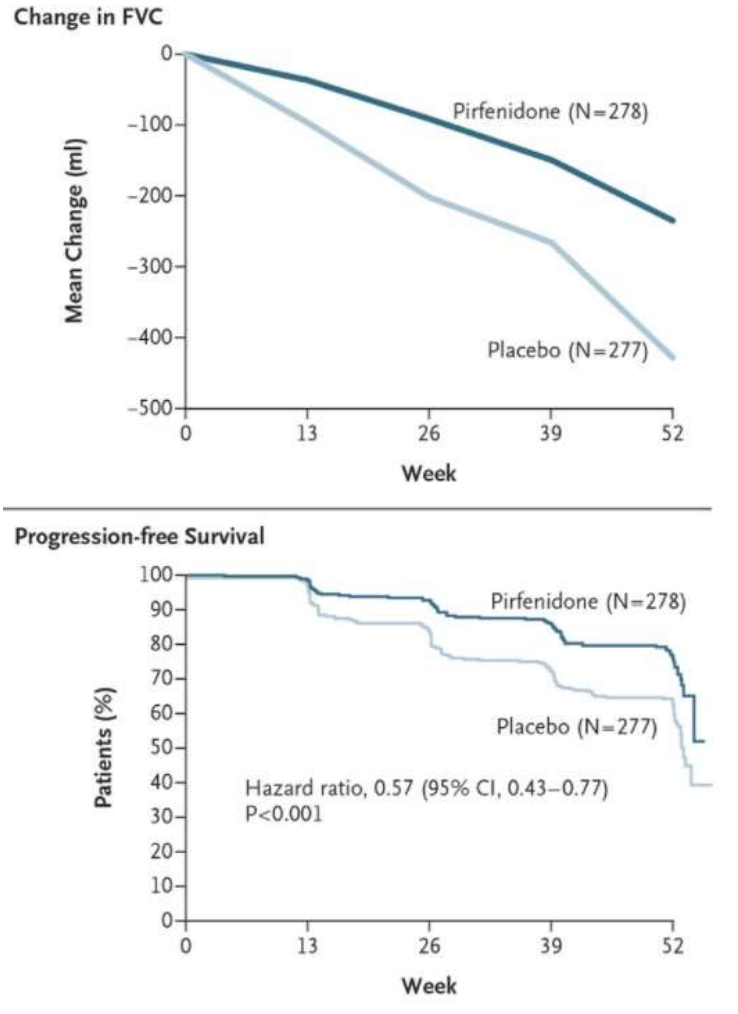

Figure 13. Effect of pirfenidone in change of FVC and progression-free survival. Adapted from King et al. (115)

Notably, the pooled analysis of CAPACITY and ASCEND data showed a reliable reduction of the proportion of patients with a ≥10% decline of FVC or death by 43.8% and increased the percentage of patients with no decline by 59.3%. Despite being unpowered for mortality outcomes, pirfenidone

treatment showed a clear trend in reducing mortality after 1 year of treatment, reporting a decrease of 48% of all-cause mortality and of 68% of IPF-related mortality (116).

The effectiveness of pirfenidone in the management of IPF has been repeatedly confirmed in large, observational real-world studies, that also underlined the good safety and tolerability profile of the drug (117–120). Pirfenidone showed to be effective also in IPF patients with more preserved or more advanced stage of disease, identified by FVC impairment (61,121), and also demonstrated to reduce the risk of acute exacerbation of disease in IPF with lung cancer patients undergoing surgical pulmonary resection (122). Notably, data analysis from National Registries or from Referral Centers demonstrated that pirfenidone maintained its efficacy in reducing the decline of FVC even after five years of treatment, supporting the long-term use of this drug in the management of disease (123,124). The results in terms of efficacy coming from European IPF Registry were substantially in line with phase III trials, showing that pirfenidone was able to halve the FVC decline rate if compared with placebo; interestingly, it appeared that some clinical and/or respiratory functional features (former smokers, age > 60 years, FVC < 80% and rapid disease progression) were associated with a higher benefit to be expected from pirfenidone treatment in terms of stabilization of disease (117). Moreover, beyond the evidences on disease progression rate, many studies demonstrated that pirfenidone therapy improves survival and progression-free survival in IPF patients (123–126).

Concerning safety and tolerability, no new safety alerts emerged and adverse events appeared to predominantly occur within the first months of treatment and may be safely managed with supportive therapies in the majority of cases (126–128). However, a significant percentage of patients is reported to not tolerate the full dose of pirfenidone due to gastrointestinal intolerance, persistent weight loss or photosensibility: in these cases, a lower dose of drug may be indicated, as a large retrospective Korean study showed no significant differences in terms of disease progression between full and low-dose of treatment (129).

Pirfenidone in PF-ILD: work in progress

PF-ILD shared not only clinical and prognostic similarities with IPF but also common biomolecular and immunological pathways, regardless the underlying disease or radiological and histopathologic patterns. Since pirfenidone has been the first drug showing a reliable efficacy in changing the progression of IPF, a similar therapeutic approach for PF-ILDs is surely reasonable. However, few solid data is currently available on this field. To date, two phase II randomized clinical trials investigated the potential effectiveness of pirfenidone in reducing FVC decline rate in patients with unclassifiable ILDs and in PF-ILDs. In the first study, pirfenidone confirmed to reduce the functional progression of disease in terms of FVC deterioration, both if performed with site or home spirometry: as well as for IPF, pirfenidone decreased the likelihood to experience a FVC decline > 5 or > 10%, but no differences were observed for different progression-free survival models (that included also death incidence) and for acute exacerbation rate, due to the paucity of these events in the entire study population (130). Regarding PF-ILDs, the RELIEF study was designed as a multicentre, double-blind and placebo-controlled phase II RCT to investigate the efficacy of pirfenidone on four diseases: CTD-ILDs, fibrotic hypersensitivity pneumonitis, asbestos-related ILD and fibrotic NSIP. To be eligible, patients were required to have a FVC between 40-90%, DLCO 10-90% and a annual decline rate of FVC > 5% in the previous 6-24 months: no radiological-documented progression of disease was required. Unfortunately, the study was prematurely stopped for futility due to slow recruitment: however, the analyses conducted on 127 enrolled patients showed that pirfenidone reached the statistical significance in reducing FVC progression regardless the diagnostic subgroups; only one death, and not related to respiratory causes, was observed in the pirfenidone arm, while five deaths (including three due to respiratory events) were reported in the placebo subgroup. No new safety alerts were found. Therefore, despite the premature termination of the study, pirfenidone appeared to be effective in reducing disease progression also in PF-ILDs, even though these preliminary data surely needs to be confirmed by larger phase III RCTs (41).

NINTEDANIB

Overview

Nintedanib is a 6-methoxycarbonyl-substituted indolinone (chemical formula: methyl (3Z)-3-[[4-[methyl-[2- (4-methylpiperazin-1-yl)acetyl]amino]anilino]-10 phenylmethylidene]-2-oxo-1H-indole-6-carboxylate; molecular formula C31H33N5O4) (Figure) . It is a multitarget tyrosine kinase inhibitor as it can competitively inhibit non-receptor and receptor tyrosine kinases: among the non-receptor tyrosine kinase, nintedanib targets are Lck, Lyn and Flt-3, belonging to the Src family, determining a predominant anti-angiogenic effect (131,132). Regarding the receptor tyrosine kinases, nintedanib acts as a competitive inhibitor of fibroblast growth factor receptor (FGF) 1, 2 and 3, platelet-derived growth factor receptor (PDGF) α and β, and vascular endothelial growth factor (VEGF) receptor 1, 2 and 3 (133). In terms of pharmacodynamics, nintedanib competitively binds to the ATP binding pocket of FGF-r, PDGF-r and VEGF-r, blocking the autophosphorylation of these receptors and eventually preventing the activation of downstream signaling cascades (134). The antifibrotic activity of nintedanib is probably explained by its pleiotropic effects on the tyrosine kinases families: in fact, both non-receptor and receptor tyrosine kinases play a crucial role in many biological processes, as they are expressed by several cell subsets (including fibroblasts and myofibroblasts) and exert a fundamental activity in the modulation of many biological processes, including proliferation, migration, recruitment, activation and apoptosis (135). The consequence of this wide inhibition exerted by nintedanib is a down-regulation of pro-fibrotic and pro-angiogenic processes, leading to the reduction of resident fibroblasts and myofibroblasts in terms of numerosity and activity, eventually causing a decrease of extracellular matrix secretion. These assumptions were finely described by Wollin et colleagues in mouse models of lung fibrosis induced by bleomycin and silica inhalation: nintedanib induced a substantial reduction of fibroblast recruitment and proliferation and significantly inhibited fibroblast-to-myofibroblast transformation. In the same study, nintedanib showed also a reliable anti-inflammatory activity, leading to a significant reduction of neutrophils

and lymphocytes percentage on BALF associated to a decrease of IL-1, TIMP-1 and lung collagen secretion. The evidence of antifibrotic and antinflammatory properties of nintedanib was further strengthened by histological sampling of pulmonary tissue, showing an important reduction of lung fibrosis extension and granuloma formation (134). These findings were confirmed by the research by Hostettler et al, which investigated the biological effects of nintedanib in lung fibroblasts isolated from IPF patients and subjects without pulmonary fibrosis. As expected, fibroblasts from IPF samples showed a significantly increased expression of VEGFR, FGFR and PDGFR, resulting in a pro-proliferative effect and a dysregulated secretion of pro-fibrotic and pro-angiogenic factors, such as inhibitors of matrix metalloproteinases (TIMPs) and collagen. Nintedanib demonstrated a reliable reduction of receptor tyrosine kinase activation, leading to a sort of restoration of pro-fibrotic/antifibrotic factors balance. These anti-proliferative and anti-fibrotic effects eventually caused a clear reduction of lung collagen production and deposition and stimulated the degradation of excessive ECM (136).

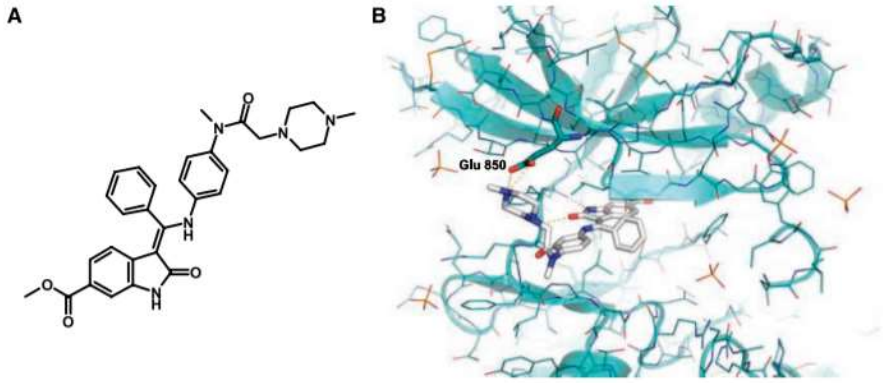

Figure 14. Chemical structure of nintedanib (A) and its X-ray structure bound in the active site of the VEGFR-2 crystal (B) (from Hilberg et al.) (131)

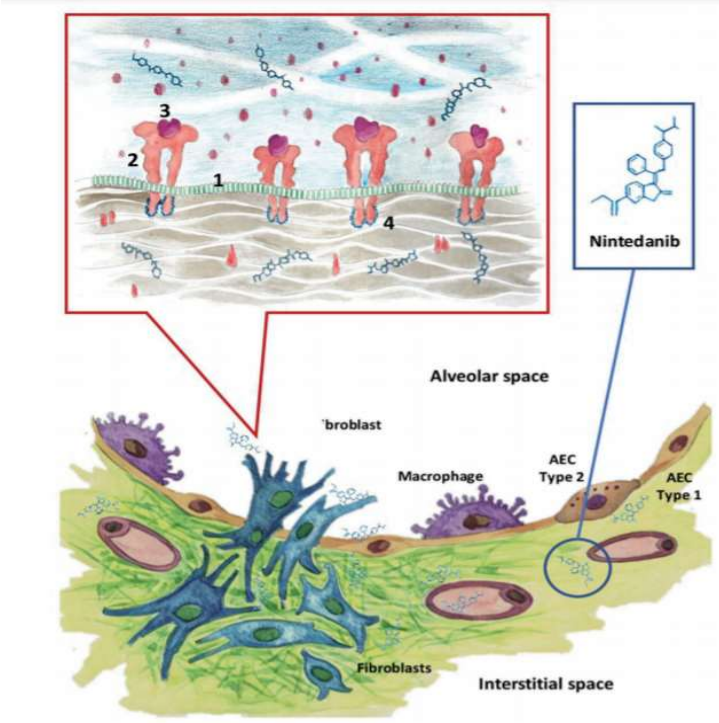

Figure 15. Mechanism of action of nintedanib in lung tissue. Nintedanib is able to reduce proliferation and activation of myofibroblasts as well as the fibroblast to myofibroblast transformation, preventing the dyregulated production and deposition of fibrotic tissue in the alveolar department. 1: cell membrane of fibroblasts and myofibroblasts; 2: receptor tyrosine kinase blocked by nintedanib (PDGFR, VEGFR and FGFR); 3: binding site of receptor tyrosine kinases; 4: downregulation of profibrotic and antiapoptotic downstream signalling cascades (figure from Varone et al.) (137)

However, despite our knowledge of the mechanism of action of nintedanib in terms of pharmacodynamics, the real impact of the drug on the complex pathophysiology of IPF and PF-ILD is far to be completely elucidated. The antifibrotic effects of nintedanib are probably not only mechanistically secondary to the inhibition of tyrosine kinases activation but may be related to a more comprehensive modulation and re-adaptation of fibroblasts cellular activity (138). This assumption is indirectly confirmed by the clinical evidence that more selective tyrosine kinase inhibitors (such as

imatinib) showed no benefit in reducing disease progression and were not effective in inducing anti-fibrotic and anti-proliferation changes in lung fibroblasts (139,140)

Effectiveness of nintedanib in IPF: randomized clinical trials and real-world evidences

Nintedanib was approved for clinical use in IPF in 2014 in the United States, in 2015 in the European Union and is available in Italy since 2016. The first randomized clinical trial investigating the safety and efficacy of nintedanib in the reduction of functional disease progression, expressed as FVC decrease rate, was the phase 2 double-blind, dose finding, placebo-controlled TOMORROW trial (ClinicalTrials.gov identifier: NCT00514683). This trial enrolled 432 patients affected by IPF with a mild-to-moderate impairment of FVC and DLCO, showing a significant reduction of FVC decline rate for 150 mg bid arm in respect with placebo after 52 weeks of treatment (60 vs 190 ml, respectively) and, as secondary outcomes, a smaller incidence of acute exacerbation of disease (141). These promising results were strengthened by the following phase 3 trials, INPULSIS-1 and -2 (NCT01335464). In these two 52-weeks parallel, multicenter and placebo-controlled RCTs, nintedanib 150 mg bid confirmed its efficacy in reducing FVC decline rate, while no solid data emerged regarding the reduction of acute exacerbation rate and the improvement of quality of life. However, the evidence that nintedanib was able to significantly slow functional disease progression led to the approval of the drug for clinical use.

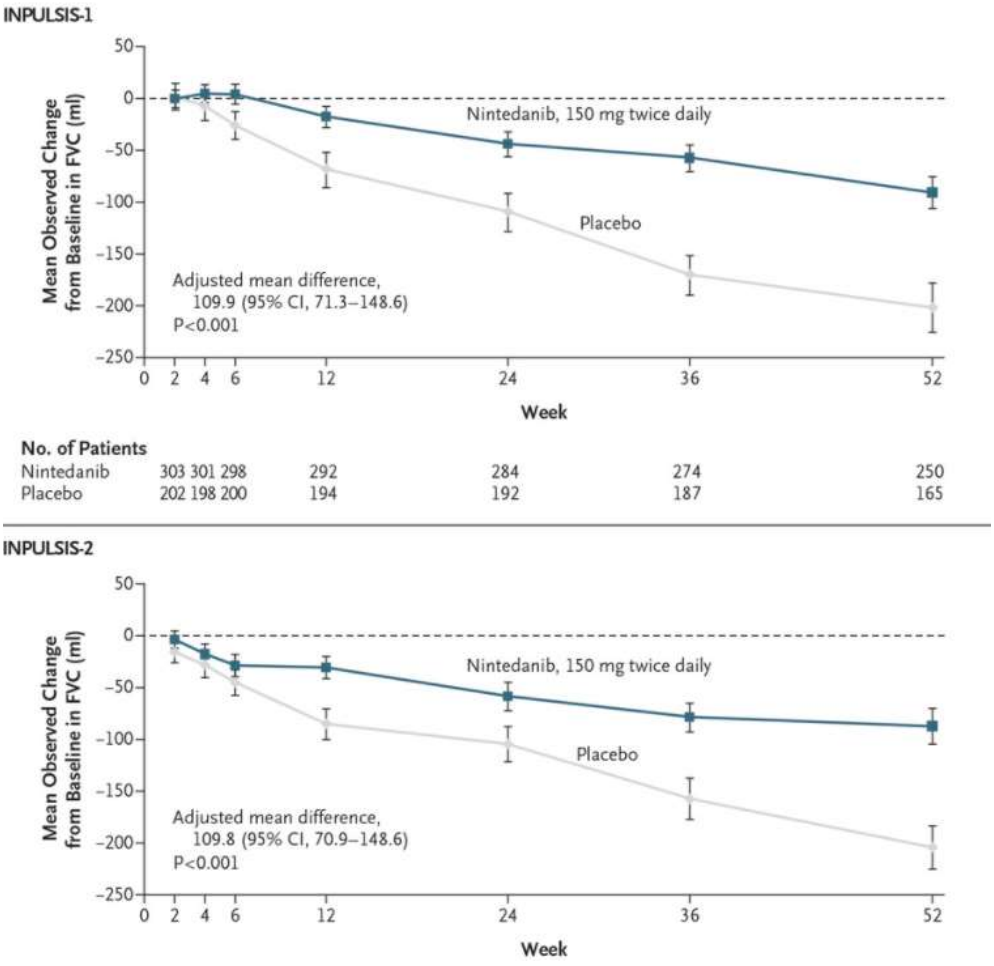

Figure 16. Mean change of FVC from baseline in the INPULSIS trial. Adapted from Richeldi et al. (142)

Post-hoc analyses of TOMORROW and INPULSIS trials demonstrated that nintedanib was equally effective in reducing the rate of decline of FVC through all pre-specified subgroups of patients enrolled in the studies: age > 70 aa, smoking status, patients with FVC > 90%, FVC < 50%, combined pulmonary fibrosis and emphysema (CPFE) and different CT patterns (definite UIP vs possible UIP without histologic confirmation) (62,143–146).

Moreover, the pooled analysis of these three trials not only confirmed the effectiveness of nintedanib in reducing disease progression and the risk of acute exacerbation but also reported a significant

reduction of mortality rate, showing for the first time that nintedanib use may improve survival in IPF patients (147).

Importantly, the results from the open-label extension trial of INPULSIS, named INPULSIS-ON (NCT01619085), showed that nintedanib maintained its efficacy in reducing disease progression for up to three years of treatment; notably, the subgroup of patients randomized in the placebo arm of INPULSIS trials reported a substantially similarity in terms of response to the treatment, further underlining the clinical effectiveness of nintedanib regardless the stage of disease (148).

Since the approval of nintedanib in clinical practice for treatment of IPF, many real-world studies evaluated the effectiveness and safety of this drug on this setting. The effectiveness of nintedanib in reducing the disease progression was widely reported and was substantially equivalent to data available from RCTs. Nintedanib also confirmed a satisfying tolerability profile and no new safety alerts emerged in the last five years: however, a little, but still significant percentage of patients (about 5-10% across longitudinal studies) experienced severe diarrhea and/or hepatotoxicity and was forced to quit permanently the treatment (149–155)

Last but not least, the improvement of survival rate and progression-free survival was undoubtedly confirmed by large, longitudinal real-life studies and was observed across different clinical (age, sex, respiratory functional parameters) and/or diagnostic subgroups (confident or working diagnosis) of patients affected with IPF (156,157).

Future is now: nintedanib in PF-ILD

To date, nintedanib is the only pharmacological drug approved for clinical use in patients affected with PF- ILDs, as recently endorsed by an European consensus statement focused on the diagnosis and management of SSC-ILD (158). In this statement, nintedanib is cited as a potential first-line therapeutic option for the management of lung fibrosis associated with systemic sclerosis.

The efficacy of an antifibrotic drug in the management of non-IPF ILD was first investigated in the SENSCIS study, a phase 3, double-blind, placebo-controlled multicenter RCTs, designed to investigate the potential effectiveness of nintedanib in slowing pulmonary disease progression in SSC-ILD patients (159). To be eligible, ILD had to affect at least 10% of the lungs, through the quantitative evaluation of chest HRCT scan by an experienced radiologists, and a mild-to moderate impairment of FVC was required at the inclusion. Notably, low-dose steroid use and/or immunosuppressant therapy (mycophenolate mofetil or methotrexate) was permitted as maintenance therapy. SSC patients treated with nintedanib showed a significant reduction of rate of decline of FVC after 52 weeks of treatment: the primary outcome was met across all the prespecified subgroups, including different background therapy, baseline respiratory functional assessment, serum autoantibody profile, lung fibrosis extension or SSC subtype (limited vs diffuse cutaneous). Interestingly, the lowest decline of FVC was observed in the subgroup of patients treated with mycophenolate and nintedanib, suggesting a potential synergistic effect of these two drugs in this setting: however, more evidences are needed to confirm this preliminary finding and, subsequently, to investigate the potential benefits of a sequential approach vs an initial combination therapy (160). Moreover, the SENSCIS trial included change in Rodnan skin score as a key secondary outcome, in order to investigate the potential effectiveness of nintedanib in improving non-respiratory features of SSC: unfortunately, results were quite disappointing, as no differences were observed between nintedanib and placebo subgroups on this field. Finally, nintedanib was not associated with a clinically significant improvement in respiratory quality of life expressed with St-George Respiratory Questionnaire (SGRQ) score: this finding was quite un surprising, since no antifibrotic treatment has never demonstrated strong evidence in improving quality of life in patients with ILD.

After SENSCIS, nintedanib proved its efficacy also in a cohort of various PF-ILDs in the INBUILD trial (40). The INBUILD trial is a phase III, double-blind and placebo-controlled RCT, designed to investigate the effectiveness of nintedanib in slowing lung fibrosis progression in patients with not-

IPF ILDs, which have shown a functional or radiological deterioration in the 24 months prior to screening. As well as SENSCIS, at baseline patients were required to have a mild to moderate impairment of FVC, associated to fibrotic abnormalities affecting more than 10% of the lungs at chest CT scan. The most common ILDs in the study were chronic hypersensitivity pneumonitis and CTD-ILDs, contributing together to the 52% of the entire population enrolled.

Similar to INBUILD and SENSCIS studies, a significant reduction of FVC decline was observed in the nintedanib subgroup in respect with placebo arm, regardless the radiological pattern (UIP or not-UIP), age, sex, race and respiratory functional assessment at screening. Accordingly, the percentage of patients experiencing a FVC decline lower than 5% or between 5 and 10% was significantly higher in patients treated with nintedanib than the placebo subgroup.

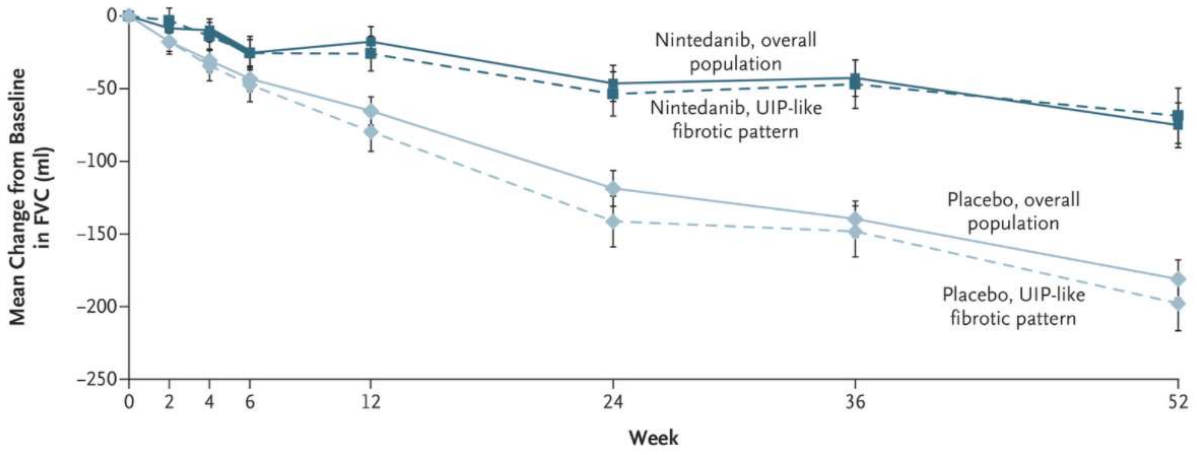

Figure 17. Nintedanib reduces FVC decline rate in PF-ILD patients. Adapted from Flaherty et al. (40)

Concerning the different ILD included in the study, a post-hoc analysis confirmed that a significant difference in annual FVC decline was observed in all diagnostic subgroups (173 patients with cHP: 73.1 ml/year; 170 patients with CTD-ILDs: 104 ml/year; 125 with idiopathic NSIP: 141.6 ml/year;

118 unclassifiable idiopathic interstitial pneumonia: 68.3 ml/year; 118 other ILDs: 197.1 ml/year) (161).

Despite the relatively short of observation time, another key secondary outcome of INBUILD trial was the risk of acute exacerbation and/or death during treatment: patients treated with nintedanib showed lower incidence in respect with placebo group, but without reaching statistical significance. The subgroup that showed the greatest benefit in terms of acute exacerbation or death incidence was composed by CTD-ILD patients.

Regarding the quality of life assessment, no differences were observed in change from baseline in K-BILD score, while a nearly significant improvement in respect with placebo was observed in other quality of life scale designed for patients with pulmonary fibrosis (Pulmonary Fibrosis Impact on Quality of Life Scale summary score and Living with Pulmonary Fibrosis dyspnea, cough and total score), suggesting a potential benefit for antifibrotic treatment also in this setting.

AIMS

The principal aim of this research is to compare the clinical effectiveness of the only two pharmacological drugs currently approved for the treatment of IPF in the real-life setting of Referral Centre for ILDs. This study will provide interesting and innovative insights for the clinical management of these patients, since it embraces almost ten years of use of antifibrotic treatment and includes also a relevant number of patients affected by non-IPF PF-ILD and familial pulmonary fibrosis. The sample size and the long time of observation allowed us to select mortality and progression-free survival as main outcomes of the study.

MATHERIALS AND METHODS

Study population

All patients treated with pirfenidone or nintedanib at the Regional Referral Centre for ILD for Siena form June 2011 to June 2020 were retrospectively enrolled in the study. Patients were selected from medical records archived in our Centre and from electronical database of Italian Medicine Agency, in which antifibrotic treatment was activated. We included in the study also patients treated with pirfenidone or nintedanib through compassionate grounds. Diagnosis of IPF and PF-ILD was performed according to international guidelines applicable at the start of treatment and underwent specialistic evaluation and discussion by multidisciplinary group for ILDs of Siena (GIM). Familial pulmonary fibrosis (FPF) was diagnosed when more than two cases of idiopathic interstitial pneumonia (IPF or non-IPF) were identified in the same family. GIM included: respiratory physicians, radiologists, pathologists and, whenever needed, rheumatologists, cardiologists or occupational physicians. All the physicians involved in GIM meetings were experienced in the diagnostic and clinical management of patients affected with ILDs. If clinical and/or radiological features couldn’t allow a confident diagnosis and histologic sampling was contraindicated or not

accepted by the patient, GIM provided a provisional diagnosis with high or low-confidence. In case of a working high-confidence diagnosis of IPF, antifibrotic treatment was proposed to the patients: if accepted, these subjects were included in the study as well. According to clinical questions and/or in case of histological sampling by surgical biopsy or explantation for lung transplant, both confident or provisional diagnoses were re-discussed in the multidisciplinary setting. In the database, we include the definitive diagnosis or and the diagnostic hypothesis with the highest confidence level made throughout the follow-up. From January 2020, nintedanib was available for the treatment of non-IPF PF-ILD through compassionate grounds: these patients were included in the study as well.

Demographic and clinical data, respiratory functional assessment, radiological and histologic features were retrospectively collected and entered in a electronical database for statistical analysis. All the available pulmonary function tests (PFTs), including DLCO assessment, performed throughout the follow-up were collected as well: if available, we included in the database also the PFTs of at least 1 year before starting antifibrotic treatment. To minimize the inter-observer and intra-observer variability and guarantee the best technical reproducibility and repeatibility, we decided to include in the database only the PFTs performed at Respiratory Diseases Unit of Siena.

Study patients were considered lost to follow-up in case of:

- Death
- Lung transplantation
- Interruption of the treatment due to any cause

Patients were excluded from the study in case of:

- Inability or refusal to provide informed consent to participate in clinical studies
- Less than one month of antifibrotic treatment
- Previous antifibrotic treatment at baseline

The principal outcome of the study was the comparison of all-cause mortality and progression-free survival between the pirfenidone and nintedanib treatment arms. Significant progression of disease was expressed as time to decline of FVC > 10% and/or time to decline of DLCO > 15%, as previously described (162). As secondary outcome, the comparison of effectiveness between the two drugs was also performed according to the following pre-specified subgroups: diagnosis (IPF and PF-ILD) and familial or sporadic ILD.

Lung function tests (PFTs)

The following lung function measurements were recorded according to ATS/ERS standards using a Jaeger Body Plethysmograph with corrections for temperature and barometric pressure: forced expiratory volume in the first second (FEV1), forced vital capacity (FVC), FEV1/FVC, total lung capacity (TLC), diffusion lung capacity for carbon monoxide (DLCO) and capacity carbon monoxide lung transfer factor/alveolar volume (DLCO/VA). All parameters were expressed as percentages of predicted reference values. DLCO assessment was not performed in patients who were on oxygen therapy.

Statistical analysis

Data was expressed as mean ± standard deviation, unless otherwise specified. Parametric tests (T-test and one-way ANOVA) were used to compare groups. Statistical analysis and graphs were performed and plotted using GraphPad Prism version 5.0 software for Windows (GraphPad Software, La Jolla, CA). Unadjusted survival and disease progression outcome estimates were obtained using Kaplan-Meier curves. Time-to decline FVC or DLCO was estimated through interpolation analysis of serial pulmonary function test performed during the follow-up. Time-to-event endpoints were compared using a two-sided log-rank test. A $p \leq 0.05$ was considered significant.

RESULTS

Study population

A total of 317 patients affected with ILD (238 males, 70.8 ± 8.6 years old) and treated with antifibrotic treatment was retrospectively recruited in the study: among these, 179 were treated with nintedanib and 138 with pirfenidone. The study population was composed by 261 subjects with IPF and 56 PF-ILD, including 17 fibrotic HP, 16 CTD-ILD (10 SSC-ILD, 5 RA-ILD and 1 Sjogren syndrome- ILD), 13 idiopathic NSIP, 6 undifferentiated ILD, 3 occupational exposure-related ILD (2 asbestosis and 1 silicosis) and 1 sarcoidosis. In the PF-ILD subgroup, 12/56 patients were treated with pirfenidone: in all these cases, diagnosis of PF-ILD was made after histologic evaluation of surgical biopsy or lung explantation (3 and 9 subjects, respectively). In the PF-ILD patients treated with nintedanib, a revision of the initial provisional diagnosis of IPF was performed in 10/42 patients, while the remaining were treated with nintedanib per compassionate grounds.

Overall, at baseline, IPF patients treated with nintedanib were significantly older than those treated with pirfenidone (73.1 ± 8.7 vs 68.1 ± 7.7 years old, $p < 0.0001$) and showed FVC% and DLCO% significantly more impaired (74.1 ± 20.2 vs 83 ± 19.7, $p=0.006$ and 42.7 ± 12.3 vs 50.1 ± 15.2, $p=0.0012$, respectively). No differences were observed between the two subgroups in terms of sex, smoking status, radiological CT pattern and medical comorbidities, as well as time from symptomatic onset and diagnosis of ILD.

Parameters	Pirfenidone	Nintedanib	p-value
IPF			
N°	126	145	
Age (yrs)	68.1 ± 7.7	73.1 ± 8.7	< 0.0001
Male gender (%)	101 (80.1)	115 (79.3)	0.9856
Smoking status (p/y)	15.5 ± 10.2	19.3 ± 12.5	0.2458
- Current/former	87 (69)	100 (68.9)	0.8845
- Never	39 (30.9)	45 (31)	0.8845
Familial IPF (%)	20 (15.8)	15 (10.3)	0.2052
Time to diagnosis (mo)	25.8 ± 32.1	22.9 ± 33.9	0.5548
Previous use of OCS or AZA (%)	34	21	0.1156
Interruption of treatment	15	9	0.2257
AE: mild/moderate/severe/fatal	70/22/15/0	95/25/9/0	0.1586
CT pattern			
UIP (%)	99	102	0.5567
Probable/possible UIP (%)	22	30	0.5567
Indeterminate UIP (%)	5	13	0.5567
Emphysema (%)	22	25	0.8264
PFTs			
FVC l	2.6 ± 0.8 (83 ± 19.7)	2.3 ± 0.7 (74.1 ± 20.2)	0.0006
FEV1/FVC	81 ± 7	81.1 ± 7.8	0.8541
TLC l (%)	4.7 ± 1.2 (79.4 ± 16.1)	4.3 ± 1.1 (72.2 ± 17.5)	0.0021
DLCO mmol/min/kPA (%)	4.0 ± 1.7 (50.1 ± 15.2)	3.2 ± 1.1 (42.7 ± 12.3)	0.0012
KCO mmol/min/kPA/ml (%)	1.1 ± 0.2 (77.6 ± 17.5)	0.9 ± 0.2 (68.2 ± 22.2)	0.0009

PF-ILD			
N°	12	34	
Age (yrs)	63.7 ± 11.1	68 ± 9.8	0.3938
Male gender (%)	7	19	0.8541
Smoking status (p/y)	12.3 ± 11.5	10.5 ± 9.4	0.4589
Current/former	6	20	0.8998
Never	6	14	0.8998
Familial disease (%)	1	4	0.7894
Time to diagnosis (mo)	15.4 ± 12.1	19.7 ± 8.6	0.2579
Previous use of OCS/AZA (%)	7	27	0.2056
Interruption of treatment	2	6	0.4698
AE: mild/moderate/severe/fatal	4/3/2/0	18/7/6/0	0.4698
CT pattern			
UIP (%)	7	13	0.3756
Probable/possible UIP (%)	5	10	0.3756
Indeterminate UIP (%)	0	4	0.3756
Emphysema (%)	4	12	0.5897
PFTs			
FVC l (%)	2.6 ± 0.7 (80.9 ± 23.1)	2.4 ± 0.9 (76.8 ± 20.7)	0.2725
FEV1/FVC	80.7 ± 8.6	80.6 ± 8.5	0.9156
TLC l (%)	5.4 ± 1.7 (88.4 ± 19.9)	4.9 ± 1.2 (84.8 ± 15.1)	0.1459
DLCO mmol/min/kPA (%)	3.7 ± 1.4 (47.9 ± 13.1)	3.9 ± 1.9 (50.6 ± 12.2)	0.5119
KCO mmol/min/kPA/ml (%)	0.9 ± 0.3 (70.1 ± 21.7)	1 ± 0.3 (75.4 ± 22.4)	0.9119

Table 1. Demographic and clinical data, radiological features and lung function assessment of pirfenidone and nintedanib subgroups. OCS: oral corticosteroids; AZA: azathioprine

Outcome analysis

At 1st March of 2021 (908.1 ± 534.2 days of observation), median of survival and time to decline of FVC > 10% o DLCO > 15% in the entire population was 1292 and 422.2 days, respectively. During the follow-up, 73 patients died (23%, 40 nintedanib and 33 pirfenidone), 12 underwent lung transplantation (3.7%, 2 nintedanib and 10 pirfenidone) and 24 interrupted antifibrotic treatment due to severe or incoercible side effects (7.5%, 15 with pirfenidone and 9 with nintedanib). Fatal or near-fatal adverse events were not observed.

Concerning survival, we didn't observe significant differences in terms of mortality between the two treatment groups in the IPF cohort (log rank test: 0.09015, p=0.7640, respectively) (Figure 18).

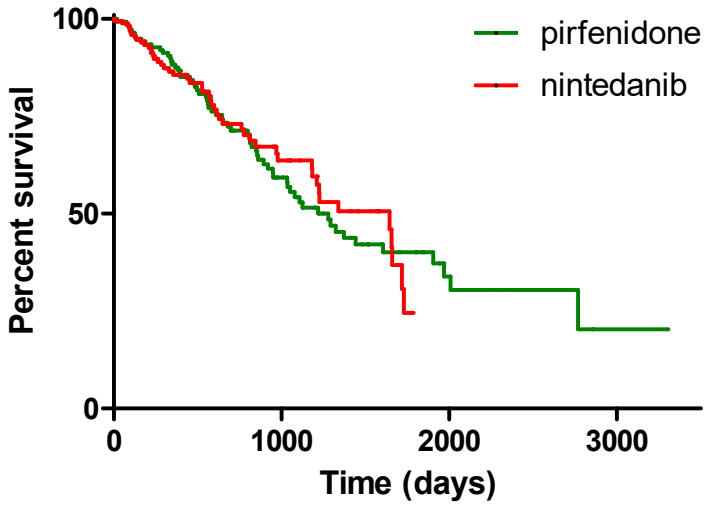

Figure 18. Kaplan-Meier survival curves for comparison of mortality between pirfenidone and nintedanib-treated subgroups.

Similarly, no significant differences were found regarding progression-free survival between patients treated with nintedanib or pirfenidone (log rank test: 0.02366, p= 0.7614) (Figure 19).

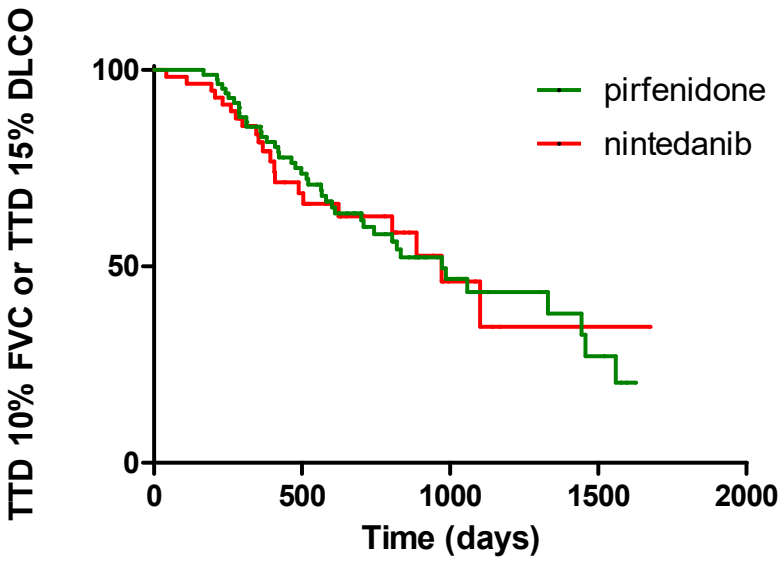

Figure 19. Kaplan-Meier curves for comparison of effectiveness in reducing functional disease progression between pirfenidone and nintedanib. TTD: time to decline

Accordingly, FVC decline rate during the follow-up was similar between patients treated with pirfenidone and nintedanib (p=0.6912 and p=0.6514 for absolute and percentage of predicted values, respectively) (Figure 20); the evaluation of annual DLCO decline rate revealed that nintedanib subgroup experienced a slower reduction in respect with pirfenidone subgroup after one year of treatment (p=0.004): however, this discrepancy progressively reduced in the following steps and became no more statistically significant from 24 months of treatment (Figure 20).

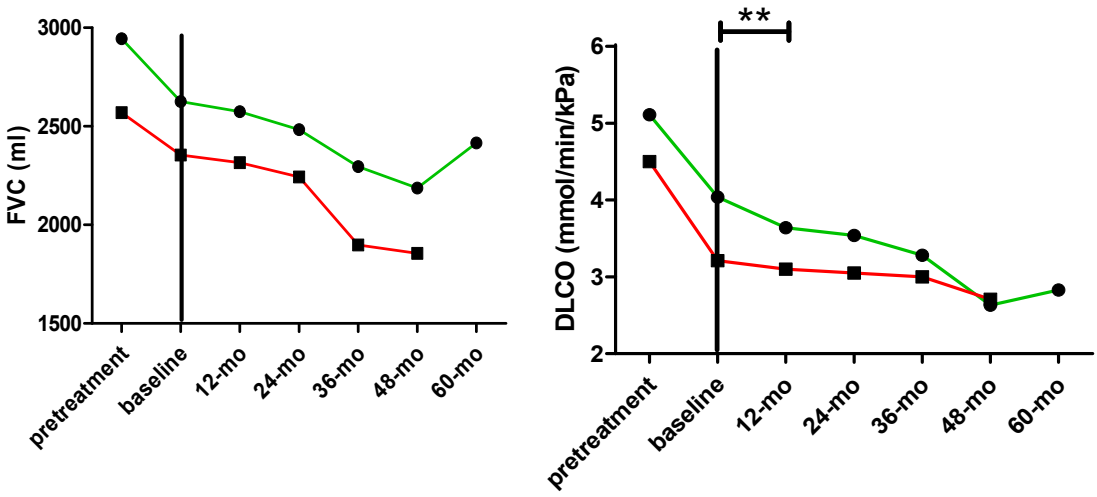

Figure 20. Comparison of FVC and DLCO decline rate in absolute values between pirfenidone (green line) and nintedanib (red line) subgroup. **: p=0.004

Pretreatment PFTs were available in 86 IPF patients (44 in the pirfenidone subgroup): in both treatment subgroups, we observed a significant reduction of FVC deterioration rate (p=0.0153 and p=0.0214 for pirfenidone and nintedanib, respectively), confirmed also by the comparison of annual time to decline of FVC before and after antifibrotic therapy (p=0.0191 and p=0.0261 for pirfenidone and nintedanib, respectively). Nintedanib showed also to significantly slow down DLCO decline rate in respect with pretreatment epoch (p=0.0081 and p=0.0256, for delta DLCO values and time to decline of DLCO > 15%, respectively); pirfenidone numerically reduced as well DLCO decline rate, but without reaching statistical significance (p=0.0864 and p=0.0654, respectively) (Figure 21)

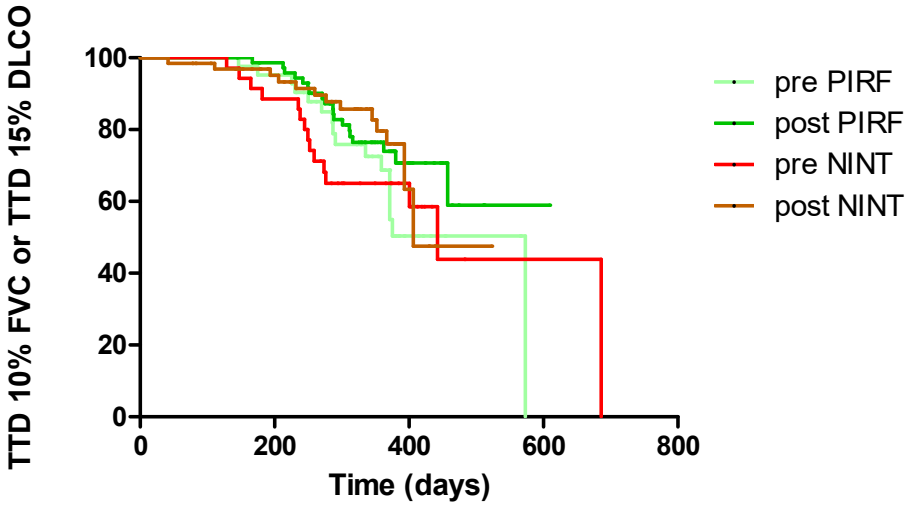

Figure 21. Comparison between progression free-survival before and after treatment with pirfenidone and nintedanib

Subgroup analysis: FPF

The study population included 35 patients affected with FPF (20 males, 68.5 ± 7.9 years old): among these, 26 patients showed a radiological pattern of typical or probable UIP and were diagnosed as familial IPF, while the remnant showed a CT pattern of NSIP or indeterminate for UIP (6 and 3 patients, respectively). In comparison with sporadic IPF, FPF patients were significantly younger (p=0.0026) and showed a higher percentage of females (p=0.0032).

Overall, FPF subgroup reported a worse survival than sporadic IPF, even if not reaching the statistical significance (log rank test: 2.901, p=0.0885). Concerning functional disease progression, antifibrotic treatment didn't appear to influence FVC neither DLCO decline rate, that remained substantially unchanged in respect with pretreatment period (p=0.2880 and p=0.6902, respectively); accordingly, we didn't observe any difference of time to decline of FVC and DLCO before and after treatment in this subgroup. If compared with sporadic IPF population, FPF subgroup showed a significantly worse progression-free survival (log rank test: 15.13, p=0.0013) (Figure 22)

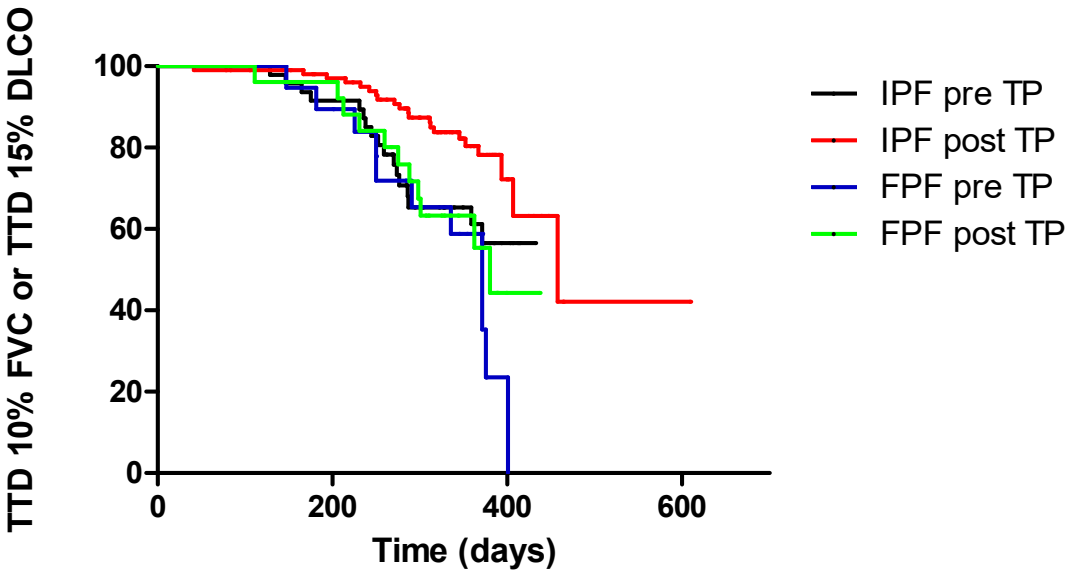

Figure 22. Kaplan-Meier curves for comparison of pre-treatment and post-treatemtn progression free-survival in IPF and FPF subgroups.

After stratification of FPF subgroup according to specific antifibrotic treatment (20 with pirfenidone), we didn't observe any significant difference in terms of survival (log rank test: 0.08808, p=0.7877), while pirfenidone appeared to be slightly more effective in preserving FVC or DLCO than nintedanib (log rank test: 2.847, p=0.0490) (Figure 23).

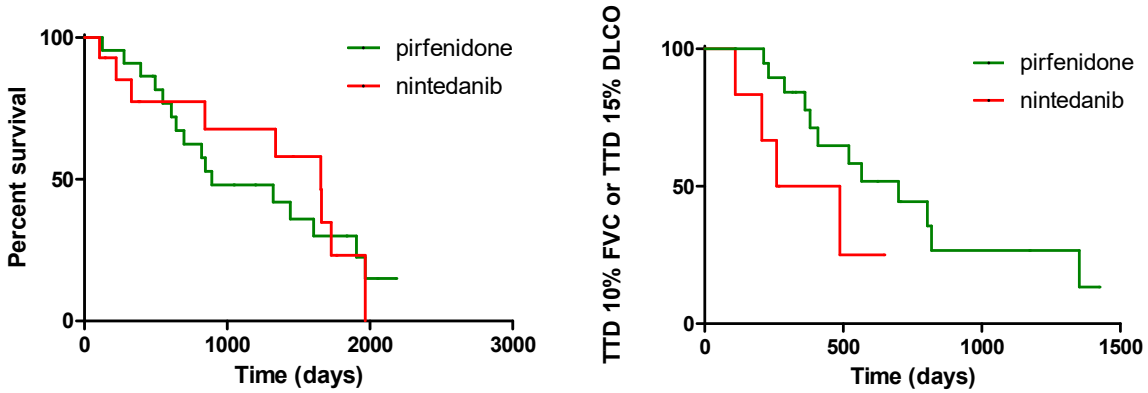

Figure 23. Kaplan Meier curves for comparison of mortality and functional disease progression in FPF patients treated with pirfenidone or nintedanib

Subgroup analysis: PF-ILD

Regarding demographic features, PF-ILD were on average significantly younger (66.6 ± 10.3 vs 74.3 ± 8.3, $p < 0.0001$) and the percentage of female patients was significantly higher than IPF cohort (p=0.0032). On the other hand, FVC and DLCO were substantially similar between the two groups at baseline (p=0.2744 and p=0.1807, respectively). As expected, only a minority (10/56 patients) showed a radiological pattern of definite or probable UIP at CT scan.

At 1st March of 2021 (463.5 ± 336.7 days of observation), median survival of PF-ILD patients was 1125 days. During the follow-up, 3/56 patients died (2 treated with nintedanib), 4 underwent lung transplantation (all treated with pirfenidone) and 5 interrupted the therapy due to adverse events (4 with pirfenidone).

In comparison with IPF cohort, we didn't observe any difference in overall survival (log rank test: 0.0697, p=0.7917); however, both pre-treatment and post-treatment time to decline of FVC or DLCO in PF-ILD subgroups were higher than in IPF population (log-rank test: 9.110, p=0.0279). Even if failing to reach statistical significance, antifibrotic treatment appeared to reduce decline rate of FVC, but not of DLCO (p=0.0956 and 0.5564, respectively) (Figure 24).

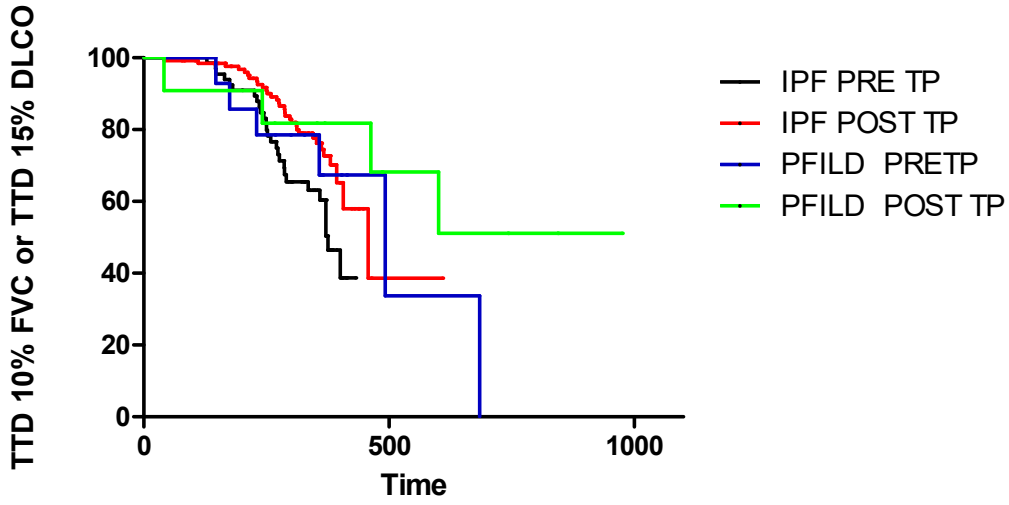

Figure 24. Kaplan-Meier curves for comparison of functional disease progression rate between IPF and PF-ILD subgroups before and after antifibrotic treatment

No differences of mortality or functional disease progression rate were observed between the PF-ILD subgroups treated with pirfenidone or nintedanib (log rank test: 0.9987, p=0.3187 and log-rank test: 0.3436, p=0.5577) (Figure 25).

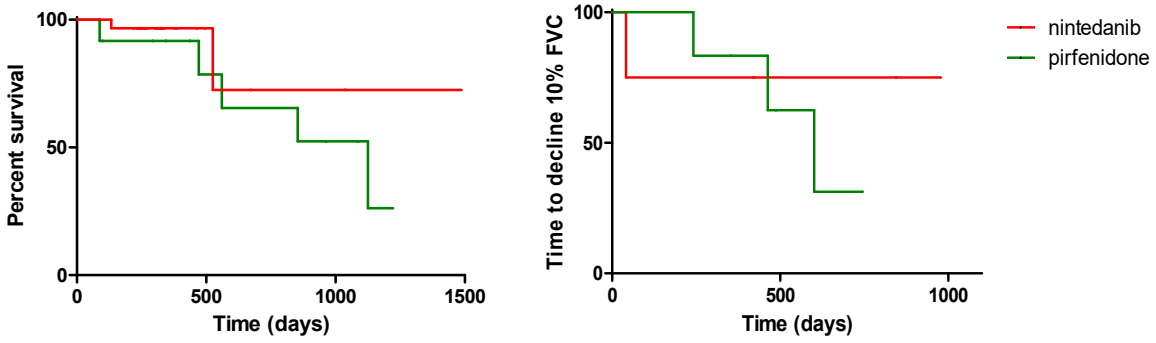

Figure 25. Kaplan Meier curves for comparison of mortality and functional disease progression in PF-ILD patients treated with pirfenidone or nintedanib

DISCUSSION

The aim of this research was to compare the only two pharmaceutical drugs currently approved for IPF in a real-life setting of a long-experienced Referral Centre for ILD. IPF, plus the other fibrotic ILD that may mimic its clinical course, represent an emerging health issue, standing the ageing and the exposure to risk factors of overall population and the relevant morbidity, hospitalization rate and mortality associated to these diseases. To date, lung transplantation is the only treatment able to "cure" diffuse fibrosing ILDs, but, unfortunately, it is a suitable option only for a well-selected minority of patients. Therefore, orally-available antifibrotic treatment plays a crucial role in the management of these patients, thanks also to the good safety and tolerability profile of pirfenidone and nintedanib (163). Although the effectiveness of these two drugs have been repeatedly demonstrated in RCTs and large multicenter observation trials (114,115,147), no solid comparative data are still available in literature.

In this study, we recruited all the ILD patients that have been treated with pirfenidone or nintedanib since 2011: our main aim was to compare the clinical effectiveness and impact of mortality of these two drugs in our quite large population and to evaluate potential differences related to specific phenotypes of disease. Moreover, according to recent guidelines endorsing a "working diagnosis" approach (1), we were able to detect a subgroup of patients with PF-ILD provisionally diagnosed with IPF and treated with pirfenidone: this allowed us to directly compare for the first time clinical outcomes in PF-ILD patients treated with pirfenidone or nintedanib.

Concerning mortality in IPF, our results showed that pirfenidone and nintedanib are substantially equal in terms of effectiveness. Medians of survival were analogue between the two subgroups and were significantly higher in comparison with data extracted from historical cohorts of patients in the pre-antifibrotic era (164,165). These findings highlighted the potential of these drugs in improving life expectancy in these patients, as already suggested by predictive models published in literature

(125,157). Our findings are particularly intriguing since they confirmed the efficacy of antifibrotic treatment in improving mortality in a real-life setting through almost ten years of observation.

In the same way, both pirfenidone and nintedanib confirmed their effectiveness in reducing functional disease progression in IPF: our findings are in line with data coming from RCTs (115,142) and they also suggest a sustained efficacy of both drugs in tapering FVC and DLCO decrease rate, since we didn't observe significant differences throughout the following years of treatment. Moreover, unlike RCTs, we included in the study population patients with a severe impairment of lung volumes or DLCO, supporting other previous reports that demonstrated an equal efficacy of antifibrotic treatment in this subgroup (121,144).

Interestingly, the only significant difference we observed between pirfenidone and nintedanib subgroup was in the comparison of DLCO decrease rate: nintedanib appeared to be more effective in stabilizing this parameter, especially during the first year of treatment. These finding may suggest a specific protective effect of nintedanib in preserving diffusion lung capacity, probably related to its antiangiogenic properties that appeared to be not expressed by pirfenidone (131,166). This assumption is also supported by the evidence that, in our population, nintedanib subgroup experienced a significant reduction of DLCO deterioration rate in respect with pretreatment trend, while pirfenidone failed, albeit slightly, to reach statistical significance on this field. However, baseline lung function data, as well as demographic features, were not homogeneous between the two treatment groups and, notably, this discrepancy tended to disappear or at least lost statistical significance during the follow-up: therefore, this aspect is worthy to be further investigated with age- and PFT-matched cohorts.

To our knowledge, this is the first study to compare the efficacy of different antifibrotic drugs in patients affected by FPF. FPF is defined as an idiopathic interstitial pneumonia affecting at least two members of the same family: unlike sporadic IPF, it may not seldomly occur in patients younger than 60 years old and often shows atypical radiological features at CT scan; moreover, in the same familial

cluster very different radiological patterns and clinical course may be observed (167–169). To date, few studies have investigated the effectiveness of antifibrotic treatment in these subjects, moreover reporting conflicting results (170,171). In our study, despite a younger age and similar functional parameters at baseline, FPF patients showed a reduced progression-free survival and a nearby significantly higher mortality risk in respect with IPF cohort. Notably, we didn't observe any modification in FVC and DLCO decline rate before and after treatment, suggesting that the effectiveness of antifibrotic therapy may be impaired in FPF subjects. Between the two drugs, pirfenidone seemed to be more effective in preserving lung function (but not in improving survival) than nintedanib: however, this comparison was limited by the small sample size (21 vs 14 patients) and, therefore, need to be confirmed in a larger cohort of patients.

Regarding PF-ILD, as for FPF, this is the first study comparing the efficacy profile of pirfenidone and nintedanib on this field. As expected, PF-ILD patients were significantly younger and showed a higher female prevalence than IPF subjects, while pretreatment FVC and DLCO decline rate, albeit clinically significant, was less pronounced. These findings are in line with literature data and mean annual FVC decrease observed in our study was similar to that reported in INBUILD trial (40). However, in contrast with INBUILD results, nor pirfenidone neither nintedanib appeared to reduce significantly the functional disease progression rate in these patients. Our findings are probably hindered by the small sample size and the limited time of observation: moreover, the entire pirfenidone and part of nintedanib subgroups were composed by subjects with a provisional diagnosis of IPF, that was later changed in view of a multidisciplinary re-evaluation due to explantation or modification of clinical/radiological status: accordingly, basal CT scans was suggestive for a definite or probable UIP pattern in all these patients. All these aspects may reasonably have influenced clinical course of PF-ILD patients and, probably, also the response to antifibrotic treatment. Interestingly, we didn't observe any significant differences in terms of survival or functional disease progression between nintedanib and pirfenidone subgroup: these findings are in line with the results reported in

the INBUILD and RELIEF trials, in which nintedanib and pirfenidone indirectly showed a similar efficacy in reducing FVC decline in absolute values (40,41).

CONCLUSION

In conclusion, our research study describes and comprehensively analyzes the almost decennial experience of our Referral Centre with antifibrotic treatment in the management of IPF and, more recently, of PF-ILD. Pirfenidone and nintedanib appear substantially equal in reducing functional disease progression in IPF and confirm their potential in improving life expectancy in these patients, while some concerns have raised for the management of FPF, in which antifibrotic treatment, and nintedanib particularly, seems to be ineffective. Regarding PF-ILD, our real-life preliminary data are surely promising and will probably contribute to "lead the way" to the antifibrotic treatment also in this field. Waiting for the new oncoming antifibrotic drugs, pirfenidone and nintedanib remained the milestones of pharmacological treatment of diffuse fibrosing ILDs and, therefore, our results provide further and intriguing insights in terms of long-term efficacy and personalization of therapy.

ACKNOWLEDGEMENTS

It's a genuine pleasure to express my deepest sense of gratitude to my mentor, Tutor and tireless guide Prof. PhD Elena Bargagli, my "North Star" as a researcher and physician.

I owe a deep sense of gratitude to my Supervisor Prof. PhD Elisa Frullanti, for her keen interest on every step of my Doctorate path and her kind helpfulness every time I needed an advice.

I feel absolutely blessed to have known and worked side by side with Dr BS Laura Bergantini and Miriana d'Alessandro, two real "forces of nature" with which I have shared (many) joys and (few) sorrows on our climb to publications.

Finally, I would like to thank with all my heart my family, the best family in the world, and Ilaria, who brings the sunshine in my life every day.

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