Idiopathic Pulmonary Fibrosis: Molecular Endotypes of Fibrosis Stratifying Existing and Emerging Therapies

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Keywords

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Abstract

Idiopathic pulmonary fibrosis (IPF) is a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown causes. Current diagnostic criteria are based on radiological, clinical, and histopathological features but, unfortunately, still many patients remain undiagnosed. Two currently approved therapies, pirfenidone and nintedanib, slow down disease progression but failed to block or revert it. On the other hand, many of the therapeutic agents tested in several clinical trials have not given satisfactory answers, probably due to the pathological heterogeneity of the disease. A growing number of studies show that IPF phenotype is the common clinical outcome of a variety of different pathophysiological mechanisms that identify disease subgroups characterised by specific genetic and molecular biomarkers (endotypes). The precision medicine approach is identifying

and analysing the complex system of genetic, molecular, environmental, and behavioural variables underlying the development of the disease and the response to therapy. These molecular pathways are potential targets for novel agents and useful diagnostic, prognostic, and theragnostic biomarkers. We outline the status of knowledge in this field by discussing the complex pathogenetic pathways underlying different disease subgroups and assessing a stratification approach to novel therapeutic agents based on these endotypes.

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Background

Idiopathic pulmonary fibrosis (IPF) is a specific form of chronic, progressive, fibrosing interstitial pneumonia of unknown cause, occurring primarily in older adults between 50 and 80 years of age, predominantly males. IPF patients complain of progressive worsening of dyspnoea and present impairment of lung function and gas exchange [1]. There is a significant heterogeneity in clinical presentation and a highly variable clinical course and response to therapies, which complicate patient management and prognostic evaluation. The disease is associated with a poor prognosis and a mortality rate higher than that of numerous cancers: the median survival is between 3 and 5 years, and the estimated 5-year survival rate is less than 30%. However, while some patients deteriorate rapidly, others follow a slower progressive functional decline, and some others experience a mix of periods of stability and steps of faster respiratory decline. The incidence of IPF seems to be increasing over the last years, with a prevalence rate in the USA estimated at 14–63 cases per 100,000 population [2, 3].

The definition of IPF requires the exclusion of other forms of interstitial pneumonia, including interstitial lung diseases (ILDs) associated with environmental exposure, medication, or connective tissue diseases [1, 4]. The diagnostic process is based on a multidisciplinary approach that comprises clinical, radiological, and (when available) histopathological evaluation. In this algorithm, chest high-resolution CT (HRCT) plays a key role since usual interstitial pneumonia (UIP) radiological pattern is sufficient to make diagnosis of IPF. If HRCT features do not fit with the criteria for the UIP pattern, surgical lung biopsy is recommended to confirm the diagnosis. However, it is necessary to underscore that the UIP pattern may also be found in a variety of conditions other than IPF, e.g. connective tissue diseases like rheumatoid arthritis, chronic hypersensitivity pneumonia, and asbestosis [5, 6]. The patients with a definite IPF diagnosis could be treated with pirfenidone or nintedanib, the currently 2 approved medications [7, 8]. The use of these drugs is associated with a slower progression of functional impairment, although the disease continues advancing despite therapy. In addition, these patients may develop adverse effects that, in some patients, can cause treatment discontinuation. Therefore, for a minority of patients, lung transplantation remains the only possibility to modify disease course [9, 10].

Since the publication of the evidence-based guidelines in 2011 [1], clinical practice has been identifying several

challenges of the diagnostic and therapeutic approach to IPF patients. In particular, a fraction of patients have an unclassifiable, progressive form of pulmonary fibrosis, which currently is a clinical management challenge. Furthermore, several patients with the features of possible UIP at HRCT cannot undergo surgical lung biopsy because of age, comorbidities, disease severity or personal preference. In some cases, even lung biopsy cannot provide a definitive diagnosis [11, 12]. All these issues, combined with an unpredictable and variable evolution of the disease and response to treatment, reflect a degree of heterogeneity among IPF patients, and suggest that several different biological factors play a role in the pathogenesis of the disease [13]. Indeed, the exact mechanism involved in the pathogenic pathways has not been fully clarified.

In the past, IPF was thought to be an inflammatory-driven disease, but the role of inflammation in IPF is still an unresolved issue. In fact, even if some features of inflammation are commonly observed in patients with IPF, e.g. enlarged mediastinal lymph nodes, recruitment of inflammatory cells in biopsies, areas of ground-glass opacity on HRCT, a combined therapy with anti-inflammatory and immunosuppressive agents has been shown to be harmful [13, 14]. The current concept is that inflammation might play a role, although it is not the *primum movens* in the pathogenesis of IPF, which appears today to be an epithelial-driven disorder [15]. There are several lines of scientific evidence supporting the concept that the pathogenetic process in IPF involves an abnormal wound-healing response, with activation and self-maintenance of many pro-fibrotic pathways, triggered by a persistent damage of alveolar epithelium cells. These injuries ultimately cause an imbalance between fibrotic and anti-fibrotic mediators, with fibroblast and myofibroblast proliferation, exaggerated deposition of extracellular matrix (ECM) and subsequently dysregulated tissue remodeling. The final outcome of this process is the derangement of the normal architecture of lung parenchyma and a consequent progressive loss of lung function [16]. In addition, evidence has emerged that the individual genetic background may contribute to disease pathogenesis: surfactant protein-related genes, telomerase complex genes, MUC5B are some examples of polymorphic loci that might increase the risk of developing IPF and may affect the natural history of the disease [17–19]. However, there is no established correspondence between genetic factors and histopathological or radiological patterns; moreover, the importance of epigenetic changes should not be underestimated, since several environmental risk factors may act through epigenetic mechanisms [12]. Neverthe-

less, despite the numerous efforts, we do not have yet an all-embracing solution to the problem of pathogenesis of IPF. Animal models with experimentally induced pulmonary fibrosis have been used to mimic the disease, but they do not replicate the IPF human pathological UIP pattern [20]. This discrepancy has limited the comprehension of pathogenic pathways and the development of new therapies, despite the many agents tested so far.

There is an urgent need to support research efforts on the pathogenesis of IPF rather than on the clinical phenotypes, by introducing the concept of IPF endotypes: the aim is to stratify patients into coherent and homogeneous genetic and molecular biomarker profiles. This approach epitomises the base of precision medicine. It has already been applied to several diseases, and it may play a central role in the future of IPF with impacts on the development of diagnostic, therapeutic, prognostic, and theragnostic approaches [10, 12, 13].

We review the existing therapeutic strategies and describe the scientific evidence on pathogenic molecular mechanisms of IPF, with the aim of identifying the molecular phenotypes of the disease and, ultimately, targeted therapies.

Precision Medicine: A Revolutionary Approach

Today we are witnessing a significant change in the history of medical research which promises to reshape the traditional approach to diseases and exponentially accelerate the development of innovative diagnostic and therapeutic strategies. Indeed, to date the current research method has been providing a slowing contribution to improve the knowledge about pathogenesis, diagnosis, and treatment [21].

In many fields of medicine, the introduction of a precision-based approach may change the scenario, providing new instruments to optimise global management of diseases. Precision medicine, as defined by the National Institutes of Health's Precision Medicine Initiative Working Group, is "an approach to disease treatment and prevention that seeks to maximise therapeutic effectiveness by taking into account these individual genetic, molecular, environmental, and lifestyle differences" [22]. In the last few years, this approach has represented a new frontier also in respiratory medicine, providing a revolutionary approach to several pathologies, such as lung cancer and asthma.

Pulmonary oncology has highlighted a major evolution through the development of specific therapies direct-

ed towards specific molecular targets, e.g. human epidermal growth factor receptor, whose mutations in non-small cell lung cancer allow patients to be treated with tyrosine kinase inhibitors [23]. In patients with asthma, the concept of Th2-like endotypes has allowed the development of personalised therapies: for instance, several trials have demonstrated the efficacy of lebrikizumab, a monoclonal antibody against IL-13, in Th2-high expression patients [24].

Precision medicine-based research aims at redefining the diagnostic, prognostic, and therapeutic management of IPF. The current approach to IPF aims to include patients with a similar clinical phenotype and without diagnostic criteria for other ILDs, into a common group, through a descriptive process. However, this approach does not consider the existence of distinct and clinically relevant subgroups of patients, characterised by genetics, molecular pathways, environmental, or behavioural factors: these would be the targets for a precision medicine-based approach. The objective of such a strategy is to identify clinically relevant endotypes, useful to reflect molecular characteristics of diseased tissue, to predict outcomes and to imagine a future in which the management of a patient with IPF may be as personalised as possible. This concept reflects the urgent need to clarify the complex pathogenesis of IPF, still largely unknown. Indeed, this clinical heterogeneity is due to a complex interaction between the various factors above mentioned, which could be grouped into 2 categories: genetic, molecular, environmental, and behavioural factors.

Genetic and Molecular Factors

Current evidence suggests that a critical role in IPF susceptibility is played by genetic predisposition. Several data support the existence of an individual susceptibility to fibrosis, reflected in familial forms of the disease, defined as the presence of at least 2 cases of the disease among first-degree relatives. A detailed analysis has identified several genetic polymorphisms associated with IPF, which could contribute to epithelial cell dysfunction, aberrant innate and adaptive immunity, and abnormal lung remodelling. However, the results of these studies were based on small patient cohorts. In the last few years, genome-wide association studies have detected several genetic loci that might confer an increased risk of developing fibrosis, e.g. polymorphisms in the MUC5B gene, found on chromosome 11 (11p15.5), or Toll-interacting protein (TOLLIP), or even the Toll-like receptor (TLR) 3 [25, 26].

Environmental Factors

Many studies indicate a contribution of environmental and behavioural factors to the pathogenesis of IPF, such as cigarette smoking, chronic microaspiration secondary to gastro-oesophageal reflux, air pollution, diet, physical exercise, professional exposures, and infections. Some of these factors determine the epigenetic modification of the genome as DNA methylation, histone modification, and non-coding microRNA (miRNA) [27]. Another research field investigates the role of lung microbial population in IPF, the so-called lung microbiome. Indeed, patients with IPF present a different composition of their microbiota, as compared to healthy individuals, although a specific microbe association has not been found yet. Patients who present an increased number of bacteria at diagnosis present a faster disease progression and a higher risk of mortality [28].

Precision medicine may represent the future of IPF management, stratifying several endotypes through a pathobiological, molecular, environmental, and behavioural approach. This process could also allow to define the aetiology behind this disease and could provide a substantial change in the disease name, by deleting the term idiopathic.

Genetic and Molecular Biomarkers

The role of molecular pathways in the pathogenesis, evolution, and diagnosis of IPF, is a recent and evolving breakthrough. It is widely accepted that IPF phenotype results from a heterogeneous pathogenetic process that involves epithelial injury, wound-healing, innate and adaptive immunity, and inflammatory infiltrates. Among these macro-categories, numerous molecules interact in different pathogenic pathways (Fig. 1) and are candidate to become targets for novel therapeutic agents. Others may become biomarkers that will enable quick and non-invasive diagnosis, estimate prognosis and response to therapy.

Dysfunctional Alveolar Epithelial Repair and Cellular Senescence

The initiation of the fibrotic process underlying IPF is not completely understood. Alveolar epithelium undergoes a different type of injury over time, and these stimuli may lead to an aberrant wound healing response that triggers fibrogenesis through pro-fibrotic molecular pathways. Cellular senescence and apoptosis are also involved in this mechanism, supporting the hypothesis that

there exists an age-dependent component in IPF pathogenesis.

miRNAs are short endogenous non-coding RNA molecules that regulate the expression of numerous genes and proteins. miRNAs can induce cellular differentiation, morphogenesis or apoptosis. Several studies show that miRNAs can alter proliferative and migratory properties of fibroblasts, can induce phenotypic changes in lung epithelial cells consistent with epithelial mesenchymal transition, and can regulate cellular senescence [29]. Many different types of miRNAs have been implicated in various cellular functions: miR-34, miR-200, miR-29, and miR-21 regulate cellular senescence [30]; miR-34 regulates cell cycle or protein expressions. Therefore, miR-34 upregulation in type II alveolar epithelial cells leads to downregulation of proteins involved in the cell cycle, resulting in cell senescence. Animal models of IPF show downregulations of miR-200 and miR-29 and upregulation of miR-21. Furthermore, TGF- β , a multifunctional cytokine belonging to the transforming growth factor superfamily, plays an important role in the upregulation of pro-fibrotic miRNAs and downregulation of anti-fibrotic miRNAs. miRNAs expressed in the serum of patients with IPF can be considered as epigenetic biomarkers useful in diagnosis and prognosis, or as therapeutic targets. Their expression is influenced by cigarette smoke, infections, genetic profile, age, and sex. Levels of miR-21, miR-155, and miR-101 in the serum of IPF patients correlate with FVC and HRTC features of IPF [31].

Reactive oxygen species (ROS), including anion superoxide (O_2^-) and hydrogen peroxide (H_2O_2), are produced intracellularly through multiple mechanisms and have a central role in host defence. Principal stimuli to ROS production include toxins like tobacco, silica, asbestos, radiation, or drugs such as bleomycin, and NADPH oxidase-4, and inadequate antioxidant defence. Oxidative stress is one important molecular mechanism underlying fibrosis in many organs and is also probably implicated in lung fibrosis in IPF, although the correlation has not yet been demonstrated. ROS production induces endoplasmic reticulum stress, promotes the apoptosis of airway epithelial cells, and enhances production of cytokines and growth factors involved in myofibroblastic differentiation and collagen deposition. There is also a probable association between ROS production and an increasing level of TGF- β . TGF- β_1 is a cytokine expressed in an inactive form in ECM, and needs multiple molecular mechanisms (ROS, proteases) for its activation [32]. TGF- β signalling participates in the wound-healing process performing a pro-fibrotic action and is involved in

Fig. 1. Pathogenic molecular pathways. AEC I, alveolar epithelial type I cells; AEC II, alveolar epithelial type II cells; CCL2, C-C motif chemokine ligand 2; CTGF, connective tissue growth factor; IL-13, interleukin-13; LOXL2, lysyl oxidase homolog 2; LPA1, lysophosphatidic acid 1; MMPs, matrix metalloproteinases; ROS, reactive oxygen species; TGF- β , transforming growth factor- β ; Th2, T-helper lymphocyte.

different kinds of lung fibrosis [33]. In fact, TGF- β induces production of NOX4 that has a central role in the pathogenesis of IPF, and genetic knockout or pharmacological inhibition of NOX4 prevents, in animal models, bleomycin-induced fibrosis.

Antioxidant defences such as catalase, glutathione, nuclear factor erythroid-2 (Nrf2) related factor-2, and superoxide dismutase are probably decreased in IPF. Catalase is a scavenger of H_2O_2 expressed in lung epithelial alveolar cells and inflammatory cells. In animal models, an intravenous infusion of polyethylene glycol catalase can block asbestosis, and intra-tracheal dose can prevent pulmonary fibrosis by inhibiting H_2O_2 production during inflammation. Superoxide dismutase decomposes superoxide radicals to H_2O_2 . Normal lungs express extracellular superoxide dismutase that, once deregulated, is im-

plicated in the pathogenesis of ROS-induced interstitial lung diseases, including IPF. Nuclear factor erythroid-2-related factor-2 is the main transcription factor of antioxidant response, and some studies describe its role in the development of pulmonary fibrosis [34].

Surfactant proteins are apolipoproteins called SP-A, SP-B, SP-C, and SP-D produced by alveolar type II cells. Mutations within genes that codify SP-A and SP-D have a key role in the pathogenesis of IPF. SP-A and SP-D confer innate immunity and promote bacteria and virus phagocytosis by macrophages. SP-A is also involved in a negative feedback to control the production of surfactant [35]. The levels of those 2 proteins are increased in IPF and are expression of type II alveolar epithelial cells hyperplasia or damage and are associated with worst prognosis. SP-A and SP-D are specific and sensible for ILDs

but cannot distinguish subtypes, and this is a major limit for their use in IPF diagnosis; however, they may be useful in early evaluation of high-risk patients. The analysis of plasma levels of SP-D, osteopontin, and MMP-7 proves useful in distinguishing IPF from others ILDs [36]. Some mutations in surfactant proteins increase pulmonary fibrosis creating endoplasmic reticulum stress, a profibrotic stimulus that promotes apoptotic pathways, inflammatory response, and induction of epithelial-mesenchymal transition.

Telomerase complex plays an essential role in maintaining the integrity of DNA especially during cell replications. The importance of telomere length is a critical factor in the pathogenesis of sporadic IPF and familial pulmonary fibrosis. Short telomeres are associated with cigarette smoke and genetic and environmental factors. Mutations in TERT and TERC genes are related to short telomeres and are associated with a rare hereditary disease called dyskeratosis congenital and approximately 1–3% of sporadic IPF. Numerous telomere alterations such TERT and TERC mutations can be also found in 7–15% of familial interstitial pneumonia [37, 38], and other rare variants in telomere-related genes are found in 15–20% of families with IPF. Telomerase mutations imply a reduction of telomere length both in lung tissue (alveolar epithelial cells) and in peripheral blood (leucocytes) of IPF patients [39], and these mutations are associated with reduced survival. Recent studies have described 2 new genetic targets involved in telomere shortening: PARN encodes an exoribonuclease implicated in mRNA processing and RTEL 1 as a regulator of telomere elongation [17].

Mucin family represents a group of glycosylated proteins which are macromolecular components of mucus secretions. Mucin 5B is a cytoplasmic protein of the secretory columnar cells of bronchi and proximal bronchioles, encoded by the MUC5B gene, providing a major contribution to lubrication and viscoelastic properties of normal lung mucus. MUC5B levels can be enhanced by many stimuli such as cigarette smoke, oxidative stress, interleukins (IL-6, IL-8, IL-13, IL-17), extracellular nucleotides, 17 β -estradiol, and epigenetic changes or DNA methylation [40]. Single-nucleotide polymorphism (SNP) in the promoter region of MUC5B (rs35705950) is a strong risk factor for developing IPF [18, 41]. Surely, MUC5B has a role in IPF pathogenesis, and this association is suggested by its higher expression in honeycombing cysts, distal airways, and respiratory bronchioles [42]. Another member of this family, Krebs von den Lungen 6 (KL-6/MUC1), is frequently elevated in the serum of IPF patients and is important both in pathogenesis and as predictor of risk

of exacerbation. Serum level of KL-6 might be a biomarker for the clinical diagnosis of IPF [43].

Integrins are a family of transmembrane receptors involved in cellular adhesion by attaching the cell cytoskeleton to ECM; moreover, they bind to the latent TGF- β complex (TGF- β -latency-activated peptide [LAP]). $\alpha\beta$ 6, $\alpha\beta$ 3, $\alpha\beta$ 5, and $\alpha\beta$ 1 are able to activate TGF- β through conformational changes; instead, $\alpha\beta$ 8 needs the cooperation of MMP14 to cleave the latent complex [44, 45]. $\alpha\beta$ 6 is implicated in TGF- β activation in the airways, initiating pro-fibrotic mechanism which leads to collagen production through an acute fibroblastic activation [46]. Blocking this integrin reduces epithelial injury and tissue fibrosis. Coherently with its action, $\alpha\beta$ 6 is overexpressed in IPF lungs and predicts worse prognosis [32, 33]. In the future, $\alpha\beta$ 6 will be used as a biomarker for the diagnosis, a prognostic marker, and a potentially therapeutic target in IPF [47].

ECM Remodelling and Fibroproliferation

ECM Remodelling

In the IPF lung, ECM deposition and cross-linking are abnormal, thus resulting in lung architecture distortion. Lung tissue fibrosis is a common end stage of different molecular mechanisms. Among these numerous pathways, there are several molecules that can represent a therapeutic target or become biomarkers useful to predict the clinical behaviour of the disease.

Matrix metalloproteinases (MMPs) are a group of zinc-dependent endoproteases involved in the degradation of ECM components that participate in ECM homeostasis, remodelling, and angiogenesis [48]. Several MMPs are upregulated during the wound-healing response, and matrix degradation seems to have a key role in IPF pathogenesis. Higher expression of several MMPs (1–9) and neoepitopes produced by their activity was identified by microarray techniques in bronchoalveolar lavage (BAL) and peripheral blood of IPF patients compared to healthy controls [49, 50]. Increased serum levels of neoepitopes are predictive of disease progression and survival [49]. A study by Morais et al. [51] evaluated MMP-1 and MMP-7 as biomarkers for differential diagnosis between IPF and other ILDs since serum levels of these 2 proteases were higher in IPF than non-IPF patients. MMP-7 dosage in peripheral blood resulted useful for the diagnosis of IPF with a cut-off value of 3.91 ng/mL. Higher blood levels of MMP-7 also have a prognostic role showing a significant correlation with disease severity measured by lung func-

tion tests [52], predicted survival [53], and poor prognosis in patients with established disease [54]. MMP-7 has also been related to HRCT abnormalities in a group of patients at risk to develop IPF [52, 53]. MMP-3 was detected in bronchiolised areas in IPF biopsy samples, and its blood concentration correlates with a faster disease progression [55].

Periostin is a secreted ECM protein that interacts with other ECM proteins through several integrins ($\alpha\beta1/\beta3/\beta5$) to support adhesion and migration of epithelial cells. Periostin forms a reticular structure connecting ECM proteins and promoting matrix deposition, mesenchymal cell proliferation, and fibrosis [56]. Periostin is secreted by fibroblasts, when stimulated by IL-13 or IL-4 and contributes to development of fibrosis in chronic asthma [57]. Periostin is overexpressed by fibroblastic foci and can be found elevated in blood samples of IPF patients. Its serum level correlates significantly with the decline in the diffusing capacity for carbon monoxide (DLCO) and vital capacity (VC) during a 6-month period, suggesting that serum periostin level may be a prognostic predictor in IPF patients [58]. A higher serum level of periostin significantly correlated with an increased honeycombing score on HRCT during a 6-month period and predicted a shortened overall survival [59].

Lysyl oxidases (LOXs) is family of copper-dependent enzymes responsible of covalent cross-linking of type I collagen. The resulting stiffness of fibrillar collagens increases local matrix structural tension and activates fibroblast and TGF- β_1 signalling. This pathway leads to a self-maintaining ECM deposition [60]. LOXL2, the most extensively studied member of this family, is secreted by activated fibroblasts and has a demonstrated pro-fibrotic action. This enzyme was associated with abnormal collagen accumulation in some neoplastic and fibrotic diseases, including IPF where it has been found elevated in lung biopsies [61]. Serum LOXL2 was studied as a potential biomarker of IPF disease activity and its higher levels have been associated with disease progression [62].

Fibroblast Activation and Proliferation

Fibrocytes are bone marrow-derived circulating mesenchymal progenitor cells that express a variety of markers such as leukocyte marker CD45, collagen-1, and hematopoietic stem cell marker CD34. Fibrocytes circulate in peripheral blood and are attracted to injured tissue by chemokines and growth factors. They can differentiate into fibroblast and myofibroblasts and secrete collagen and other ECM proteins, participating in fibrosis genesis [63]. Moeller et al. [64] demonstrated that circulating fi-

brocytes were significantly elevated in patients with stable IPF compared with healthy controls, and their number was an independent predictor of early mortality and worse survival with a cut-off value of >5%. In 2012, Fujiwara et al. [65] confirmed the presence of a higher level of circulating fibrocytes in IPF patients and demonstrated that their number correlated significantly with the pulmonary function test (FVC and DLCO) and disease activity, evaluated through measurement of serum levels of sialylated carbohydrate antigen (KL-6). Fibrocytes express chemokine receptors such as CXCR4 and CCR7, in particular CXCR4 is chemokine CXCL12's receptor, and the biological axis CXCL12/CXCR4 could mediate the contribution of fibrocytes to pulmonary fibrosis [66]. CXCL12 is increased in blood samples from IPF patients and shows a negative correlation between its plasma levels and lung function (DLCO and oxygen saturation on exercise). CXCL12 is strongly expressed by alveolar epithelial cells of IPF lungs and has been found elevated in BAL fluid in 40% of IPF patients.

Autotaxin is a secreted enzyme that converts lysophosphatidylcholine into lysophosphatidic acid (LPA), a lipidic signalling molecule involved in wound healing processes, homing of fibroblasts into the sites of injury, adhesion, and proliferation [67, 68]. It has also a role in increasing vascular permeability, favouring blood coagulation and collagen deposition. LPA stimulates its G-protein receptors (GPCRs) LPA1–5; among this family, LPA1 plays a role in fibroblast recruitment and vascular leakage, as demonstrated by Tager et al. [69] in 2008. In the same study, the authors found increased levels of LPA in the BAL fluid from IPF patients compared to normal controls. Moreover, another member of this family, receptor LPA2, is expressed in lung epithelial cells and activates $\alpha\beta6$ -mediated TGF- β through the interaction with Rho kinase (RHOK). In murine models, knocking out LPA1 and LPA2 seems to be protective from bleomycin-induced lung fibrosis [69, 70].

However, GPCRs do not seem to be the only molecular pathway in LPA signalling: in fact, these molecules can stimulate other receptors from tyrosine kinase family as mitogen-activated protein kinase, phosphatidylinositol 3-kinase (PI3K) and Janus kinase. These receptors activate various cellular pathways which produce several effects like increasing cell growth, proliferation and survival. Worth noting is the pathway activated by PI3K, through its interaction with protein kinase B (Akt), which blocks apoptosis [71]. This signalling pathway has been correlated with a poor prognosis and the risk of exacerbation [72].

Connective tissue growth factor (CTGF), also known as CCN2, is a matricellular protein that connects cell surfaces with ECM. CTGF is involved in wound healing processes through its role in cell adhesion, migration, proliferation, and angiogenesis [73]. Its overexpression, in cooperation with TGF- β , is profibrotic and exacerbates ECM production [74]. Higher BAL fluid levels of this protein have been found in IPF patients compared with healthy controls [75]. Some studies confirm an interaction between TGF- β and CTGF in fibrosis pathogenesis and increasing extent of bleomycin-induced fibrosis in mice [76].

An alternative mechanism involved in fibroblast activation is galectin-3, a galactoside-binding lectin that participates in the pathway of TGF- β -induced pulmonary fibrosis, stimulating myofibroblast activation and differentiation, collagen synthesis, and macrophage activation. Some authors studied galectin-3 pro-fibrotic action in different organs (liver, kidney). In 2012, Mackinnon et al. [77] showed that pulmonary fibrosis was less severe in animal models deficient in galectin-3. In the same paper, authors investigated galectin-3 as a biomarker of disease activity. They found an increased level of this protein in the BAL fluid and serum of stable IPF patients who undergo a further rapid increase during acute exacerbations of IPF [77].

Fibulin-1 is a glycoprotein produced by lung fibroblasts, which is found in ECM, blood, and human embryos, where it is necessary for alveolar septa morphogenesis [78]. Due to its established role in the pathophysiology of airway fibrosis in patients with chronic asthma, fibulin-1 was supposed to be involved in the mechanisms of fibrogenesis and progression of IPF through a dysregulated expression [79]. In a recent study, Jaffar et al. [80] have shown that fibroblasts of IPF patients produce more fibulin-1. Serum and tissue levels of this protein were increased in IPF compared with controls and correlated negatively with lung function (FVC).

Osteopontin is a glycoprotein biosynthesised by a variety of cell types, including lung fibroblasts, that mediates several biological functions: chemotaxis, cell adhesion, and tissue reparative processes [81]. A higher expression of osteopontin has been identified by microarray techniques in bleomycin-induced lung fibrosis in mice [82], and it seems to play a pro-fibrotic role [83]. Pardo et al. [84] showed that the osteopontin gene (SPP1) is upregulated in IPF lungs and its product is overexpressed by alveolar epithelial cells. Furthermore, the level of osteopontin in BAL in IPF patients was significantly elevated compared with healthy controls.

Immune Dysregulation and Inflammation

Inflammation and immune dysregulation have been considered key drivers in IPF. Clinical trials showed the lack of benefit from immune-modulatory and anti-inflammatory therapies [85, 86]; nonetheless, genetic and molecular studies suggest that inflammation and immune dysregulation have to be considered, at least, a collateral mechanism in the pathogenesis of IPF.

Innate Immunity

TOLLIP is an inhibitory adaptor protein that interacts with several components of the TLR and regulates the innate immune responses mediated by this family of receptors and the TGF- β signalling pathway. TLRs are non-catalytic receptors expressed in sentinel cells (macrophages, dendritic cells) that recognise structurally conserved molecules derived from microbes and activate immune cell responses. TOLLIP is encoded by the TOLLIP gene, and 3 single nucleotide polymorphisms (SNPs) have been found more frequently in IPF patients than healthy controls. Two of them (rs111521887, rs5743894) seem to be implicated in disease pathogenesis and are associated with IPF risk, and one (rs5743890) protects from the development of IPF but correlates also with mortality [25]. Among the TLR family, TLR3 recognises double-stranded RNA triggering innate antiviral immunity and is involved in activation of pro-inflammatory cascades [87]. The TLR3 polymorphism (L412F) decreases receptor activity in primary fibroblasts, reducing cytokines and deregulating fibroproliferative response as observed in TLR3 knockout compared with wild-type mice. O'Dwyer et al. [88] showed that a reduced TLR3 activity promotes progressive IPF, and L412F polymorphism was associated with disease progression and increased risk of mortality. Furthermore, this polymorphism could be a promising therapeutic target in IPF.

Adaptive Immunity

Infiltrating T cells are the prevalent immune population in IPF lung biopsies compared with normal lung specimens, and their presence has been found prominent in proximity to active fibroproliferative foci [89, 90]. The magnitude of intrapulmonary T-cell density in IPF correlates with the disease severity and prognosis [90], and more specifically increased CD8+ infiltration is associated with a progressive disease [91]. Circulating T cells in IPF patients are also deregulated, for example CD4+ CD25+ FOXP3+ regulatory T cells (Tregs), which con-

trol self-tolerance and prevent the development of autoimmune diseases, are reduced in the blood of IPF patients [92].

T cells participate in IPF pathogenesis mediating epithelium injury and subsequent epithelial cell apoptosis, a frequent finding in IPF lungs [93], and producing mediators that can activate, recruit, and/or alter functions of other immune effector cells including macrophages, neutrophils, and B cells [94]. Among T-cell mediators, there is a great interest around IL-13, a Th2 cytokine that has effects on B cells and monocytes. Usually, it promotes IgE class switching and inhibits inflammatory cytokine production, and, more recently, it was discovered that IL-13 is a regulator of the ECM and plays a role in inducing tissue fibrosis in schistosomiasis and asthma. Its overexpression has a direct pro-fibrotic action in animal models [95] and, on the other hand, its downregulation inhibits the development of fibrosis [96, 97]. Wilson and Wynn [98] demonstrated that an elevation in IL-13 level is involved in lung fibrosis, and several studies showed higher levels of this cytokine in lungs of IPF patients [99, 100]. These findings correlate directly with the rate of disease progression [97] and inversely with percent predicted FVC and DLCO [100]. IL-13 pro-fibrotic activity works in synergy with IL-4 [96], but its role is controversial. In order to get a better understanding of this relationship, Liu et al. [101] used the bleomycin model and showed that IL-4/IL-13 double knockouts and IL-13 single knockout had a similar attenuation of bleomycin-induced lung fibrosis, suggesting that IL-13 plays a key role. Comparable results have been obtained by Kolodsick et al. [95] by using a fluorescein isothiocyanate-induced lung fibrosis model.

Autoimmunity

Within lymphoid aggregates identified in lung biopsies from IPF patients [55, 102], B cells play a role in the pathogenesis of the disease. CXCL13 is a chemokine that regulates B-cell trafficking to lymphoid aggregates and inflammatory foci, and higher serum and lung tissue levels of this mediator correlate with lymphoid infiltration in IPF, and they are predictive of a poor prognosis [55, 103]. Furthermore, chronic lung injury exposes intracellular proteins by stimulating the production of autoantibodies against epithelial-derived antigens in IPF lungs [104], and these antibodies (i.e., antibodies to heat-shock protein 70) seem to be associated with a more progressive decline that was assumed to be secondary to a complement-dependent cytotoxicity [105].

Monocyte/Macrophage Activation

Monocyte chemoattractant protein-1 (MCP-1/CCL2) is a chemokine that regulates migration of monocytes, memory T cells, and dendritic cells to the site of injury. This process is implicated in routine immunological surveillance of tissues and in response to inflammation. CCL2 and its receptor are involved in the pathogenesis of various diseases, included IPF, where circulating CCL2 levels and fibroblast responsiveness to CCL2 are increased [99]. Monocytes can differentiate into various cell types, for example pro-fibrotic macrophages (M2 macrophages) that produce TGF- β and IL-13 and are predominant in IPF lungs [106]. Moreover, M2 macrophages secrete cytokine CCL18 to stimulate fibroblast proliferation and collagen production [106]. In IPF patients, serum CCL18 levels >150 ng/mL correlate with a more progressive disease [107].

Existing Therapies

Disease-Modifying Drugs

Current IPF therapies, pirfenidone and nintedanib, do not fully distinguish the pathobiological insights of translational science. Indeed, approved drug studies for IPF were not designed to individually customise treatment (Table 1). In the 2015 IPF treatment guidelines update, one drug was not recommended rather than the other, while this choice should be evaluated in each single IPF patient. Recently, the launch of a new clinical trial was announced, with the aim to investigate the effects of nintedanib on blood biomarkers. It is a 12-week, double-blind, randomised, placebo-controlled, parallel-group trial, followed by a single active arm phase of 40 weeks, evaluating the effect of 150 mg twice daily on changes in biomarkers of ECM and on FVC impairment (INMARK 1199.227 trial). It is expected that further studies will be performed taking into consideration the need to identify different IPF endotypes, based on biomarker profiles.

Pirfenidone is an orally administered pyridone derivative with anti-fibrotic, anti-inflammatory, and anti-oxidant properties [4]. Its effect consists of modulating cytokines and growth factors – TGF- β , basic fibroblastic growth factor, and TNF α among others [108]. Pirfenidone has been approved by the European Medicines Agency in March 2011. Several trials have shown its efficacy in mild-moderate IPF patients. The mechanisms of action of this drug have not yet been fully understood. It has been proven to induce a downregulation of L-arginine-arginase pathway activity that is involved in colla-

Table 1. Drug list with mechanisms of action

Drug	Mechanism of action	Trial
Pirfenidone	Downregulates cytokines and growth factors as TNF α , TGF- β Anti-inflammatory and anti-oxidant properties	NCT00287716 (CAPACITY 1, 2): phase 3 completed NCT01366209 (ASCEND): phase 3, completed NCT00080223: phase 2, completed NCT00287729: phase 3, completed NCT02141087: approved for sale
Nintedanib (BIBF 1120)	Inhibits several tyrosine kinases as PDGF receptors α , β , VEGF receptors 1–3, FGF receptors 1–3, FMS-like tyrosine kinase 3, and the non-receptor tyrosine kinases Src, Lyn, and Lck	NCT01335464 (INPULSIS – 1): phase 3, completed NCT01335477 (INPULSIS – 2): phase 3, completed NCT01619085 (INPULSIS – ON): phase 3, active NCT02598193 (studied with pirfenidone): phase 4, recruiting patients NCT02579603 (studied with pirfenidone): phase 4, active NCT02606877 (studied with pirfenidone): phase 4, recruiting patients
Integrin antagonists STX 100 (BG00011) GSK3008348	Antagonize α v β 6 integrin activation of the TGF- β ₁ pathway	NCT01371305: phase 2, recruiting patients NCT02612051: phase 1, completed
GLPG1690 (autotaxin inhibitor)	Reduces the production of extracellular lysophosphatidic acid (LPA) and background inflammation, inhibiting autotoxin	NCT02738801: phase 2, recruiting patients
CC-90001	Inhibits the MAPK 8 (JNK) pathway	NCT02510937: phase 1, phase 2, recruiting patients
BMS-986020 (anti-LPA1)	Promotes fibroblasts apoptosis and reduces collagen deposition	NCT01766817: phase 2, completed
GSK2126458	Reduces mitogenic fibroblast responses through inhibition of the PI3K/Akt/mTOR pathway	NCT01725139: phase 1, completed
KD025 (Slx-2119)	Reduces IL-21 and IL-17 levels	NCT02688647: phase 2, ongoing
PRM-151	Inhibits the differentiation of circulating monocytes into M2 pro-fibrotic macrophages	NCT02550873: phase 2, ongoing
Aerosol IFN- γ	Promotes Th-1 phenotypes and inhibits Th-2 differentiation	NCT00563212: phase 1, ongoing
Rituximab (anti-CD20)	Inhibits CD20 reducing B-cell activity	NCT01969409: phase 2, recruiting patients NCT01266317 (studied in combination therapy): phase 1, phase 2, ongoing
Dabigatran	Reduces pro-thrombotic state in IPF Inhibits thrombin-induced fibroblast proliferation	NCT02885961: design phase
Anti-IL-13 Tralokinumab (CAT354) QAX 576 Lebrikizumab Anti-CCL2 CNT0888	Antagonize fibroblast activation TGF- β -induced, inhibiting IL-13 and CCL2	NCT01629667: phase 2, terminated NCT02036580: phase 2, completed NCT01266135: phase 2, terminated NCT00532233: phase 2, completed NCT01872689: phase 2, recruiting NCT00786201: phase 2, completed
FG-3019 (anti-CTGF)	Blocks fibrosing process avoiding the interaction between TFG- β and CTGF	NCT01890265: phase 2, active
TD 139 (anti-galectin-3)	Regulates TGF- β function	NCT02257177: phase 1, phase 2, completed
Tipelukast (MN-001)	Antagonizes leukotriene receptor, inhibits phosphodiesterase and 5-lipoxygenase, downregulates pro-fibrotic (LOXL-2, collagen type 1, TIMP-1) and pro-inflammatory (CCR2, MPC-1) genes	NCT02503657: phase 2, recruiting patients
IW001 (collagen V)	Induces tolerance to collagen to autoreactive T cells	NCT01199887: phase 1, completed

gen production in IPF lungs. The anti-fibrotic property of pirfenidone also acts by depressing TGF- β_1 production [2] downregulating its gene as shown in animal models [109]. Furthermore, pirfenidone was able to stop the production of lung interleukins (IL-1 β , IL-6, IL-18, IL-12p40) and MCP-1, usually increased in bleomycin-induced fibrosis. Moreover, its anti-fibrotic effect is exerted through suppression of the lung stroma cell-derived factor (SDF-1 α), IFN- γ and lung basic-fibroblast growth factor [3].

Nintedanib, previously named BIBF 1120, is an intracellular inhibitor of various tyrosine kinases which prevents development of fibrosis in animal models. Nintedanib has effect on various molecular signalling pathways implicated in pathogenesis and progression of fibrosis [110]. For example, it inhibits some tyrosine kinases receptors (PDGF α - β , VEGF 1–3, FGF 1–3), FMS-like tyrosine kinase 3 and non-receptor tyrosine kinases Src, Lyn, and Lck [111]. Nintedanib also influences fibroblast proliferation, migration and differentiation to myofibroblasts, and the secretion of ECM since it has an indirect effect on TGF- β through the FGF inhibition [112]. In animal models of lung fibrosis, treatment with nintedanib is preventive and therapeutic through the inhibition of mRNA expression of TGF- β that gives anti-fibrotic and anti-inflammatory effects [113]. Moreover, it has recently been demonstrated by experimental data that nintedanib exerts anti-fibrotic activity, including downregulation of ECM production (fibronectin and collagen 1a1), independently from TGF- β signalling [114].

There are no direct comparative studies between pirfenidone and nintedanib; however, 3 combination studies (NCT02579603, NCT02598193, NCT02606877) are ongoing, investigating the possible use of pirfenidone in addition to nintedanib in sequential or combined regimens [115].

Emerging Therapies

Targeting ECM Remodelling and Fibroproliferation

BG00011 (anti- $\alpha\beta_6$). Partial inhibition of $\alpha\beta_6$ integrin mediated by antibodies represents a therapeutic strategy, since, in murine models, it prevents bleomycin-induced pulmonary fibrosis without exacerbating inflammation [116]. The humanised monoclonal antibody against $\alpha\beta_6$ integrin, STX-100 (BG00011) has to be tested in a phase 2 trial (NCT01371305, currently recruiting patients) for establishing safety and tolerability in IPF patients. While writing, a new trial on inhibition of integrins in IPF is ongoing. GSK3008348 is a novel drug which an-

tagonises integrin $\alpha\beta_6$. It is the first trial on this molecule and has the purpose of studying pharmacokinetics, safety, and tolerability of a single daily nebulised drug dose (NCT02612051).

GLPG1690 (Autotaxin Inhibitor). Inhibiting autotaxin enzymatic activity is a therapeutic strategy aimed at reducing the production of extracellular LPA. A phase 1 trial has demonstrated the safety, tolerability, and favourable pharmacokinetics of this drug. A phase 2 trial is currently ongoing with the aim to compare orally administered 600 mg of GLPG1690 versus placebo for 14 weeks. The end points of this trial are: changes in inflammatory index levels and improvement in lung function tests (NCT02738801).

BMS-986020 (LPA1). LPA1 antagonism reduces pro-fibrotic and pro-inflammatory mediators in mice bleomycin model [117]. A phase 2 trial is evaluating safety and efficacy of BMS-986020 (AM152), an anti-LPA1 receptor drug. The dose of 600 mg is orally administered once or twice daily for 26 weeks compared with placebo (NCT01766817).

CC-90001 (C-Jun NH2-Terminal Kinase Inhibitor). C-Jun NH2-terminal kinase (JNK) is a member of the mitogen-activated kinase and has a role in induction and prevention of apoptosis. Subsequently, it has been shown that in human lung fibroblasts TGF- β_1 downregulates VEGF-D expression through both TGF- β receptor and JNK signalling pathway [118]. Actually a phase 1/2 trial is ongoing with the aim to evaluate the safety, tolerability, pharmacodynamics, and pharmacokinetics of a JNK inhibitor (CC-90001) in IPF patients (NCT02510937).

GSK2126458 (PI3K Signalling Inhibitor). The use of pan-PI3 kinase (PI3K)/mammalian target of rapamycin (mTOR) in IPF is an example of the potential role of existing oncology drugs as novel therapeutic agents in IPF patients. GSK2126458 inhibited PI3K signalling and functional responses in IPF-derived lung fibroblasts, through blocking Akt phosphorylation in cells derived from tissue and BAL of IPF patients [119]. A double-blind, placebo-controlled, parallel study of GSK2126458, a PI3K/mTOR inhibitor, has been completed (NCT-01725139, waiting for results). The kinase mTOR is a PI3K involved in cell growth, motility, proliferation, and survival. Sirolimus, also known as rapamycin, is an anti-biotic and immunosuppressive drug and seems to have a strong anti-fibrotic action. This drug was tested on bleomycin-induced fibrotic mice lungs, and it has been proven to be effective in decreasing levels of cellular inhibitor of metalloproteinase-1 (TIMP-1) and metalloproteinase-9 (MMP-9) [120]. However, it has shown to also have

pro-fibrotic effects, mediated by the PI3K-pathway [121], and this dual effect suggests that we should use it carefully in IPF. A trial is now ongoing (NCT01462006) to evaluate sirolimus tolerability and its property to reduce the number of circulating fibrocytes in IPF patients.

KD025 (RHOK Inhibitor). KD025 (Slx-2119) is a selective inhibitor of Rho-associated protein kinase 2 which is an effector of system of Rho GTP-ases, basic for cytoskeletal activity, motility, and cell contraction [122]. In phase 1 trial on healthy subjects, KD025 reduces the levels of IL-21 and IL-17 secreted by T cells, but it does not seem to reduce the response to stimulus of IFN- γ [123]. Currently, a phase 2 trial is ongoing to test the safety and efficacy of oral administration of 400 mg once a day for 24 weeks (NCT02688647).

FG-3019 (Anti-CTGF). An anti-CTGF antibody was studied in murine bleomycin-induced fibrosis model. It inhibited collagen deposition and demonstrated a reduction of histological signs of fibrosis [124]. A human anti-CTGF antibody, FG-3019, may be potentially used to inhibit pulmonary fibrosis. Its efficacy and safety are going to be evaluated in a phase 2 trial in IPF patients (clinicaltrials.gov identifier NCT01890265). An open-label phase 2 study to evaluate the safety and tolerability of intravenous infusions every 3 weeks for 45 weeks (NCT01262001) is ongoing. Preliminary analysis showed that FG-3019 was safe and overall well tolerated. An open-label phase 2 trial has evaluated the safety and efficacy of 2 doses of FG-3019 administered by intravenous infusion every 3 weeks for 45 weeks in IPF patients and showed positive data for the use of FG-3019 [125].

PBI-4050. PBI-4050 is another anti-fibrotic antibody that demonstrated efficacy in reducing CTGD and collagen deposition in preclinical studies. A phase 2 open-label single-arm trial is ongoing, exploring safety and tolerability of oral administration of 800 mg per day of PBI-4050 in 40 adult IPF patients (NCT02538536). PB-4050 was shown to inhibit CTGF and collagen I mRNAs expression in bleomycin-induced lung fibrosis models [126].

TD139 (Anti-Galectin-3). TD139 is an anti-galectin-3 drug administered via dry-powder inhaler. TD139 has been studied in a phase 1/2 trial (NCT02257177) to assess its safety, tolerability, pharmacodynamics, and pharmacokinetics in healthy human volunteers and IPF patients.

Dabigatran. It is known that IPF patients have a prothrombotic state 4 times higher than healthy controls, and this finding is directly related to poor prognosis [127]. Although trials, such as ACE-IPF, on the effective-

ness of warfarin in IPF were not encouraging, there are more expectations on the use of direct thrombin inhibitors like dabigatran [128]. This drug showed, in vitro, a dose-related inhibition property on lung fibroblast proliferation and collagen gel concentration, both thrombin-induced. Moreover, dabigatran stopped differentiation of fibroblast in myofibroblast resulting in an anti-fibrotic effect [129]. In bleomycin-induced pulmonary fibrosis animal model, dabigatran has been shown to have anti-fibrotic and anti-inflammatory effects reducing collagen, CTGF, and α -SMA expression [130]. A new trial on the coagulation cascade and use of dabigatran in IPF is currently in the planning phase, and it is hoped it downregulates inflammation activity (NCT02885961).

Targeting Immunity and Inflammation

Dasatinib and Quercetin. Dasatinib is a platelet-derived growth factor receptor and Src-kinase inhibitor. In an animal model, this drug attenuated fibrosis progression through reduction of mesenchymal cell proliferation, myofibroblast differentiation, and interstitial collagen deposition [131]. It had already been shown that in models of acute silicosis it reduces remodelling through inhibition of pro-inflammatory pathways and stimulation of anti-inflammatory and anti-fibrotic cells [132]. Another therapeutic option was proposed: use of dasatinib associated with quercetin (a flavonol able to prevent the oxidation of lipoproteins expelling free radicals and linking metal ions). In mice with induced fibrosis, quercetin causes a decrease in lung injury [133]. A study was designed to evaluate the association of dasatinib and quercetin in reducing the expression of pro-inflammatory cells in skin biopsy (NCT02874989). It is a phase 1 open-label human pilot study and is recruiting patients.

Lebrikizumab (Anti-IL-13) and CNTO 888 (Anti-CCL2). Several trials have been designed to test the efficacy of anti-IL-13 monoclonal antibodies. A phase 2 study designed to establish safety, tolerability, pharmacokinetics, and efficacy of anti-IL-13 antibody QAX576 was completed but is still without results (NCT01266135). Tralokinumab (NCT01629667), a human antibody for IL-13, was studied in a phase II trial that has recently been terminated, showing negative results. A phase 2 trial about lebrikizumab, another human recombinant antibody against IL-13, is currently recruiting participants (NCT01872689). The safety and efficacy of the anti-CCL2 antibody, CNTO888, was tested in a phase 2 trial that has recently been terminated (NCT00786201), and results are awaited.

PRM-151 (Serum Amyloid P). Serum amyloid P, also known as endogenous blood plasma protein pentraxin (PTX-2) is involved in wound healing and inhibits the differentiation of circulating monocytes into M2 pro-fibrotic macrophages and fibrocytes. It regulates circulating bone marrow-derived mesenchymal progenitor cells, whose levels are elevated in IPF patients with a poor prognosis. PTX-2 levels are lower in patients with fibrotic diseases [134]. In mice models and in lung fibrosis with TGF- β overexpression, PTX-2 intravenously administered is correlated with reduction of fibrosis [135]. Safety, tolerability, and pharmacokinetics of a single ascending intravenous dose of PRM-151, a recombinant human form of PTX-2, was studied in a phase 1 trial. It has shown a reduction of fibrocyte numbers 24 h after dose administration [136]. A phase 2 randomised, double-blind, placebo controlled trial is now ongoing for patients with IPF to test the efficacy and safety of PRM-151 administered every 4 weeks for 6 months (NCT02550873).

Aerosolised IFN- γ . IFN- γ is a cytokine which promotes differentiation of T-helper lymphocytes towards Th-1 phenotype. Inflammation in IPF seems to be triggered by Th-2 lymphocytes, so IFN- γ is supposed to reduce inflammation [137]. Aerosol therapy seems to be a feasible way of administering IFN- γ , thus reducing systemic side effects. The most common side effect was cough during inhalation [138]. A phase 1 trial on subcutaneous therapy with IFN- γ is ongoing (NCT00563212).

Rituximab (Anti-CD20). In ILD, there is often a dysfunction of B-lymphocytes against the self-matrix component. Rituximab, a monoclonal antibody directed to CD20 protein on the cell surface of B lymphocytes, seems to have some benefit in severe non-IPF disease. It would improve FVC value and DLCO stability over time [139, 140]. A trial is currently recruiting patients to investigate the effects of autoantibody reduction (NCT01969409), and another is ongoing on combined therapy including the use of rituximab in acute exacerbations of IPF (NCT01266317).

IW001 (Type V Collagen). Collagen V is a fibrillar-structured collagen that is involved in tissue compliance and elasticity and may play a role in the development of fibrotic lung diseases. Lung injury causes a T-cell-dependent immunity response that brings a release of collagen V fragments [141]. In animal models, collagen V-induced tolerance abrogates the bleomycin-induced fibrotic process and determines a reduction of TGF- β -associated fibrotic effects [142]. A phase 1 study has demonstrated the safety and tolerability of IW001 (collagen V) when ad-

ministrated orally once a day for 24 weeks in IPF patients, with no unexpected adverse events [143].

Tipelukast. Tixelukast (also known as MN-001) is a molecule already tested in asthma patients. It seems to have a dose-dependent efficacy and has different targets: it antagonises leukotriene receptor, inhibits phosphodiesterase and 5-lipoxygenase, and downregulates both pro-fibrotic (LOXL-2, collagen type 1, TIMP-1) and pro-inflammatory (CCR2, MPC-1) genes [144]. Currently, there is just one phase 2 trial recruiting patients for testing efficacy, safety, and tolerance of tiixelukast 750 mg twice per day in IPF patients versus the placebo arm (NCT02503657).

Conclusions

Basic, translational, and clinical IPF research is a rapidly growing field. It has been extensively demonstrated that pulmonary fibrosis is the product of distinct molecular pathways that lead to a common phenotype, and the current diagnostic criteria seem to be inadequate to describe the heterogeneity underlying this phenotype. Moreover, the 2 therapies currently approved, pirfenidone and nintedanib, are broad-spectrum inhibitors of several molecular pathways and should be considered as a starting point in the understanding and treatment of this disease. Over the next few years, according to the principles of precision medicine, the re-definition of disease subgroups taking into consideration molecular, behavioural, and environmental criteria will prove essential. The aim of this process is to: (1) add a further non-invasive diagnostic step that reduces the number of patients that still remain undiagnosed and be able to include non-IPF patients, who are now unclassifiable, in some endotypes; (2) stratify patients into subgroups which share the main pathogenic mechanism; (3) recruit more accurately patients for clinical trials in order to allow better associations of novel agents with specific molecular subgroups; (4) identify a definite disease process to allow clinicians to choose which drug should be administered first in each endotype, by configuring first- and second-line therapies or combination therapies; (5) obtain reliable biomarkers to assess prognosis and, especially, the response to a chosen anti-fibrotic treatment. In the coming years, this innovative research strategy could revolutionise the current landscape of IPF. Scientists and all stakeholders involved in the diagnostic and therapeutic process of IPF will face the challenge to reinterpret the pathophysiology to obtain therapies that can consider-

ably influence the mortality from IPF. The detailed knowledge of molecular pathogenic pathways may also subvert the concept of irreversibility of IPF, and disease regression will no longer be a utopia.

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