# Insights into interstitial lung disease pathogenesis

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## Abstract

This review summarises some of the key features of interstitial lung diseases (ILDs) from a translational science point of view and brings insights into potential therapeutic options. Genetic predisposition and environmental factors like smoking, pollution and infections significantly impact the onset, progression and treatment response in ILDs, highlighting the need for personalised management. Fibroblasts are central to ILD pathology, influencing the tissue microenvironment, immune cell interactions and extracellular matrix (ECM) production, making them critical therapeutic targets. Monocyte-derived M2 macrophages drive fibrosis in idiopathic pulmonary fibrosis by secreting cytokines and remodelling the ECM. Understanding macrophage subtypes and their dynamics offers new therapeutic possibilities. Chronic type 2 immunity contributes to fibrosis, emphasising the need to enhance protective markers in order to even out the balance shift of pathological immune responses in ILD treatments. Serum biomarkers like Krebs von den Lungen-6 (KL-6), surfactant protein (SFTP) D, matrix metalloproteinase-7 (MMP-7), and C-C motif chemokine ligand (CCL)-18 are valuable for diagnosing and predicting ILD progression, although more research is needed for clinical application. Animal models, especially bleomycin-based models, offer insights into ILD pathology, but challenges like lung hyperinflation highlight the need for careful model selection and translational research to bridge preclinical and clinical findings.

## Educational aims

- Genetic and environmental factors affect the onset, progression, and treatment response in ILDs.
- Fibroblasts and macrophages are critical in ILD pathology, presenting new therapeutic opportunities.
- Serum biomarkers are promising for diagnosing and predicting ILD progression.
- We need to select ILD models for translational research to bridge preclinical and clinical findings.

## Introduction

Interstitial lung diseases (ILDs) constitute a group of heterogeneous pulmonary disorders with varied aetiologies encompassing genetic factors, environmental exposures or unknown origins. The processes taking place in ILDs insidiously alter the structure of the lung parenchyma, including alveoli, alveolar ducts and bronchioles, thus transforming it into fibrous tissue that may lead to respiratory failure, the need

for transplantation or even death [1]. Available epidemiological data show a wide variation in the incidence of ILDs across age, gender, ethnicity and geographical regions [1]. Currently, two antifibrotic treatments that can slow down disease progression are available, pirfenidone and nintedanib. However, it remains that no medication has been found that can cure the disease or reverse the fibrotic lesions. Therefore, there is the utmost need for further research and discovery of novel therapeutic targets that may lead to better outcomes and quality of life.

## Mechanisms driving ILDs: from genetics to environmental factors

Recent evidence has shown the role of genetics in patients with ILDs, shedding light on the pathogenesis of this challenging group of chronic lung disorders [2, 3]. The term familial pulmonary fibrosis (FPF) is used when two or more first- or second-degree family members are diagnosed with fibrotic ILD [2]. Approximately one quarter of families with idiopathic pulmonary fibrosis (IPF) carry an associated gene mutation, whilst a relative of a patient with FPF has 10% probability to develop pulmonary fibrosis [2, 4]. Using whole genome sequencing, it was detected that 5% of individuals with sporadic disease had a genetic predisposition [4].

Two major groups of genes associated with FPF have been the most studied: those implicated in telomere homeostasis, telomere-related genes (TRGs); and those related to surfactant homeostasis, surfactant-related genes (SRGs) [5]. Overall, TRG mutations are the most common in adults, and SRG mutations in childhood. Although international guidelines do not clearly suggest genetic sequencing in FPF cases, genetic testing is increasingly present in clinical practice in ILD centres [6]. A recently published European Respiratory Society statement on FPF focused on TRGs and SRGs [2], while statements for other mutations such as lysosome-related mutations or interferonopathies are lacking, mainly due to the rarity of these cases and consequently, resulting in limited evidence in the literature [2].

Regarding telomeres, they consist of non-coding DNA (TTAGGG) at the end of the chromosomes, protecting genome integrity. The telomere complex, which is responsible for the addition of the telomeric repeats, consists of the enzyme telomerase transcriptase, telomerase RNA component and dyskerin protein complex. Telomeres also consist of a six-protein complex component called shelterin that protects telomeres, and includes TRF1, TRF2, RAP1, POT1, TPP1 and TIN2 [7]. Telomere length is expected to decrease with age, but telomere length below a critical point comprises a hallmark of aging and is associated with cellular senescence and stem cell exhaustion it may also be related with organ dysfunction beyond the lung – dyskeratosis congenita, bone marrow failure and liver disease [7]. Shorter telomere length has been detected in blood leukocytes, alveolar epithelial cells and fibroblasts, both in familial and sporadic cases of ILD [2, 8, 9]. Interestingly shorter telomere length has been identified in 15% of ILD patients without a TRG mutation [10]. People carrying a TRG mutation have shorter telomeres compared to age matched controls, irrespective of symptoms, while half of all ILD patients older than 60 years have telomere length below the 10th percentile [11]. Importantly, short telomere length has been associated with worse clinical outcome in fibrotic ILDs [12–14], increased fibrosis [15, 16] and poor tolerance to immunosuppressive treatments [17–20].

Pulmonary surfactant is synthesised and secreted by alveolar type II (AT2) epithelial cells and consists of a unique mixture of phospholipids and proteins that are essential for reducing alveolar surface tension and preventing alveolar collapse [21]. SRGs include *surfactant protein (SFTP)-A1*, *SFTPA2*, *SFTPC*, NKX2.1 and ATP binding cassette family A member 3 (*ABCA3*), which have higher penetrance compared to TRGs, but low frequency in FPF cases (approximately 5%) [2, 21]. SRG mutations are associated with accumulation of misfolded proteins, inducing endoplasmic reticulum stress and AT2 cell apoptosis. The lipidic transporter ABCA3 is involved in the transport and storage of surfactant proteins into lamellar bodies in AT2 cells. *NKX2.1*, also known as thyroid transcription factor 1, is a transcription factor that regulates the expression of surfactant proteins A, B and C, as well as ABCA3 [22–24].

Single nucleotide polymorphisms are single base pair changes in the DNA sequence that represent DNA polymorphisms and may affect the gene function and the activity of the encoded protein [25]. Increased risk of IPF development has been linked to polymorphisms in the promoter of the gene encoding salivary mucin 5b (*MUC5B*), and the Toll interacting protein (rs5743890; TOLLIP) [26]. The MUC5B rs35705950 polymorphism is associated with both familial and sporadic pulmonary fibrosis and is characterised by high prevalence and low penetrance [27]. Whether its existence is related to worse prognosis is under investigation, though it may serve as a promising target for precision medicine [28–30].

The current theory of pulmonary fibrosis pathogenesis is based on recurrent triggers in a genetically predisposed epithelium leading to epithelial damage, secretion of profibrotic mediators and, finally,

activation of fibroblasts. Genetic predisposition has been found in a wide spectrum of fibrotic ILDs [30–32]. It is crucial to note that even when an underlying cause has been identified [33, 34], a genetic predilection may influence the disease development, response to treatment and prognosis [30, 35, 36].

Bronchial and alveolar epithelium is in continuity with inhaled air; thus, environmental factors can act as the triggers for ILD development [3]. Cigarette smoking represents a well-established factor of ILD pathogenesis [37–39]. Regarding air pollution, there is evidence showing that an increase in nitrogen dioxide and particulate matter (PM) with aerodynamic diameter <2.5 µm ($PM_{2.5}$) and <10 µm ($PM_{10}$) exposure is associated with an increased incidence of IPF, acute exacerbation or decline in respiratory function [40–45]. Combining genetic background with environmental exposures, it has been shown that individuals with a high polygenic risk score involving 13 single nucleotide polymorphisms and who were exposed to airborne pollutants had the highest risk of IPF incidence compared to low air pollution and low polygenic risk score [42]. The effect of environmental exposures has been suggested to be through epigenetic alterations; DNA methylation, histone modifications and noncoding RNAs (particularly microRNAs) [46], telomere shortening [47], or through overwhelming ciliary and macrophage clearance mechanisms leading to oxidative stress [48].

Host environment, possibly linked with micro-aspiration, gastro-oesophageal reflux and viral exposures has also been correlated with an increased risk of ILD [36, 49]. The microbiome represents another persistent exposure to the lung and airways. Patients with IPF have an increased bacterial burden and loss of microbiome diversity, although the underlying mechanisms that drive disease pathogenesis remain unknown [50]. The genetic and environmental factors implicated in ILD pathogenesis are summarised in figure 1.

Type 2 immunity, driven by cytokines like IL-4, IL-5 and IL-13, is traditionally linked to allergic inflammation and defence against helminths. More recently, type 2 immunity has been recognised for its role in tissue repair and regeneration [51]. However, chronic or dysregulated type 2 responses can contribute to fibrosis. While older studies have reported elevated type 2 cytokines and eosinophils in bronchoalveolar lavage fluid (BALF) from patients with sarcoidosis, IPF, and systemic sclerosis-associated ILD (SSc-ILD) [52], the precise role of type 2 immunity in ILD progression remains unclear and has been largely underexplored in recent research. Notably, in SSc, type 2 markers were previously shown to correlate with ILD severity [53].

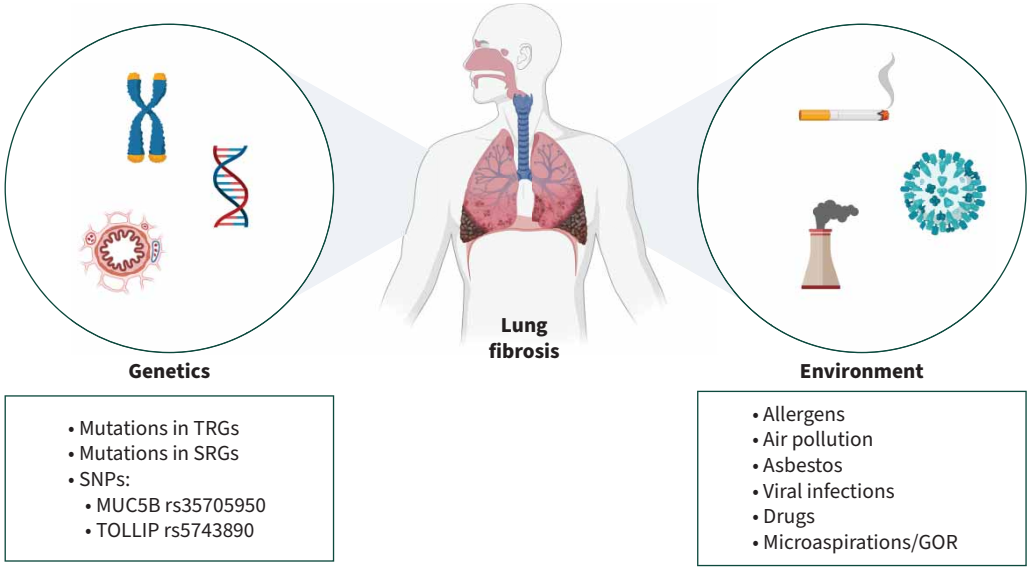


**FIGURE 1** The major genetic and environmental factors impacting the development and progression of interstitial lung disease. Recently detected genetic predisposition involves mutations in telomere-related genes (TRGs) and in surfactant-related genes (SRGs), as well as single nucleotide polymorphisms (SNPs) in the MUC5B and TOLLIP genes. There are also some ultra rare genetic disorders that are not included in the figure. Environmental factors also influence disease development, including air pollution, exposure to allergens, occupational diseases and inhalation of several particles, but also viral infections, drugs and aspirations mainly associated with gastro-oesophageal reflux (GOR). Figure created using BioRender.

## Fibroblasts are key orchestrators of the tissue microenvironment in ILDs

Several advances have been made in the context of fibroblast biology and fibroblast-immune cell interactions, providing a multi-faceted view of ILD pathogenesis [54] (figure 2). Fibroblasts and myofibroblasts themselves play a central role in IPF and ILD in general, by producing extracellular matrix (ECM) components, particularly collagens, that accumulate in the interstitial lung space. These cells respond directly to mechanical cues in the fibrotic microenvironment, which influences their activation, survival and ECM production. Recently, new insights have been generated revealing additional mechanisms by which fibroblasts can amplify the fibrotic cascade and drive ILD pathogenesis. Specific examples include, but are not limited to, fibroblast-immune cell interactions [54], distinct matrix producing fibroblast phenotypes [55, 56], fibroblast metabolism [57] and fibroblast extracellular vesicle-mediated communication [57].

Multiple ECM-producing fibroblast subsets have been identified in the lung with distinct spatial localisation [55]. These subsets differ in their contribution to fibrosis, indicating that fibrosis is not a uniform process, but rather a spatially organised response driven by specific cell populations. Certain fibroblast subsets are more active or invasive, *e.g.* collagen triple helix repeat containing 1 (CTHRC1$^{+}$), depending on their lung region (peri-bronchial, adventitial or alveolar) [55], further highlighting the importance of spatial cellular dynamics in fibrosis. Specific fibroblast lineages (Scube2$^{+}$) have been described within the alveolar regions that actively participate in both initiating and sustaining fibrosis [56]. These unique fibroblast subpopulations are also found in the human IPF lung and produce ECM proteins such as collagen 1A1 (COL1A1) and CTHRC1, in addition to inflammatory mediators Secreted frizzled related protein (SFRP)-2, CXC-chemokine motif ligand (CXCL)-12, CXCL14, contributing to persistent lung scarring [56], underscoring fibroblast heterogeneity in human disease. Certain alveolar fibroblast subsets appear to play a key role in disease progression, *e.g. via* transition from resting to inflammatory to

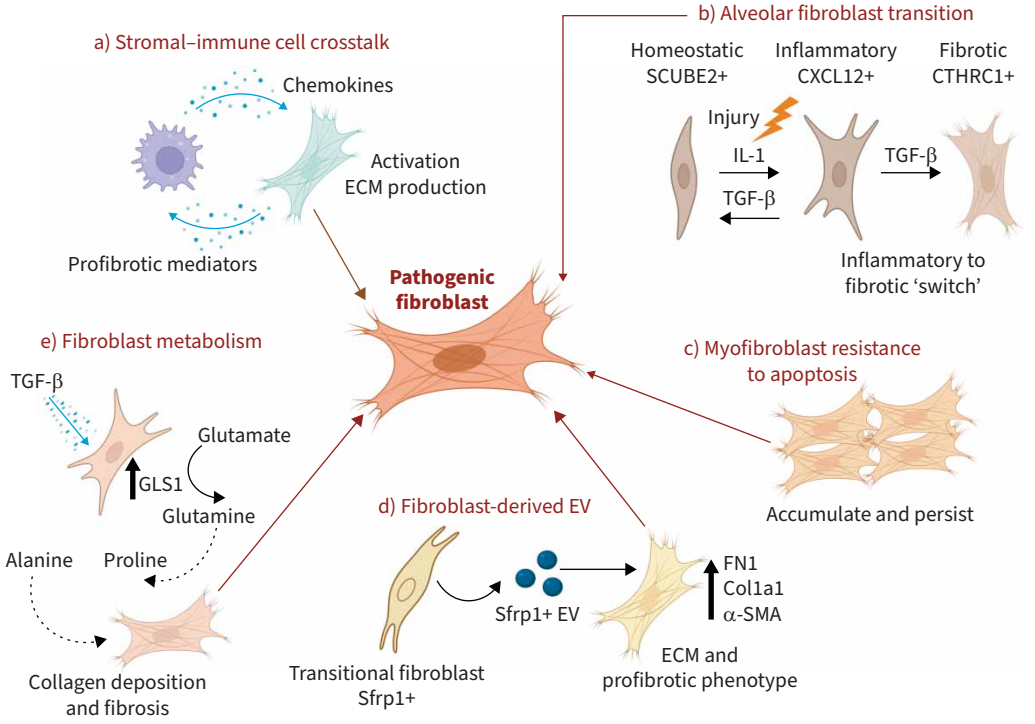


**FIGURE 2** Pathogenic fibroblasts arise *via* multiple cellular and molecular mechanisms in interstitial lung disease (ILD). a) Bidirectional communication between macrophages and fibroblasts *via* the release of chemokines and pro-fibrotic mediators drives myofibroblast activation and increased deposition of extracellular matrix (ECM). b) Scube2-expressing alveolar fibroblasts are the origin of both inflammatory (CXCL12+) and fibrotic (CTHRC1+) fibroblast subsets in the lung. c) Apoptosis-resistant myofibroblasts accumulate and persist in the injured lung. d) Fibroblast-derived extracellular vesicles (EVs) are released from SFRP1+ transitional fibroblasts and exert pro-fibrotic effects. e) Transforming growth factor-β (TGF-β) increases GLS1 expression driving conversion of intracellular glutamine levels to glutamate, which supports alanine and proline biosynthesis, necessary for the TGF-β–induced collagen response. IL: interleukin. Figure created using BioRender.

pathogenic fibrotic fibroblasts. Therefore, targeting these specific fibroblast lineages could present a more refined therapeutic approach to reduce fibrosis without disrupting essential lung tissue maintenance.

It is also important to note that myofibroblasts in the fibrotic lung have a distinct survival advantage as they are highly resistant to apoptosis, making the persistence of these cells a major barrier to fibrosis resolution in IPF. A recent study has shown that inhibiting p38α mitogen-activated protein kinase (MAPK) activity within myofibroblasts enables fibrosis resolution by promoting de-differentiation and restoration of apoptosis in mice [58]. Modulation of the MAPK pathway may effectively deactivate myofibroblasts, which are otherwise resistant to apoptosis in fibrotic tissue [58]. This may be a promising strategy to reverse lung fibrosis in IPF, instead of merely slowing its progression.

Further complexity is added by the identification of present $SFRP1^+$ transitional fibroblasts, a unique subset that bridges the diversity among fibroblast lineages and marks cells with invasive potential in fibrosis [59]. Interestingly, fibroblast derived extracellular vehicles, also contain SFRP1, which modulates the Wnt signalling pathway, a known regulator of fibrosis [60]. Fibroblast-derived extracellular vehicles can act as carriers of pro-fibrotic signals by transmitting molecular cues *in vitro* to precision cut lung slices and epithelial organoids [60]. This induces pro-fibrotic phenotypes, for example elevated α-SMA, COL1A1 and reduced epithelial progenitor function, respectively. Notably, ECM production by fibroblasts requires a high metabolic demand. Metabolic pathways play a considerable role in supporting fibrotic processes, offering a promising target for therapeutics. In fibrotic tissue, fibroblasts rely on glutamine metabolism that is facilitated by the enzyme glutaminase-1 (GLS1) [57]. Modulation of pyruvate metabolism alters fibroblast sensitivity to GLS1 inhibition and effectively reduces their ability to sustain fibrosis [57]. Interventions that disrupt the specific metabolic dependencies of pro-fibrotic fibroblasts could limit their activity and proliferation in IPF.

## Macrophages play a key role in the pathophysiology of IPF

Macrophages are central to the immune response in IPF pathology, with distinct subtypes contributing to initiating and sustaining fibrotic signalling and tissue remodelling. Lung macrophages include self-renewing, tissue-maintaining resident alveolar macrophages, which differ from infiltrating monocyte-derived macrophages recruited during injury. Additionally, the lung contains interstitial macrophages, a type of tissue-resident macrophage that, unlike alveolar macrophages, shows transcriptional similarity to interstitial macrophages in other organs. In IPF, increased monocyte counts are significantly associated with decreased clinical outcomes, including mortality [61]. The persistence of monocyte-derived macrophages in IPF drives much of the fibrotic tissue remodelling that occurs in the lung, and it is also linked to IPF initiation, progression and resolution. In murine models, deleting monocyte-derived macrophages has been shown to reduce fibrosis severity, highlighting their causal role [62].

Recruited monocyte-derived macrophages have historically been subdivided into M1-like or M2-like phenotypes in IPF. The M1-like macrophages are driven by Th1-cell cytokines interferon-gamma (IFN-γ), IL-1β and tumour necrosis factor-alpha (TNF-α), as well as by lipopolysaccharide (LPS). The M2-like macrophages are on the contrary driven by the Th2 cytokines interleukin (IL)-13, IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF). The usefulness of the M1-like and M2-like macrophage paradigm has become less clear in recent years but remains widely used in both experimental and clinical settings. Lung fibrosis caused by excess IL-1β is dependent on IL-17A, with evident IL-17A and IL-1β increase found in BALF of IPF patients [63]. Furthermore, a decreased IL-1Ra/IL-1β ratio is present in serum and BALF of IPF patients, an imbalance which may have a pathogenic consequence in IPF [64]. Moreover, IL-1β secretion requires the inflammasome NLR family pyrin domain containing 3 (NLRP3) and absent in melanoma 2 (AIM2)-dependant inflammasome activation, which is localised to alveolar macrophages in IPF. Similarly, study observing knockout of NLRP3-dependant inflammasome in mice showed fibrotic-resistance, highlighting potential therapeutic avenues *via* blockade of toll-like receptors and damage-associated molecular patterns, acting upstream of inflammasome activation in alveolar macrophages [65].

During normal healing, Th2-cell cytokine macrophage activation aids wound healing *via* secretion of TGF-β, VEGF, fibroblast growth factors (FGF) and platelet-derived growth factor (PDGFα), and thus regulates epithelial cell proliferation, fibroblast activation, angiogenesis and ECM deposition for scar tissue formation. However, this macrophage activation is highly correlated with IPF manifestation. Here, activated macrophages secrete CCL18 which has been found elevated in the BALF and serum of IPF patients, where it correlates with increased mortality and number of exacerbations [65]. Collagen production is a key feature of tissue remodelling in the IPF lung. CCL18 recruits T lymphocytes, leading to increased collagen production by pulmonary fibroblasts. Monocytes and lymphocytes, with elevated chemokines and

cytokines, drive fibroblast activation and transformation into myofibroblasts, thus advancing fibrosis [54, 62]. The AP-1 transcription factor Fos-related antigen-2 (Fra-2), which drives type VI collagen expression and lung fibrosis in mice, is upregulated in M2-like macrophages and is increased in IPF lung sections. Fra-2 and collagen VI are co-expressed in human IPF lung macrophages and specifically increased in ILD, indicating this combination of markers could serve as a specific fibrotic disease biomarker [35, 66].

The contextual association of a sustained Th2 immune response and subsequent activation of M2-like macrophages in IPF creates potential for their use as disease biomarkers. Chitinase-3-like protein 1 (CHI3L1), expressed in alveolar macrophages, promotes alternative activation of M2-like macrophages and fibroblast proliferation in bleomycin-challenged mice [67]. Serum expression of CHI3L1 predicts worsening of pulmonary function in IPF patients and may serve as another clinically useful biomarker. However, to date, clinical trials targeting canonical Th2 cytokines such as IL-13 have so far failed to show efficacy for IPF [68, 69]. This lack of clinical efficacy suggests that greater investigation into the spectrum of clinically relevant subtypes of macrophages is warranted.

Recently, new paradigms of macrophage activation have been characterised. The CX3CL1–CX3CR1 (fractalkine – CX3C motif chemokine receptor 1) ligand-receptor axis in IPF suggests that CX3CL1 production may recruit CX3CR1-expressing monocyte-derived macrophages to the lung [70]. Furthermore, single-cell RNA sequencing has uncovered distinct and clinically relevant macrophage subsets that may be integral to fibrosis progression. The macrophages expressing secreted phosphoprotein 1 ($SPP1^{+}$) increase in the lungs of IPF patients, localise to fibrotic foci in fibrotic lung areas, thus promote further macrophage proliferation, while simultaneously elevated serum levels of SPP1 are linked to disease severity [71].

Understanding the spatial distribution of these functionally distinct macrophage subtypes is critical, as the proportions of these mixed populations in certain spatial settings may drive the differentiation of IPF disease. Further still, a question of how specific macrophage subtypes in these mixed populations evolve and their impact on patients' respective disease progression is yet to be fully understood. Further discoveries incorporating the temporal-spatial distribution of macrophages during disease progression may provide crucial insights in designing disease-modifying therapeutics to treat fibrosis.

## Diagnostic and prognostic biomarkers of common ILDs

The diagnosis and monitoring of ILD requires a comprehensive approach. In this process, assessments such as both pulmonary function tests, which rely on patient cooperation and high-resolution computed tomography, exposing the patient to radiation, are usually required. In some cases, invasive diagnostics are also necessary. Given these limitations, increasing attention is focused toward identifying blood serum biomarkers that could be used in routine clinical practice [72, 73]. For example, potent inducers of fibroblast-myofibroblast activation involved in collagen synthesis (PRO-C3 and PRO-C6) have been shown to predict progression by measuring serum levels in the PROFILE IPF cohort [15].

Molecules referred to as protein biomarkers can be categorised into groups based on the types of cellular mechanisms involved in fibrotic lung injury [74]. The most extensively studied biomarkers are indicators of alveolar epithelial cell injury and impairment, including KL-6, SFTPA and SFTPD.

KL-6 is a mucin-like high-molecular-weight glycoprotein released from type II pneumocytes and bronchial epithelial cells [75]. In studies focusing on the most thoroughly examined group of ILD patients, those with IPF, a significant negative correlation between KL-6 serum levels and the percentage of predicted forced vital capacity (FVC) as well as diffusing capacity of the lung for carbon monoxide ($D_{LCO}$) has been described [76]. In the literature, KL-6 blood concentration was predictive of mortality and its temporal changes may reflect disease progression [77, 78]. Furthermore, studies have indicated the utility of this biomarker in predicting acute exacerbations in IPF [79].

KL-6 serum levels are also elevated in patients with connective tissue disease (CTD)-associated interstitial lung disease (CTD-ILD) compared to healthy controls and CTD patients without ILD, across various CTD subtypes [80]. In SSc-ILD, the most extensively studied CTD-ILD, KL-6 has been identified as a biomarker for assessing the severity of the lung fibrosis. Its concentration negatively correlates with lung function (as measured by FVC and $D_{LCO}$) and positively correlates with the extent of lung fibrosis quantified through high-resolution computed tomography [80, 81]. The literature is inconsistent regarding the biomarker potential of KL-6 as a predictor of SSc-ILD progression. While many studies suggest this association [82–84], a large multicentre study did not confirm such a relationship [85]. Preliminary studies also described the potential utility of KL-6 measurements in evaluating the response to immunosuppressive therapy. However, these data are insufficient to translate this knowledge into clinical practice [74].

In addition, SFTPA and SFTPD, the lipoprotein complexes secreted by AT2 cells and club cells, may play a significant role in the assessment of ILD [86]. Among patients with IPF or SSc-ILD, elevated levels of SFTPD (and SFTPA, particularly in IPF) were observed compared to individuals without ILD [87–89]. Higher serum concentrations of these biomarkers may also correlate with reduced survival in IPF patients. However, the strength of these findings varies across studies [89]. Interestingly, in IPF, lower baseline concentrations of SFTPD were associated with future declines in FVC and total lung capacity [90]. Similarly, in studies on polymyositis/dermatomyositis-associated ILD, baseline SFTPD levels were lower in survivors compared to non-survivors. Though, it was noted that biomarker levels varied depending on the presence of anti-MDA5 and anti-ARS antibodies. Research on the diagnostic or prognostic value of SFTPD in SSc-ILD remains inconclusive [91].

Another group of biomarkers include molecules reflecting fibrogenesis, fibroproliferation and matrix remodelling. Among these, matrix metalloproteinases (MMPs), particularly MMP-7, are the most extensively studied. Serum concentrations of MMP-7 are elevated in both IPF and SSc-ILD patients compared to non-ILD cohorts [92, 93]. MMP-7 may be useful in prognosis prediction, particularly in IPF, as its serum concentration correlates with lung impairment and all-cause mortality. Incorporating MMP-7 into prognostic models alongside other biomarkers, such as KL-6 or clinical parameters may enhance predictive accuracy [74, 92, 94].

Chemokines, indicative of immune dysregulation and inflammation, particularly CCL18, are of significant interest. CCL18 is secreted by alveolar macrophages and contributes to the activation of fibroblasts, promoting collagen synthesis in fibrotic lung diseases [94]. The serum concentration of this molecule is elevated in many idiopathic interstitial pneumonias, particularly in hypersensitivity pneumonitis [95]. CCL18 holds prognostic value among patients with both IPF and SSc, as longitudinal levels have been shown to correlate with lung function and mortality [96, 97]. Furthermore, in the faSScinate study, evaluating early SSc-ILD treatment with tocilizumab demonstrated that CCL18 levels differentiated the impact of treatment and placebo on %FVC [98].

Among other molecules that may serve as biomarkers useful for the diagnosis or monitoring of ILD, several proteins have been identified such as club cell secretory protein (CC16), CCL2, disintegrins and metalloproteases, circulating fibrocytes, osteopontin, fibulin-1, heat shock protein (HSP) 47, autoantibodies to HSP 70, lysyl oxidase-like protein 2, insulin-like growth factor-binding proteins, chitinase-3-like protein 1, adhesion molecules, CXCL13, CXCL4, B lymphocyte stimulator, B-cell activating factor, VEGF and endothelin-1. However, data regarding these potential biomarkers remain limited and further research on larger cohorts is required to draw conclusions.

## Translational aspects of current preclinical models of ILD and how they resemble human ILD pathology

In order to investigate potential drug targets in ILDs, reliable and robust model systems are crucial. There are numerous *in vitro* and *ex vivo* models that have recently been developed to mimic specific characteristics of the ILD pathophysiology in the laboratory, mostly for drug discovery and testing purposes, reviewed in detail by Kolanko *et al.* [99]. Here we particularly focused on animal models and their translational value in replicating human ILD pathophysiology. Especially when specific pathways or cell types are targeted, translational experimental animal models are needed, which strive for utmost resemblance of human ILD. To mimic diseases such as IPF or nonspecific interstitial pneumonia, it is important that the animal models display progression of fibrosis, as well as relevant hallmarks of the disease process. When models fail to mimic disease pathophysiology, there is a risk of identifying promising new drugs that will subsequently fail in clinical trials. This issue might have contributed to the fact that only a few drugs derived from ILD animal studies have been successful in targeting important aspects of the disease in the clinic. Additional complication that hampers the immediate translation of ILD models into the clinic is the lack of accurate and sensitive readouts for the pathophysiology and treatment response of ILDs [100–102].

The most frequently published animal model of ILDs, usually implemented to mimic human IPF, is single-dose bleomycin, administered *via* the intratracheal route in rodents [103, 104]. This conventional model is transferred easily and reproduced across different research sites [105]. The bleomycin model has also previously been raised in an European Respiratory Society statement as an important tool in modelling pulmonary fibrosis and acute lung injury [106]. While the acute model comprises an initial inflammatory phase, many researchers may only be interested in the following phase of fibrosis progression and will thus only evaluate later stages of this model. Although these later stages fit the current overall research interest in the underlying mechanisms of epithelial damage and aberrant repair, the inflammatory phase might

reveal important knowledge of the early mechanisms that eventually lead to fibrosis. In addition, the low-grade inflammation seen in patients with nonspecific interstitial pneumonia or other ILDs such as CTD-ILD or drug-induced-ILD (DIILD) [107], implies that the acute experimental bleomycin model is useful for studying the transition from inflammation to fibrogenesis. When administering bleomycin locally *via* the intratracheal route, pathologically large lung volumes are observed in rodents as a compensatory mechanism to increase lung capacity for non-aerated lung regions [108]. Therefore, this model has been under debate lately, as the induced hyperinflation results in enlarged lungs thus expressing properties opposite of a truly restrictive disease model [100]. Due to this lung volume compensation occurring, cautious interpretation of the obtained data with this model is needed as results may be influenced by the applied normalisation per lung volume/weight. Another concern with the single dose intratracheal model is that some studies have shown that the induced ILD-like symptoms can resolve over time. Although other studies show that the induced ILD in animal models will not resolve in elderly mice when challenged with bleomycin [109, 110]. Interestingly, it has also been suggested that this observed resolution of the disease is due to a survival bias [111], as only animals with moderate disease burden are kept throughout the study, while animals presenting severe lung injury are taken out of the study beforehand due to humane endpoints. Hence, disease can resolve in those animals that initially had less disease burden. Other alternative bleomycin models have emerged lately, in an attempt to create sustained ILD with fibrogenesis as endpoint upon initial lung injury and inflammation. Such models may involve repeated intratracheal doses given to mice over several days instead of the single-dose approach [112]. The benefit of such repeated exposure enables sustained and stable disease, which would allow for more accurate therapeutic evaluation. Other type of chronic models, systemically induced using intraperitoneal injections or subcutaneous administration, not only indicate the induction of sustained ILD but also trigger pathology that better resembles the clinical situation compared to the single dose exposure [113–116]. When the rodents receive bleomycin systemically, the metabolism of the inducing agent is different than when introduced locally into the lungs. The systemic administration of fibrosis-inducing agents creates a pathology that rather closely resembles human disease with dense fibrotic lesions appearing mainly in the peripheral lung areas [117].

In contrast to the induction of stable fibrosis generation to mimic human ILD, the exacerbation models include several exposures and addition of peak dose on top of the initial challenge, to create an acute disease model similar to end-stage IPF [118]. Compounds other than bleomycin are also commonly used to induce ILD, such as silica, quartz [119] or asbestos [120]. Specific mimicry of DIILD models has also been attempted, involving drugs known to cause DIILD including amiodarone and methotrexate [121, 122]. Also, overexpression of TGF-β is another approach to model lung fibrosis [123].

Different type of ILD models and administration routes may be optimal for specific research questions. If the purpose is to create a model for evaluation of excessive collagen production, the single-dose intratracheal model may as well be sufficient. The same applies to imaging biomarker testing in experimental models, when employing computed tomography (CT) or magnetic resonance imaging (MRI). Every disease model may look different in the scanner, therefore, applying the well-known single-dose intratracheal bleomycin model will allow for testing of imaging biomarkers and evaluating lung lesions by distinguishing between inflammation and fibrosis. Moreover, having lung lesions present with known pathology is advantageous for exploring different CT protocols or setting up new MRI-sequences, as well as for evaluating the imaging modalities' sensitivity to detect lesions early on [124]. The timing is also of great importance when investigating therapeutic effects [125]. In general, the most optimal rodent ILD model would be an ILD model where fibrotic lesions are homogeneously and continuously present and disease burden does not shift or resolve over time. Regardless of the type of experimental model chosen to deepen our knowledge of ILD pathology, we must aim for a translational approach in that particular model system [126].

Overall, the lack of an accurate animal model of pulmonary fibrosis hampers our understanding of disease pathogenesis. The future readouts must be evaluated carefully when aiming to investigate symptoms or biomarkers of disease onset and progression. More longitudinal readouts should be included, such as non-invasive imaging for model evaluation or characterisation, in addition to the current gold standard readouts such as histopathology, hydroxyproline or even lung function assessments. Choosing the correct readout in the carefully chosen model is an important step before moving into clinical trials. With the emerging imaging technologies and the increasing machine learning approaches to process images, future studies may be more reliant on imaging biomarkers of ILD pathology, as imaging provides spatial information, both in terms of the disease burden and lesion magnitude [127] (figure 3). To provide precision medicine options in patients with ILD, recent evidence supports focusing not merely on the phenotype of the disease, but rather on the genotype. Furthermore, potential treatable traits include host factors, such as targeting the microbiome, apart from the obvious clear suggestion of smoking cessation

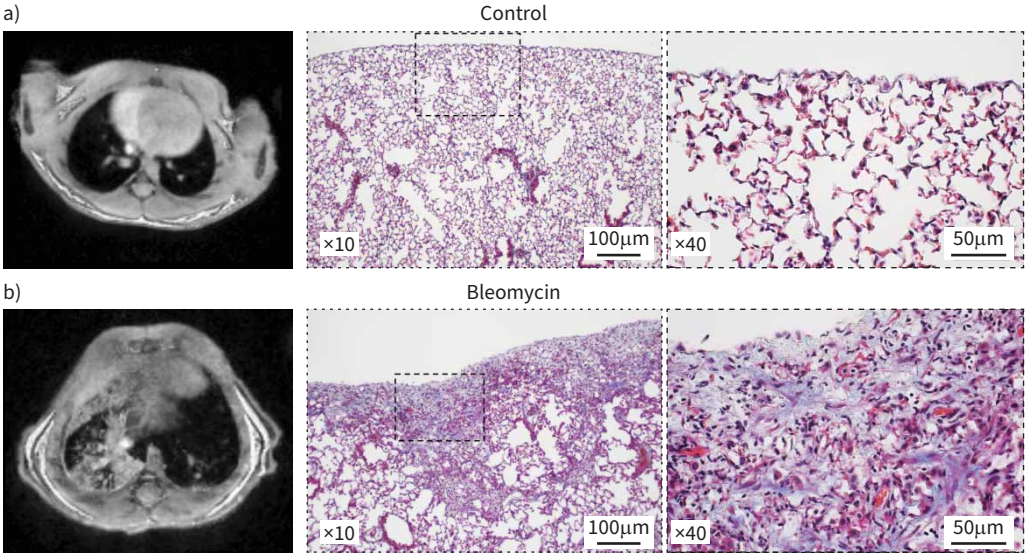


**FIGURE 3** Representative images from a control group (panel a) and the bleomycin-exposed group (panel b), showing magnetic resonance imaging (MRI) scans and histology assessment by Masson's trichrome stained lung tissue sections. The MRI was acquired in the transversal plane using the Rapid Acquisition with Refocusing Echoes (RARE) sequence combined with the Ultra Short Echo (UTE) sequence of 1 ms, in a chronic bleomycin model with systemic exposure (intraperitoneal) for 4 weeks (scans performed at 6 weeks post-dosing initiation, thus 4 weeks dosing plus 2 weeks rest). The figure was reproduced and modified from [114] with permission.

and avoiding allergens, pollution and other recognised exposures. As a respiratory community, we need to strongly highlight the crucial role of air pollution in both the development and progression of chronic lung diseases.

## Future steps in our understanding of ILDs

Genetic predisposition combined with environmental factors (*e.g.* smoking, pollution, infections) plays a crucial role in the onset and progression of ILDs, as well as the response to treatment. The interplay between these factors is complex and underscores the need for a more personalised approach in clinically managing ILDs. Fibroblasts contribute to the pathogenesis of ILD not only by modulation of ECM, but also *via* localisation to distinct microenvironmental niches. Thus, targeted therapies for ILD could be designed and refined to address fibroblast subsets according to their localisation and role in fibrosis. Macrophages, particularly monocyte-derived M2 macrophages, are key players in IPF progression, driving fibrosis through cytokine secretion, fibroblast activation and ECM remodelling. New technologies and deeper insights into macrophage subtypes and their spatial-temporal dynamics offer promising avenues for developing disease-modifying therapies in IPF. Moreover, understanding the balance between protective and pathological type 2 immunity responses is crucial for developing targeted therapies for ILDs. There are some promising biomarkers that, subject to further research and testing, could be used to assess disease severity and predict outcomes with the potential of replacing or complementing current diagnostic methods, thus reducing the need for invasive procedures and improving patient management. However, to advance our understanding of ILD pathophysiology, in addition to current *in vitro* and *ex vivo* models recapitulating the human ILD pathophysiology, ILD animal models that match the research goal and clinical characteristics will be crucial. Ultimately, translational approaches remain important for bridging the gap between preclinical studies and clinical applications in ILD research.

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