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Pulmonary fibrosis: emerging diagnostic and therapeutic strategies

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Abstract:	Fibrosis is the concluding pathological outcome and major cause of morbidity and mortality in a number of common chronic inflammatory, immune-mediated and metabolic diseases. The progressive deposition of a collagen-rich extracellular matrix (ECM) represents the cornerstone of the fibrotic response and culminates in organ failure and premature death. Idiopathic pulmonary fibrosis (IPF) represents the most rapidly progressive and lethal of all fibrotic diseases with a dismal median survival of 3.5 years from diagnosis. Although the approval of the antifibrotic agents, pirfenidone and nintedanib, for the treatment of IPF signalled a watershed moment for the development of anti-fibrotic therapeutics, these agents slow but do not halt disease progression or improve quality of life. There therefore remains a pressing need for the development of effective therapeutic strategies. In this article, we review emerging therapeutic strategies for IPF as well as the pre-clinical and translational approaches that will underpin a greater understanding of the key pathomechanisms involved in order to transform the way we diagnose and treat pulmonary fibrosis.

Pulmonary fibrosis: emerging diagnostic and therapeutic strategies

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Abstract

Fibrosis is the concluding pathological outcome and major cause of morbidity and mortality in a number of common chronic inflammatory, immune-mediated and metabolic diseases. The progressive deposition of a collagen-rich extracellular matrix (ECM) represents the cornerstone of the fibrotic response and culminates in organ failure and premature death. Idiopathic pulmonary fibrosis (IPF) represents the most rapidly progressive and lethal of all fibrotic diseases with a dismal median survival of 3.5 years from diagnosis. Although the approval of the antifibrotic agents, pirfenidone and nintedanib, for the treatment of IPF signalled a watershed moment for the development of anti-fibrotic therapeutics, these agents slow but do not halt disease progression or improve quality of life. There therefore remains a pressing need for the development of effective therapeutic strategies. In this article, we review emerging therapeutic strategies for IPF as well as the pre-clinical and translational approaches that will underpin a greater understanding of the key pathomechanisms involved in order to transform the way we diagnose and treat pulmonary fibrosis.

Keywords

Fibrosis, idiopathic pulmonary fibrosis, fibroblast, collagen, anti-fibrotic agent, fibrogenesis

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1. Introduction

Pulmonary fibrosis is considered to be one of the most irreversible forms of fibrosis across different organs, despite the lung possessing potent reparative capacity. Idiopathic pulmonary fibrosis (IPF) is the most common and fatal form, inexorably progressing to secondary respiratory failure, with a median survival of 3-5 years from diagnosis (Podolanczuk, Thomson, et al., 2023). While the aetiology of IPF remains elusive, current thinking revolves around a complex interplay between genetic predisposition and environmental exposures in predominantly elderly male individuals, leading to chronic progressive pulmonary fibrosis (PPF) (Raghu, Remy-Jardin, et al., 2022) (Figure 1). The most well studied genetic risk factors include genes associated with epithelial function, such as *MUC5B* (mucin 5B) and *SFTPC* (surfactant protein C), genes responsible for maintaining telomere homeostasis, such as *TERT* (telomerase reverse transcriptase) and *TERC* (telomerase RNA component), genes associated with the immune system, e.g. *TOLLIP* (toll-interacting protein) or the inflammasome e.g. *DPP9* (dipeptidyl peptidase 9), and with cell-cell adhesion, like *DSP* (desmoplakin), or the RhoA pathway, like *AKAP13* (A-kinase anchoring protein 13) among others (Allen et al., 2017; Fingerlin et al., 2013; Mathai et al., 2016; Noth et al., 2013). More recently, a genetic *locus* associated with *DEPTOR*, a gene involved in several cellular functions including negative regulation of mTOR (mechanistic target of rapamycin), has also been associated with increased risk of developing IPF (Allen et al., 2020). Environmental factors linked to IPF pathogenesis include cigarette smoke, occupational exposure to certain substances in the workplace, such as wood, metal and silica dust, air pollution, particularly particulate matter and nitrogen dioxide, gastroesophageal reflux disease (GERD) and viral infections (Raghu & Meyer, 2012; Wolters et al., 2018).

The picture remains complex at a cellular level, where IPF is characterised by micro-anatomical redistribution and dysfunction of several cell types. In terms of cell composition, the distal parenchyma in IPF, is enriched with epithelial cell populations that typically reside in the airways. Aberrant basaloid cells have recently been identified as a unique epithelial feature of IPF (and chronic obstructive pulmonary disease) expressing basal cell, senescence and epithelial to mesenchymal transdifferentiation (EMT) markers and appear to overlay fibrotic foci (also known as fibroblastic foci), the histological hallmark of the disease (Adams et al., 2020). Fibrotic foci comprise activated and hyper-synthetic fibroblasts and

myofibroblasts embedded in a collagen-rich extracellular matrix (Kuhn & McDonald, 1991). Peribronchial vascular endothelial cells, normally restricted to the bronchial circulation, are observed in areas of remodelling and aberrant angiogenesis (Adams et al., 2020). Type 2 alveolar epithelial cells (AEC2), the stem cell progenitors of the adult lung, display evidence of senescence, with shortened telomeres and a compromised ability to regenerate a functional alveolar epithelium that is able to support efficient gas exchange (Platé et al., 2021). Senescence is also a feature of IPF fibroblasts which are further resistant to apoptosis and unable to efficiently support epithelial regeneration (Álvarez et al., 2017; Ng-Blichfeldt et al., 2019; Ramos et al., 2001). In terms of the IPF immune cell compartment, profibrotic macrophage populations dominate the immune cell landscape in IPF (Adams et al., 2020; Habermann et al., 2020). These together with T cells, may secrete profibrotic and inflammatory cytokines which further promotes the recruitment of other immune cells. B cells have also been found to accumulate in the IPF lung and produce antibodies against self-antigens, suggesting that autoimmunity may be a feature and also play a role in the disease (Desai et al., 2018). The spatially and temporally heterogenous cellular landscape of the IPF lung is therefore complex and future therapies will need to potentially target multiple cellular mechanisms.

Since being first described almost 80 years ago, considerable advances have been made in the diagnosis and prognosis of IPF. The current diagnostic guidelines recommend the use of a combination of clinical evaluation, high resolution computer tomography (HRCT) imaging, and non-invasive tests, such as pulmonary function tests and blood tests, for most cases (Raghu et al., 2018). In IPF, patients present with a definite or probable usual interstitial pneumonia (UIP) pattern on HRCT. A definite UIP pattern is characterised by reticulation and honeycombing, with or without traction bronchiectasis in a predominantly subpleural and basal distribution (Podolanczuk, Thomson, et al., 2023). The standard method of assessing severity and response to treatment is lung function, including forced expiratory volume (FEV1), forced vital capacity (FVC) and diffusing capacity of the lung for carbon monoxide (DLCO). While surgical lung biopsies (SLB) are recommended in specific and limited cases (Raghu et al., 2018), in 2022, a conditional recommendation was made to regard transbronchial lung cryobiopsy as an acceptable alternative to SLB in centres with appropriate expertise (Raghu et al., 2022).

In terms of the development of anti-fibrotic therapeutics, the approval of two agents, pirfenidone (Esbriet) and nintedanib (Ofev), which slow disease progression, represented a turning point for the treatment of IPF. However, these two drugs do not halt or reverse the disease and present considerable side effects (Pleasants & Tighe, 2019), so that new therapeutic approaches are urgently required. Nintedanib/Ofev, is now approved for the treatment of progressive pulmonary fibrosis (PPF), where progression is assessed by a combination of worsening radiological, lung function and symptomatic parameters.

Recently, the James Lind Alliance, a priority setting partnership bringing together patients, carers and clinicians, has published the top 10 priorities in PPF. These include improvement in accuracy and time taken in the diagnosis of PPF and the development of new treatments that can slow, halt or reverse disease progression, convey fewer side effects and importantly improve survival (https://www.jla.nihr.ac.uk/priority-setting-partnerships/progressive-pulmonary-fibrosis/). In this review, we will describe emerging strategies that have the potential to be transformative for the future diagnosis and treatment of IPF.

2. Emerging diagnostic and prognostic strategies

2.1 Imaging

Central to the diagnosis of IPF is the interpretation of an HRCT scan by an experienced radiologist. Yet important challenges remain that can delay accurate diagnosis, expose patients to unnecessary and risky investigations and increased anxiety linked to frequent misdiagnoses, and put financial pressure on healthcare resources. Some of these challenges are for instance the substantial variability in diagnostic agreement even between experienced radiologists and the unavailability of experienced radiologists, especially at community hospitals (*Clinical Radiology Census Report*, n.d.; *Europe's Looming Radiology Capacity Challenge: A Comparative Study*, 2016). The development of robust and reliable computer based and machine learning algorithms to aid the interpretation of HRCT scans are paving the way for effective solutions to some of these challenges.

2.1.1 Computer-aided quantitative analysis

The recent application of computer-based CT analysis allows for the extraction and analysis of quantitative parenchymal features from CT images, that leads to greater precision than standard visual scoring. These CT parenchymal features, which may have no visual correlate can be used to evaluate disease severity and risk of mortality. One such example is the quantitation of pulmonary vessels (arteries and veins) and associated structures (perivascular fibrosis), collectively termed vessel-related structures (VRSs), which cannot be achieved by the human eye. By measuring pulmonary VRSs, the Computer-Aided Lung Informatics for Pathology Evaluation and Rating (CALIPER) software can outperform current gold standard measures of outcome in IPF, by strongly predicting survival and likelihood of forced vital capacity (FVC) decline with effects enhanced over functional indices in patients with less extensive disease (Jacob et al., 2017, 2018). This can have important decision-making implications with regards to medication, referral for transplantation or palliative care pathways. VRS scores have been able to identify patients with IPF who may reach drug trial endpoints, and this has the potential to significantly impact the requisite sample size in IPF drug trials, reducing it by 26%, with important cost implications. Importantly, an implication of this is that VRS scores can identify patients in whom antifibrotic medication prolongs life and reduces FVC decline (Jacob et al., 2018).

2.1.2 Machine learning

Deep learning (DL) is a subset of machine learning, which in turn is part of the broader field of artificial intelligence (AI). DL is particularly suited to large sets of data such as images, that may have weak or even no labels. Rather than being taught specific rules, a deep learning algorithm may identify relationships between input and output autonomously. This approach relies on initial training with images that have been classified by an expert, with inference performed on new images. This approach has been successfully applied in the context of fibrotic lung disease by Walsh and colleagues who developed a deep learning algorithm trained to classify HRCT scans from patients with fibrotic lung disease according to the 2011 ATS/ERS/JRS/ALAT evidence-based guidelines for diagnosis and management of IPF and the 2018 Fleischner Society criteria for usual interstitial pneumonia. The algorithm achieving almost instantaneous, human-level performance based on comparison with 91 specialised thoracic radiologists. However, this study also highlighted some limitations of this specific

algorithm, such as the limited number of scans in the training set, and the potential bias introduced by the fact that the training set was labelled by one specialist only (Levin, 2018). A limitation of all algorithms aimed at recognising UIP is that an independent gold standard for its diagnosis does not exist. In fact, radiological diagnosis cannot be standardised against multidisciplinary diagnosis as it is a part of it, and it cannot be standardised against surgical lung biopsy (SLB) as these are only indicated when HRCT is non-definitive. In this specific study, the algorithm was validated by outcome, since UIP is associated with increased mortality. Despite its limitations, this algorithm showed remarkable performance, supporting the diagnostic process and could relieve the burden on already stretched healthcare resources.

2.1.3 Functional imaging by hyperpolarized ^{129}Xe MRI

While HRCT is currently the gold standard for lung imaging in IPF, it presents its own limitations, namely the use of radiation limiting the number of scans and indications to scan, and the lack of information on lung function, such as ventilation or gas exchange. Moreover, structural alterations such as honeycombing and traction bronchiectasis, used in UIP pattern recognition, are apparent when the disease has already progressed. Spirometry, commonly used to assess pulmonary function, only provides a global assessment and does not provide regional information. Hyperpolarized (HP) xenon 129 (^{129}Xe) magnetic resonance imaging (MRI) has shown potential for evaluating global and regional gas exchange. When inhaled, HP ^{129}Xe has the property to dissolve into the lung tissue and bind to red blood cells (RBC). It can therefore be used as a probe for functional assessment of alveolar-capillary diffusion, ventilation and intra-acinar gas diffusion (Weatherley et al., 2019) because it has three distinct corresponding spectral peaks that can be measured (Mata et al., 2021).

A recent preliminary study showed that HP ^{129}Xe-MRI could evaluate functional measures of gas transfer and ventilation to accurately identify patients with progressive PF. This novel imaging approach was also able to identify differences in gas transfer in regions of nonfibrotic lung in patients with progressive fibrosis compared with those with stable fibrosis (Hahn et al., 2022). However, in another study, ventilation or oxygen enhanced-MRI biomarkers were not able to discriminate between the different types of interstitial lung disease (ILD) (Tibiletti et al., 2023). Further studies, such as the current study being carried out within the UK

interstitial lung disease-long COVID (UKILD-long COVID) study (Wild et al., 2021), will be helpful to establish whether the regional information uniquely provided by HP ^{129}Xe-MRI combined with the option to perform more frequent and earlier scans may be useful for monitoring disease progression on a regional level as well as for characterizing disease phenotypes in the future. It is also worth commenting that MRI techniques present intrinsic downsides which require careful consideration. These include cost of the procedure, anxiety and discomfort to patients associated with the prolonged time spent in the noisy and enclosed space of the scanner, risks associated with strong magnetic fields (especially in people with pacemakers and cochlear implants).

2.2 Quantitative analysis of lung sounds

Velcro-like crackles on chest auscultation are a common and early indication of IPF and they closely correlate with the extent of several HRCT features, including honeycombing and reticulation. Their detection plays an important role in triggering further clinical investigations in patients presenting with chronic respiratory symptoms (Sgalla et al., 2018). Electronic stethoscopes are inexpensive and widely available tools that permit point-of-care digital recording of lung sounds, and provide a useful tool for longitudinal monitoring of acoustic features of lung sounds. A recent study by Sgalla and colleagues combining longitudinal recording of lung sounds with MatLab-based algorithms, showed that a set of acoustic features of lung sounds represent a reproducible metric which significantly changes over the course of a 12-month period and is associated with established measures of disease severity and clinical deterioration (Sgalla et al., 2019). Larger cohort studies are required, especially in the context of clinical trials, to validate the potential of using quantitative recording of lung sounds as a clinical biomarker for diagnosis and disease progression. The recent advances in AI to further enable this technology may present further opportunities to enhance the use of lung sounds for patient management and disease monitoring.

2.3 Breathomics

Volatile organic compounds (VOCs) are molecules that can be found in exhaled breath and bodily fluids. Breath is the major source of VOCs, and healthy breath contains close to 1500 identifiable VOCs. Exogenous VOCs are derived from the environment, while endogenous VOCs result from physiological and pathological processes in the body, such as metabolic

activity and inflammation. While VOCs are typically measured using gas chromatography or mass spectrometry, the development of eNose devices has opened avenues for the use of real-time point-of-care VOCs patterns (breathprints) recognition for the diagnosis of IPF in clinical practice. Several cohort studies using different eNose devices have shown that breathprints differed significantly between patients with ILD and healthy controls (100% accuracy), IPF and healthy controls (98.5% accuracy) and IPF and non-IPF ILD (91% accuracy) (Ibrahim et al., 2021; van der Sar et al., 2021).

2.4 Blood biomarkers

The enduring search for reliable blood biomarkers in fibrotic lung disease, in particular for IPF, has culminated in the discovery of biomarkers with potential diagnostic and prognostic value. While no IPF biomarker is currently sufficiently validated to be used in routine clinical practice, some are showing considerable promise and are being increasingly incorporated as exploratory endpoints in clinical trials to provide evidence of target engagement and proof of principle of a potential anti-fibrotic effect. A number of these may also hold promise to serve as early endotyping biomarkers to identify patients with progressive disease and poor prognosis and who therefore could particularly benefit from early antifibrotic treatment. However, at the time of writing there was no substitute for FVC as an endpoint in pivotal clinical studies in IPF or any other fibrosing interstitial lung diseases.

With regard to the current evidence, IPF biomarkers fall into three broad mechanistic categories: i) epithelial cell dysfunction and senescence (*e.g.* surfactant protein (SP)-A, SP-D, *MUC5B*, Krebs von den Lungen-6 (KL-6), telomerase reverse transcriptase (TERT) and the tumour markers, CA-125, CA-19 9), ii) aberrant immunity (*e.g.* C-C Motif Chemokine Ligand (CCL)-18, heat-shock protein (HSP)-70, YKL40); and iii) abnormal lung remodelling (*e.g.* matrix metalloproteinase (MMP)-7, lysyl oxidase-like (LOXL)2, ECM synthesis and degradation biomarkers), as recently reviewed by Maher et al (Maher et al., 2022).

Among the promising biomarkers representing epithelial cell dysfunction, MMP7 is consistently elevated in patients with IPF compared with healthy controls. MMP7 levels above a threshold level (12.1 ng/mL) are predictive of all-cause mortality and correlate with poorer transplant free survival. MMP-7 concentrations negatively correlate with DLCO, and

positively with a mortality risk scoring system (GAP; that combines age, gender, FVC) and DLCO (Tzouvelekis et al., 2017). MMP7 has also been identified as a diagnostic and prognostic biomarker in combination with other proteins. High plasma concentrations of MMP7, vascular cell adhesion molecule (VCAM)-1, S100A12, intercellular adhesion molecule (ICAM)-1, and IL-8 predicted poor overall survival, transplant-free survival, and progression-free survival in an IPF derivation cohort of 141 patients, while all five were predictive of poor transplant-free survival; MMP7, ICAM-1, and IL-8 of overall survival, and ICAM-1 of poor progression-free survival in the validation cohort (101 subjects) (Richards et al., 2012). Another study showed that a panel including MMP7, surfactant protein D (SP-D) and osteopontin (OPN) enhanced diagnostic accuracy in IPF versus alternative idiopathic ILDs when plasma levels of at least one analyte in the panel exceeded a threshold (White et al., 2016). In combination with ICAM-1, OPN and periostin (POSTN), MMP7 was recently found to be superior to the clinical GAP score in predicting progression at 12 months (Clynick et al., 2022). MMP7 and SP-D were able to distinguish IPF patients from controls and to predict outcome in the Prospective Observation of Fibrosis in the Lung Clinical Endpoints (PROFILE) cohort (Maher et al., 2017). The same study showed that baseline values of SP-D and the tumour marker CA19-9 were significantly higher in patients with progressive versus stable disease; and increasing concentrations of the tumour marker CA-125 over 3 months were associated with increased mortality. Both CA19-9 and CA-125 are felt to be markers of epithelial damage in IPF (Maher et al., 2017).

Biomarkers representing abnormal lung synthesis and remodelling, based on the detection of ECM neoepitopes were investigated in the PROFILE study and revealed that several neoepitopes (BGM, C1M, C3M, C3A, C6M, ELM2, VICM) in addition to C-reactive protein degraded by MMP-1/8 (CRPM) were able to distinguish IPF patients from healthy controls. Six neoepitopes (C1M, C3A, C3M, C6M, CRPM, and VICM) at baseline were significantly higher in patients with progressive IPF than in those with stable disease. When assessed longitudinally, concentrations of six neoepitopes (BGM, C1M, C3A, C3M, C6M, and CRPM) were significantly higher in patients with progressive versus stable IPF by 6 months. Baseline concentrations of C1M and C3A correlated with increased mortality. The rate of change between baseline and 3 months of BGM, C1M, C3M, C5M, C6M and CRPM was strongly predictive of overall survival, and the increased risk was proportional to the magnitude of change in neoepitope

concentrations, with strongest association being with CRPM (Jenkins et al., 2015). Markers of collagen type 3 (PRO-C3) and 6 (PRO-C6) synthesis were also elevated in IPF compared with healthy controls at baseline, and in progressive versus stable disease (Organ et al., 2019).

3. Current standard of care treatments for IPF

With an ever-increasing aging population, the incidence and prevalence of progressive fibrotic lung disease are predicted to significantly increase, substantially adding to the existing global burden of morbidity and mortality. This leaves a pressing unmet clinical need for effective therapies that improve quality of life and prognosis for patients suffering from PPF. In this section we will review the existing anti-fibrotic agents and explore emerging therapies which are progressing to clinical testing.

The approval of the antifibrotic agents, nintedanib and pirfenidone, represented a pivotal moment for the treatment of IPF, with the former recently being approved for all progressive pulmonary fibrosis. Although, both drugs individually exert pleotropic actions, they have near identical efficacy, with regards to slowing FVC decline. The mechanism of action of pirfenidone in IPF remains unclear, but pre-clinical studies demonstrated that it exerts inhibitory effects on inflammatory, antioxidant and profibrotic pathways, such as fibroblast proliferation, TGFβ1 driven myofibroblast transformation and pro-inflammatory cytokine release (Aimo et al., 2022). Post-hoc analysis of pooled data from ASCEND (NCT01366209) and the preceding CAPACITY 1 and 2 trials (NCT00287729 and NCT00287716), revealed a reduction in all-cause mortality of almost 50% (King et al., 2014). However, a systematic review, observed that pirfenidone does not improve the risk of acute exacerbations, which have a significant impact on prognosis (Petnak et al., 2021). Pirfenidone is currently licensed for the treatment of IPF for patients with a predicted FVC between 50-80%. The main side effects are gastrointestinal symptoms such as nausea and weight loss, and photosensitivity. However, current evidence suggests that less than 20% of patients discontinue treatment due to side effects. At the time of writing, the ELEVATE trial (NCT05321420) is currently recruiting patients to evaluate the efficacy, tolerability, safety, and dose response of LYT-100, a selectively deuterated form of pirfenidone with a different pharmokinetic profile which leads to fewer gastrointestinal side effects.

The second approved anti-fibrotic agent, nintedanib is an intracellular triple tyrosine kinase inhibitor of platelet-derived growth factor receptor (PDGFR) α and β, fibroblast growth factor receptor (FGFR) 1, 2, and 3, and vascular endothelial growth factor receptor (VEGFR) 1, 2, and 3, and also exerts a variety of antifibrotic effects (Rangarajan et al., 2016). The clinical approval of nintedanib came from the seminal INPULSIS phase III trials (1 and 2 - NCT01335464 and NCT01335477) which demonstrated a 50% reduction in FVC decline compared to placebo and again pooled post-hoc analysis of the INPULSIS trials showed there was a trend towards reduction in all-cause mortality, with a 30% reduction with nintedanib versus placebo (p=0.0954) (Richeldi et al., 2014, 2016). Side effects were limited to 15% of patients, with the main adverse event being diarrhoea. Expansion of the indication for nintedanib has been continuously investigated, and nintedanib has also now been approved for systemic sclerosis (SSc)-associated ILD (SSc-ILD), based on the positive outcome of Safety and Efficacy of Nintedanib in Systemic Sclerosis (SENSCIS) trial (Distler et al., 2019). The safety of combining nintedanib with pirfenidone therapy has been assessed in two clinical trials. These revealed acceptable tolerance compared to single agent treatment but further investigation is required to evaluate improvement in therapeutic efficacy (NCT02598193, NCT02579603) (Flaherty et al., 2018; Vancheri et al., 2018).

Although the approval of nintedanib and pirfenidone marked a turning point for both the treatment of IPF and the future development of anti-fibrotic agents for other fibrotic conditions, there remains a pressing need to develop treatments that halt progression or even reverse disease and reduce the rate of acute exacerbations, to significantly impact on the quality of life and survival of patients with IPF. Lung transplantation remains the only curative treatment but is an unrealistic option for many in this commonly aged and multi-comorbidity afflicted patient population. Below, we discuss potential future treatment strategies which at the time of writing, were being investigated in clinical trials (Table 1).

4. Emerging therapeutic strategies for IPF

The two current standard of care treatments for IPF, nintedanib and pirfenidone, emerged from campaigns aimed at targeting key profibrotic-signalling axes in order to control aberrant fibroblast function and control excessive ECM deposition. Many of the therapeutic agents

currently under clinical evaluation in IPF are still based on a similar tenet of interfering with the fibrogenic cascade. Our biological understanding of the key pathomechanisms underlying IPF is continuously evolving, and this is also reflected by emerging therapeutic strategies. These include strategies to address perturbed epithelial repair, as well as immune dysfunction, in particular with respect to macrophage phenotypes, in order to address the complex interconnected cross-talk which perpetuates abnormal cellular behaviour and thereby promotes fibrogenesis within the fibrotic niche in IPF.

4.1 Targeting pro-fibrotic signalling axes

Multi-tyrosine kinases

The oral multi-kinase inhibitor TAS-115, which similarly to nintedanib inhibits PDGFR and VEGFR, as well as colony stimulating factor-1 receptor (FMSR), hepatocyte growth factor receptor (HGFR), and other receptors competitively inhibited by adenosine triphosphate, reduces the profibrotic function of lung fibroblasts *in vitro* and inhibits the development of pulmonary fibrosis and collagen deposition *in vivo.* An exploratory phase II clinical trial of TAS-115 revealed acceptable safety and tolerability, with a signal towards decreased rate of lung function decline (Nishioka et al., 2023).

Rho kinases

Rho associated coiled coil-forming protein kinases (ROCK) facilitate the cellular response to injury through reorganisation of the actin cytoskeleton. Transcriptomic studies have shown that ROCK1 and 2 mRNA levels are increased in fibrosis with ROCK inhibition abrogating α-SMA protein expression in vivo and that selective inhibition of ROCK ameliorated bleomycin-induced lung fibrosis *in vivo* (Knipe et al., 2015, 2018). KD025, an oral inhibitor of ROCK2, has demonstrated good safety and tolerability and reduction in lung function decline over 24 weeks in a phase II clinical trial (NCT02688647).

SRC kinases

The SRC family of non-receptor protein tyrosine kinases are crucial in mediating key pro-fibrotic processes such as myofibroblast activation and epithelial-mesenchymal transition. A recent study adopting an *in-silico* data approach, identified saracatinib, a potent Src kinase inhibitor that was previously developed in the oncology setting, as a potential target in IPF.

Pre-clinical studies have shown that this compound exerts anti-fibrotic effects in vitro and in vivo (Ahangari et al., 2022) and at the time of writing, phase I and II trials were actively recruiting in IPF (NCT04598919).

c-jun N-terminal kinases

c-jun N-terminal kinase (JNK)s are part of the mitogen activated kinase (MAPK family) and also influence key profibrotic processes, such as fibroblast proliferation, and differentiation. *In vivo* studies have demonstrated that genetic deletion of JNK decreases profibrotic gene expression and lung fibrosis (Alcorn et al., 2009). CC-9001, a JNK inhibitor, was reported to be safe and well-tolerated in a phase Ib IPF trial and at the time of writing, recruitment has recently completed for a phase II trial (NCT03142191) (Popmihajlov et al., 2022) but the further clinical evaluation of this inhibitor is currently on hold due to business decisions.

Lysophosphatidic acid pathway

The Lysophosphatidic acid (LPA) pathway was proposed as a potential target in IPF on the basis of its pleotropic pro-fibrotic effects, including promoting fibroblast survival, proliferation, migration, differentiation, and profibrotic cytokine release. LPA is a lipid mediator that signals through specific G protein coupled receptors and is produced by autotaxin (or lipophosphase D), which hydrolyses lysophosphatidylcholine into the signalling molecule, LPA. Autotaxin levels are increased in the lungs of patients with IPF and in pre-clinical animal models (Montesi et al., 2014; Tager et al., 2008). Genetic deletion of the LPA receptor or autotaxin from bronchial epithelial cells or macrophages is protective in the bleomycin mouse model (Oikonomou et al., 2012). These pre-clinical data set the scene for the evaluation of GLPG1690, a potent selective oral inhibitor of autotaxin. Despite a promising phase I trial demonstrating good tolerability and efficient target engagement (van der Aar et al., 2019), two phase III ISABELLA trials (NCT03711162 and NCT03733444) were discontinued on the recommendation by the independent data monitoring committee that the toxicity profile did not support continuation (Maher et al., 2023). At the time of writing outcome data for two alternative autotaxin small molecule inhibitors, BBT-877 (NCT05483907) and the allosteric inhibitor BLD-0409 (NCT05373914) were not yet available.

Agents directed against LPA receptor 1 (LPA1) have also been developed. Although the phase II, multicentre, randomized, double-blind, placebo-controlled trial of the LPA1 antagonist, BMS-986020, demonstrated a significant reduction in the rate of FVC decline, there was reported treatment related hepatobiliary toxicity and cholecystitis leading to early termination of the study (Palmer et al., 2018). BMS-986278, a potent, next generation LPA1 receptor antagonist is structurally distinct and has demonstrated no hepatobiliary toxicity events in vitro and in vivo. A phase II randomised double blind, placebo-controlled trial was recruiting (NCT04308681) at the time of writing.

Integrins αvβ6 and αvβ1

Integrins play a key role in cellular adhesion and communication within the fibrotic microenvironment. The αvβ6 integrin is involved in the activation of latent TGFβ1 in response to epithelial injury and has emerged as a key target in IPF. αvβ6 levels are increased in lung tissue from IPF patients and correlate with poorer prognostic outcomes, so that this integrin might also hold prognostic value (Saini et al., 2015). The first agent targeting the αvβ6 integrin was the monoclonal antibody, BG00011 (Biogen) which showed promising data in the bleomycin model (Horan et al., 2012). However, a phase IIb clinical trial was terminated early due to increased mortality, exacerbations and a lack of clinical benefit (Raghu, Mouded, et al., 2022). The adverse side effect profile of this agent may in part be explained by the homeostatic role of αvβ6 in regulating macrophage function and consequent overwhelming inflammatory events in response to lung injury and the prolonged mode of action of an antibody-based approach (Sime & Jenkins, 2022).

The αvβ1 integrin has also emerged as playing a critical role in tissue fibrosis (Reed et al., 2015). It is highly expressed on activated fibroblasts, directly binds to the latency-associated peptide of $TGF\beta_1$ and also mediates $TGF\beta_1$ activation. At the time of writing, the outcome of the phase II (INTEGRIS_IPF) trial of the small molecule dual inhibitor PLN-74809/ Bexotegrast, targeting both αvβ6 and αvβ1, was not available but a press release suggests it has favourable safety and tolerability profiles. Pliant announced that the BEACON-IPF multinational randomized placebo-controlled phase IIb trial is due to commence in mid-2023.

Galectin-3

The β–galactoside binding lectin, galectin 3, is widely expressed in human tissues and promotes pro-fibrotic signalling via its ability to cross-link key pro-fibrotic cell surface receptors, including TGF-beta receptors and integrins. Galectin 3 levels are increased in bronchoalveolar lavage fluid and serum of IPF patients, with further increases observed in IPF exacerbations. Genetic deletion of galectin-3 in murine models leads to a reduction of fibrosis *in vivo* (MacKinnon et al., 2012). GB0139 (formerly TD139), an inhaled small molecule inhibitor of galectin 3, which regulates the expression of TGF receptors on the surface of AECs and ameliorates TGFβ1 induced fibrosis *in vivo*, was shown to be well-tolerated and reduce plasma levels of pro-fibrotic mediators, such as CTGF in a phase II trial.(Hirani et al., 2021). The results of a subsequent phase IIb trial, evaluating the effect on FVC decline in IPF as a primary outcome, were not yet available at the time of writing (NCT02257177).

Phosphodiesterase inhibitors

Phosphodiesterase (PDE)-4 plays a key role in signal transduction by regulating cellular concentrations of cyclic nucleotides. Oral selective inhibitors of the phosphodiesterase enzyme, PDE4, prevents the hydrolysis of cAMP, leading to increased levels of cAMP, which in turn antagonises profibrotic signalling cascades and ECM synthesis. An oral preferential inhibitor of phosphodiesterase PDE4B, BI1015550, was assessed in a phase II trial over 12 weeks, as monotherapy or in combination with antifibrotic therapy, and prevented lung function decline in patients with IPF compared to placebo. However adverse events, commonly mild diarrhoea, were frequent in the treatment group and more so in the group receiving BI1015550 on top of standard anti-fibrotic therapy (L et al., 2022). At the time of writing, two phase III trials were actively recruiting to evaluate BI1015550 in patients with IPF and progressive fibrosing ILD (NCT05321069) (NCT05321082).

Sildenafil is an oral PDE5 inhibitor and pulmonary vasodilator that is already approved for pulmonary arterial hypertension. The STEP-IPF and INSTAGE trials tested combination therapy of sildenafil and nintedanib in IPF but did not show a superior effect on 6 minute walk distance and breathlessness scores compared to nintedanib alone (Kolb et al., 2018; Network, 2010). Recently, an inhaled form of the prostacyclin analogue, treprostonil, was investigated in patients with varied interstitial lung diseases and associated pulmonary hypertension

(INCREASE study) (Waxman et al., 2021). There was an observed improvement in 6 minute walk distance at 4 months. This early clinical data is now being followed by the TETON programme, consisting of two replicate 52 weeks randomised, double blind phase 3 trials, that will evaluate the effect of inhaled treprostonil on FVC decline at week 52 in patients with IPF (NCT04708782, NCT05255991) (Nathan et al., 2022).

4.2 Targeting senescence

IPF is an age-related disorder and exhibits the fundamental hallmarks of aging – including telomere shortening, stem cell exhaustion, mitochondrial dysfunction and cellular senescence. Senescent cells that undergo stress, such as DNA damage, incur irreversible cell cycle arrest and develop a senescent associated secretory phenotype (SASP), resulting in the release of pro- fibrotic, -inflammatory and -apoptotic factors to create a cytotoxic microenvironment. These senescent cells, including AEC and fibroblasts, are known to persist and accumulate in fibrotic mouse models *in vivo* and in IPF (Schafer et al., 2017). Senolytics, which selectively induce apoptosis in senescent cells, present an attractive treatment strategy to potentially target the senescent phenotype in IPF and other age -related diseases. Dasatinib, a known chemotherapy agent for chronic myeloid leukaemia, is a multi-tyrosine kinase inhibitor and broadly targets multiple SRC kinases. Querceptin is a natural nonspecific kinase inhibitor, targeting downstream PI3K/AKT pathways such as HIF related senolysis. The synergistic combination of these senolytic agents (D and Q) resulted in downregulation of SASP profibrotic pathways *in vivo* and significantly improved lung function and fibrosis in mouse models of pulmonary fibrosis (Schafer et al., 2017). Two phase I randomised control trials of combined D and Q, were effective at eliminating senescent cells, showed no serious adverse events but there were increased reports of cough and GI symptoms. Large, randomised placebo- controlled trials are now required to confirm whether senolytics hold promise as a novel therapeutic approach in IPF (Justice et al., 2019; Nambiar et al., 2023).

4.3 Targeting the immune system in IPF - time to revisit?

The historical paradigm for the pathogenesis of IPF assumed that this condition arises as a result of an initial inciting inflammatory or immune response followed by aberrant wound healing responses leading to excessive matrix deposition. For decades, broad immunosuppressive therapy was the standard of care in IPF. However, in the aftermath of

the PANTHER trial (NCT00650091) ("Prednisone, Azathioprine, and N -Acetylcysteine for Pulmonary Fibrosis," 2012), where treatment with steroids and azathioprine was demonstrated to result in a higher incidence of death, immunosuppressive therapy was discontinued as standard of care in IPF. The evidence for immune dysregulation as a key mechanism driving IPF was recently reviewed (Shenderov et al., 2021). There is now emerging evidence suggesting that a more nuanced and targeted immuno-modulatory approach might be required. Recent genetic studies indicate an increased risk of severity of disease is associated with polymorphisms in immune-related genes encoding TLR3, toll-interacting protein (TOLLIP), and IL-1RA among others (Shenderov et al., 2021). Furthermore, given the broad mechanism of actions of nintedanib and pirfenidone, these agents may in part exert their beneficial effects through their immunomodulatory properties. For example, pirfenidone has been shown to regulate the immune-crosstalk between T cells and dendritic cells (van Geffen et al., 2021). The anti-inflammatory and immunomodulatory effects of oral selective PDE4 inhibitors, underpin their use in inflammatory and auto-immune disease. In terms of the encouraging results of the BI1015550 phase II trial, it is possible that this agent might exert its potential beneficial effects via a combination of anti-inflammatory and immuno-modulatory properties, in addition to its anti-fibrotic effects (L et al., 2022).

Emerging data from in vivo studies, also suggests that fine-tuning the distribution of different immune cell subset phenotypes can promote resolution of fibrosis. Polarisation of macrophages to the M2-like phenotype and differentiation of T cells towards Th17 and CD8 T cell subsets favours a fibrotic phenotype, while increased expression of T helper 1 and tissue resident memory CD4+ T cells appear to be protective (Shenderov et al., 2021). Supporting the notion of an aberrant immunophenotype driving pulmonary fibrosis, a recent scRNA-seq analysis of the peripheral immune system in IPF has recently revealed that levels of classical monocytes and T regulatory cells (Treg) are increased in progressive IPF (Unterman et al., 2023). As described earlier, single cell RNA-sequencing studies in IPF lung tissue have also identified profibrotic macrophage populations, that potentially work in concert with immune cells, such as T cells, to promote a fibrotic microenvironment (Adams et al., 2020). A detailed understanding of the role of innate and adaptive immunity in IPF may allow the development of targeted approaches to reconfigure subtle immune cell subset imbalances and re-establish immune homeostasis and abnormal cellular cross-talk within the IPF fibrotic niche.

4.4 Fibrometabolism- an emerging therapeutic field

Growing evidence suggests that alterations in metabolism play an influential role in the pathogenesis of fibrosis, most notably in IPF. Metabolic networks are extensively regulated to facilitate tissue-specific programmes and robustly maintain homeostasis. Metabolic reprogramming in response to several cues, including genomic alterations, the microenvironment, and oxidative stress allow cells to mount an appropriate cellular response to a specific stimulus or stressor. The rewiring of metabolic networks, or metabolic reprogramming, is a hallmark of cancer and the field of "fibrometabolism" is now an emerging and exciting avenue of research in IPF, which we have recently reviewed (Selvarajah et al., 2021). We and others have recently demonstrated that fibroblasts fine-tune their cellular metabolic networks to support the hyper-synthetic needs of myofibroblasts. There is now growing optimism for the implementation of novel metabolism-targeting therapeutic strategies, with several trials reporting good tolerability and efficacy in the oncology setting (Selvarajah et al., 2021), opening an opportunity to repurpose these agents as potential standalone therapeutics or, similarly as in cancer, as adjuvant therapies that could potentially sensitize key cells to currently approved or emerging therapies. We will briefly review the current evidence supporting a potential role for targeting the mTOR and adenosine monophosphate (AMP)–activated protein kinase AMPK metabolic axis in this disease context.

mTOR and AMP act as key nutrient and cellular energy sensing kinases and master regulators of cell metabolism. The interplay between these two signalling pathways is increasingly recognized to play a pivotal role in directing the reconfiguration of metabolic networks during the fibrogenic response to tissue injury. We have shown that mTOR, and more specifically the mTORC1/4E-BP1 signalling axis, plays a pivotal role in mediating the fibrogenic response to TGFβ1 stimulation in fibroblasts originating from multiple organs, including the stromal reaction in cancer (Woodcock et al., 2019). We further identified a critical role for mTORC1 in promoting the metabolic adaptations required to supply glucose-derived glycine to meet the amino acid requirements associated with the hyper-synthetic phenotype of TGFβ1–activated fibroblasts (Selvarajah et al., 2019). In terms of the potential translational evidence to support a role for this axis in IPF, omipalisib, a dual PI3K- mTOR inhibitor, was found to induce an exposure-dependent reduction in ^{18}F-FDG uptake in fibrotic areas of IPF patients (Lukey et al.,

2019). Moreover, as previously mentioned, *DEPTOR* which encodes the dishevelled, Egl-10 and Pleckstrin domain–containing mTOR-interacting protein, a major endogenous inhibitor of mTOR activity, has recently been identified as a IPF risk gene. The IPF risk allele at this locus is further associated with decreased gene expression of *DEPTOR* in lung tissue and may therefore lead to unopposed mTOR activation (Allen et al., 2020).

In contrast to mTOR which is activated in energy-replete conditions, AMPK is phosphorylated in response to depleted energy levels and is a critical upstream inhibitor of mTORC1 signalling through the phosphorylation of the tuberous sclerosis complex (TSC). Metformin, a biguanide, used for the treatment of type 2 diabetes, facilitates AMPK activation through inhibition of electron transport chain (ETC) complex I and has been observed to prevent α smooth muscle actin (SMA) and ECM production in TGFβ1–stimulated fibroblasts (Rangarajan et al., 2018; Sato et al., 2016). Moreover, metformin prevents the development of experimental fibrosis and promotes the reversal of established fibrosis in the bleomycin model (Sato et al., 2016). It is worth commenting that a post-hoc analysis of metformin versus non-metformin in the placebo arms of three IPF trials revealed that metformin had no significant effect on disease progression, lung function decline, and mortality in IPF (Spagnolo et al., 2018). However, given potential pitfalls associated with the post-hoc analysis of clinical trials which were not designed to test the hypothesis *a priori*, a future interventional trial will be required to provide a more definitive answer.

4.5 Gene therapy

Gene silencing using nucleic acid-based therapeutics is an emerging potential therapeutic approach in IPF, to precisely regulate the expression of specific genes to achieve highly specific and therapeutic effects. This approach has been successfully applied *in vivo* to attenuate a wide range of fibrosis related processes, including myofibroblast differentiation, ECM synthesis and EMT (Ruigrok et al., 2021). However, there remain considerable challenges, including overcoming issues with immunogenicity, difficulties with optimal transfection efficiency and accurate delivery to the target tissue. Among the oligonucleotide technologies, miR-29 has shown to target mRNAs, collagen I and II, IGF1 and CTGF, with low levels observed in fibrotic pathologies affecting multiple organs, including the lungs. Decreased miR-29 levels are also associated with increased mortality in IPF cohorts (Chioccioli

et al., 2022). A first-generation synthetic oligonucleotide mimic of miR-29b, Remlarsen/ MRG-201 has been shown to abrogate fibrosis in the bleomycin model (Montgomery et al., 2014). However, miRNA gene therapy has revealed issues with target tissue engagement and subsequent systemic toxicity and efforts to mitigate these consequences include changing drug delivery. MRG-229, a next generation synthetic oligonucleotide, which has improved stability and potential for targeted delivery, abrogates fibrogenic responses in TGFβ1 induced fibroblasts, PCLSs and *in vivo* bleomycin studies. Furthermore, they are shown to exhibit favourable toxicology profiles in animal models, offering potential promise as a new approach in IPF (Chioccioli et al., 2022). Furthermore, TRK-250, is an inhaled single stranded oligonucleotide that produces a silencing RNA (siRNA), that targets human TGFβ1 mRNA and reduces the expression of TGFβ and inhibits collagen production *in vivo* (Shibata et al., 2019). A phase I placebo-controlled, double-blind trial assessing the safety and tolerability of single and multiple inhaled doses in patients with IPF has just completed recruitment (NCT03727802).

4.6 Establishing regenerative capacity – stem cell therapy

Due to their capacity for self-renewal, multi-differentiation and low immunogenicity, stem cells have heralded a new era for the field of regenerative medicine, although very few stem cell treatments have yet to reach clinical translation and approval. Recurrent injury to the alveolar epithelium is largely felt to be the initiating event in IPF and leads to the generation of an abnormal hyperplastic alveolar epithelium with diminished stem cell capacity and ultimately compromised epithelial integrity in IPF. In view of this, there is growing momentum to harness the regenerative capacity of stem cells, such as embryonic and induced pluripotent stem cells (iPSCs), endogenous lung progenitor cells, and mesenchymal cells, for the future treatment of IPF.

Pre-clinical studies in bleomycin-challenged mice, transplanted with human embryonic derived AEC2s, showed recovery of body weight and blood oxygen saturation, reduced collagen deposition, improved survival and no evidence of teratoma formation (Wang et al., 2010). To overcome potential ethical considerations surrounding the use of embryonic derived stem cells, iPSCs, which are programmed from somatic cells and gain self-renewal capabilities, have also been investigated in this model. Transplantation of CD-166+ lung

epithelial cells derived from iPSCs, not only alleviate bleomycin-induced acute lung injury but also lead to an improvement in lung function and survival (Soh et al., 2012). In the context of endogenous progenitor cells, intrathecal transplantation of isolated AEC2s has been observed to abrogate and reverse fibrosis in the bleomycin model (Serrano-Mollar et al., 2007). At the time of writing, the open label trial, HALT IPF (NCT04262167) is recruiting patients to investigate the effect of intravenously administered human autologous lung spheroid stem cells, generated from transbronchial biopsies. The efficacy of autologous lung stem cells administered endobronchially is also being assessed in 20 patients with IPF (NCT02745184).

Mesenchymal cells (MSCs), which are derived from bone marrow, fat, umbilical cord blood or placenta, have the ability to differentiate into different cell lineages, are less immuno- and tumourigenic, and avoid ethical considerations surrounding the use of embryonic derived cells. MSCs exert immunomodulatory and anti-inflammatory effects in preclinical studies in IPF and have progressed to clinical testing. The phase I AETHER trial (NCT02013700) intravenously infused allogenic bone marrow derived MSCs into patients with mild to moderate IPF with safety and tolerability endpoints being met at 60 weeks (Cheng et al., 2022; Glassberg et al., 2017). Emerging data also suggests that stem cell conditioned medium containing extracellular vesicles can replicate the therapeutic effects of stem cells, is anti-fibrotic *in vivo* and compared to other stem cells convey a low risk of tumorigenicity and immune rejection(Cheng et al., 2022). There remain questions however regarding the current evidence to support the use of stem cell therapy in IPF, especially as the above clinical studies are likely underpowered to detect significant changes in outcomes such as FVC decline and there are practical concerns regarding feasibility and the optimal stage of disease at which to intervene.

5. Paving the way for future novel therapeutic targets – emergence of human-based models for the study of PF

IPF is a complex and heterogeneous disease, potentially with multiple endotypes. Major challenges to successful patient management include a lack of effective means to diagnose patients early, identify those that carry a higher risk of progression, as well as robust approaches to evaluate disease progression and response to treatment.

Refining our understanding of IPF pathomechanisms is going to be paramount to address these challenges. There is little doubt that the commonly used animal model of bleomycin-induced lung injury and fibrosis has had a major impact on our understanding of the pathways leading to the transient accumulation of excessive extracellular matrix (ECM). The recent introduction of models based on multiple intra-pulmonary instillations have also brought the bleomycin model a step closer to representing some of the key pathological features of IPF (Redente et al., 2021). In recent years, the application and refinement of single cell RNA-sequencing (scRNA-Seq) techniques to explore transcriptional perturbations at single cell resolution has transformed the way we think about the lung, its cellular components and their interactions with each other and with the ECM in health and in disease. These studies have revealed major new insights into the complexity of cellular heterogeneity in IPF; the presence of multiple epithelial cell phenotypes, including the appearance of aberrant basaloid cells which are not present in the healthy lung; multiple myeloid cell phenotypes, including pro-fibrotic macrophages and T cell exhaustion (Adams et al., 2020; Habermann et al., 2020). Our concept of fibroblasts as the key effector cells of the fibrogenic response is also evolving and we know have a better appreciation of fibroblast heterogeneity, including the identification of multiple collagen-producing subpopulations, such as a subpopulation, characterized by expression of Cthrc1 (collagen triple helix repeat containing 1) which express the highest levels of collagens (Tsukui et al., 2020).

In terms of current approaches to investigate the key pathomechanisms underlying IPF, monocellular, two-dimensional, metabolically artificial in vitro models are increasingly felt to have limitations in terms of representing the complexity of the cell-cell and cell-matrix interactions underlying the development of fibrosis. Advances in the generation of more complex human-based model systems to better reproduce the physiological and pathological tissue microenvironment of IPF, the so-called fibrotic niche, are likely to have a major impact on our current understanding of the key pathomechanisms underlying this condition and will be paramount to the identification of novel drug targets. These will now be briefly described in turn, for a more detailed review of recent advances in 3D in vitro models in IPF, please see recent reviews (Jeong et al., 2022; Vazquez-Armendariz et al., 2022).

5.1 Alveolar organoids

Organoids are self-organising three-dimensional (3D) structures derived from self-renewing and differentiating stem or progenitor cells grown in Matrigel, that replicate some of the physiological and pathological features of their primary tissue (Corrò et al., 2020). Human alveolar organoids are derived from AEC2s and depending on the culture conditions, AEC2s can give rise to organoids alone (Katsura et al., 2020; J. H. Kim et al., 2021; Youk et al., 2020), or require the presence of a mesenchymal feeder layer (Barkauskas et al., 2013), and can differentiate into alveolar epithelial type 1 (AEC1) cells. Alveolospheres have been particularly helpful for enabling mechanistic studies of the epithelium in the distal lung as they overcome some of the difficulties surrounding the in vitro expansion and cryopreservation of AEC1 and 2 cells. They have been particularly instrumental in providing key insights into alveolar regeneration, epithelial-mesenchymal cross talk and more recently of viral infections, e.g. SARS-CoV-2 (Katsura et al., 2020; Youk et al., 2020). The main limitations of the model relate to the access to suitably sourced healthy and diseased tissue, and the fact that once differentiated, the apical surface of the alveolosphere is located on the inside, which in turn influences the preferential delivery of mediators and compounds to the basal surface over the apical side. Once alveolospheres have been generated and expanded, they can be enzymatically dissociated and cells can be transferred to air-liquid-interface in a 2D transwell culture system to obviate this limitation.

In terms of further improvements, current research efforts are centred around improving the throughput of the model and perfecting cell culture conditions to allow for the introduction of immune cell types in co-culture systems. Unfortunately, there are currently still no effective protocols for transfection or transduction of alveolospheres, and therefore gene editing is still not available for organoids generated from human-derived AEC2. However, major advances in the development of protocols to generate alveolar organoids derived from genetically-editable (e.g. CRISPR-Cas 9) human induced pluripotent stem cells (iPSCs) are now paving the way for disease modelling, as well as the future functional regeneration of the distal lung. iPSCs can also be derived from adult patient cells, including from patients with monogenic lung disease or, in the case of PF, a genetic predisposition to developing lung disease (Strikoudis et al., 2019). These approaches hold much promise for a future precision medicine-based approach, including personalised drug screens.

5.2 Precision cut lung slices (PCLS)

The application of precision cut lung slices (PCLS) in IPF research is also offering considerable potential to bridge the translational gap between current cell-based model systems and early-clinical trial as part of pre-clinical target validation studies. PCLS are 100-500 µm-thick slices of lung parenchyma that can be maintained in culture for up to 14 days, cryopreserved for future use (although this has been shown to alter certain aspects, such as metabolism), and used as an *ex-vivo* model for mechanistic, pathobiology and toxicology studies (Dean & Königshoff, 2021). The main advantage of PCLS is that they faithfully recapitulate physiological cell diversity, ratio and cell-cell and cell-environment interactions, in a manner that has been impossible to achieve so far *in vitro* (Evans & Lee, 2020; Liu et al., 2019). Using PCLS, it is also possible to investigate mechanisms involved in repair and regeneration following lung injury. For example, the acid injury and repair (AIR) model involves applying hydrochloric acid to a restricted area of tissue to produce a spatially limited injury (S. Y. Kim et al., 2020). The same model could also be used to investigate pro-repair agents, also exploiting the semi-high-throughput scalability of PCLS.

PCLS have been particularly useful when they are directly derived from diseased tissue, and IPF PCLSs have been used to evaluate the therapeutic effect, pharmacology, and mechanism of action of potential antifibrotic agents, including the PI3K/AKT/mTOR inhibitor, GSK2126458 (omipalisib) (Mercer et al., 2016), the dual ATP-competitive mTOR inhibitor CZ415 (Woodcock et al., 2019), the αvβ6 inhibitor GSK3008348 (John et al., 2020), and the antifibrotic agent epigallocatechin gallate (EGCG) (Wei et al., 2021). It is not yet possible to genetically manipulate PCLS ex-vivo but it is possible to generate PCLS from mice via intra-pulmonary instillation of a lacZ-expressing adenovirus. LacZ transgene expression in PCLS was observed in bronchiolar and alveolar cells after 4 days in culture (McBride et al., 2000). Similarly to organoids, the main drawback of human PCLS is access to fresh human tissue, particularly PF tissue. In an attempt to reproduce the disease processes and scale up the PCLS IPF model for future drug testing, it is now possible to induce several features which mimic the fibrogenic response by applying a profibrotic cytokine cocktail (e.g. TGFβ1, lysophosphatidic acid receptor (LPA), and interleukin-1 (IL-1)), in PCLS obtained from control tissue over the course of a few days (Alsafadi et al., 2017).

5.3 Lung-on-a-chip

Lung-on-a-chip models use microfluidic devices and cellular components to replicate physiological lung functions such as stretch associated with breathing. An early example of this model was developed over a decade ago and consisted of a device comprising an air-filled, alveolar cell-lined chamber and a fluid-filled, endothelial cell lined chamber which were separated by a porous membrane to mimic gas exchange. Vacuum was applied to the two side-chambers in a cyclical manner to mimic stretch associated with breathing movements (Huh et al., 2010). More recently, the ECM component was added to lung-on-a-chip models, including the fibrosis-on-a-chip system which comprises arrays of membranous 3D microtissues formed through cell-mediated ECM remodelling, recapitulating key biomechanical properties of both healthy and fibrotic lung alveolar tissues. Transition from physiological to the tissue stiffness associated with fibrotic tissue is achieved by TGFβ1 treatment. Proof of principle studies that this model might be useful for drug discovery was provided by demonstrating that pirfenidone and nintedanib both restored tissue compliance (Asmani et al., 2018; Asmani & Zhao, 2021). Since personalised lung-on-a-chip models have already been established using patient cell or tissue samples, for instance for the study of chronic obstructive pulmonary disease (Benam et al., 2015), the fibrosis-on-a-chip system may similarly open up future avenues for personalised drug screening, as well as more reliable mechanistic studies in a model system that more faithfully reproduces the breathing and gas exchange properties of the lung. The high failure rate (70%) of orally inhaled drugs in clinical trials may be related to the fact that commonly used 2D in vitro and rodent in vivo models are poorly representative of the human respiratory physiology and biology (Movia & Prina-Mello, 2020). The emergence of these complex 3D *in-vitro* and *ex-vivo* models that better recapitulate the physiological and pathological characteristics of human tissue have the potential to i) expedite our fundamental understanding of the pathomechanisms underpinning PF, therefore improving drug target selection, ii) allow for patient specific and endotype specific studies that will bring us closer to personalised medicine development.

6. Challenges and future perspectives in the clinical development of IPF therapeutics

There remains a pressing need for treatments that have a significant impact on mortality and quality of life in patients with progressive pulmonary fibrosis. Outcomes for patients remain worse than for many cancers. Unfortunately, numerous clinical trials have failed to deliver

effective therapies due to either not achieving statistical significance on clinical endpoints or deemed to have unacceptable safety profiles. Recent clinical trials to note, include the biological therapies, pentraxin and pamrevlumab. PRM-151 is a recombinant form of the human pentraxin 2 protein, despite significantly slowing FVC decline in phase II trials (NCT02550873) (Raghu et al., 2019), a recent phase III randomised double-blind placebo-controlled trial (STARSCAPE - NCT04594707) was terminated by the sponsors as the futility outcome analysis suggested that the primary endpoint would not be met. Similarly, pamrevlumab (FG-3019), a monoclonal antibody that targets CTGF and consequently prevents activation of downstream profibrotic signalling, had shown promise in the recent phase II PRAISE trial by significantly slowing lung function decline and reducing lung fibrosis HRCT scores. Despite these promising data, the sponsor of two phase III trials (ZEPHYSUS-1 AND 2), examining the efficacy and safety of pamrevlumab in IPF (NCT03955146 and NCT04419558) has recently reported that ZEPHYSUS-1 did not meet the primary endpoint based on a change from baseline in FVC at 48 weeks. Based on these disappointing results, the ZEPHYSUS-2 study will now be discontinued.

Several factors need to be considered for future clinical trials, in light of the failure of these recent phase 3 trials despite promising preceding clinical data. With nintedanib and pirfenidone being standard of care in the treatment of IPF, future phase 2 trials, need to be sufficiently powered to assess efficacy, safety and importantly tolerance with the backdrop of concomitant antifibrotic use. Additionally, time-efficient and patient relevant clinical trial outcomes such as assessing FVC decline over a shorter time period of 3 months rather than 12 months and utilising endpoints such as all-cause mortality and hospitalisation, need to be taken into consideration in the design of future clinical trials in IPF. Furthermore, patient selection and subgroup analysis throughout the drug development process, needs to take account of differences in biological sex, race, ethnicity, and geographic variation which all contribute to the heterogeneity and complexity of IPF with regards to pathogenesis, response to treatment and mortality (Podolanczuk, Richeldi, et al., 2023).

Understanding the complexity and heterogenous nature of IPF, has highlighted the need for individualised management strategies that include diagnostic, treatment, and prognostic classification. The integration of multi-omic based biomarkers and AI enabled- approaches

hold great promise for personalised medicine to drive effective treatment strategies in progressive pulmonary fibrosis. Subtyping patients based on validated biomarker, imaging and AI parameters will enable enriched cohorts for personalised medicine-led drug design. Conceptual shifts in trial design methodology are also emerging including identifying clinically relevant, patient-reported and practical endpoints, as well as moving towards efficient trial platforms. REMAP-ILD, a randomised, embedded, multifactorial adaptive platform trial for interstitial lung disease, is an international adaptive platform clinical trial, to accelerate the assessment of therapies for PPF by testing several interventions with multiple patient strata simultaneously as was done so successfully in the COVID-19 RECOVERY trial.

The success of nintedanib and Ofev may in fact be attributed to their pluripotent effects rather than targeting a single fundamental fibrogenic process in IPF. The continued exploration of agents which may target multiple collaborating mechanisms may therefore be warranted. However, it is unlikely that such agents will address all the key processes underlying the development of fibrosis, so that a strategy involving combination therapy, as used in the treatment of cancer, may need to be considered for the treatment of complex progressive fibrotic lung diseases. With significant amelioration of lung function decline with ofev and nintedanib as standard of care, the success of future novel pharmacological agents in IPF, will also depend on an efficient synergism with existing antifibrotic agents to produce superior outcomes without increasing undesirable side effect profiles. Inhaled agents (e.g. GB0139) may overcome some of the challenges by avoiding systemic exposure. The emergence of novel nanoparticle-based inhaled drug delivery strategies to potentially offer a more site-specific, targeted approach to achieve efficacious anti-fibrotic therapy with more tolerable side effect profiles may further hold promise (Ghumman et al., 2021).

IPF is typically diagnosed at later stage and therefore conveys worse outcomes. As in cancer screening, a key research priority should centre on identifying patients with early fibrosis and at risk of progressive fibrotic lung disease. Lung cancer screening cohorts, which are enriched with patients at risk of IPF (smoking, age), have identified participants with incidental early fibrosis or interstitial lung abnormalities (ILAs). This presents a unique opportunity to screen for participants with early fibrosis or undiagnosed pulmonary fibrosis, understand the evolution and risk of progressive fibrotic lung disease and potentially intervene early to improve prognosis. In addition to detecting early disease and patients at risk of progression,

there is growing research momentum to concentrate on the management of other factors that significantly affect outcomes in PPF, such as exacerbations, lung cancer, pulmonary hypertension and co-existing co morbidities.

7. Conclusion

The last decade has seen major advances in our understanding of IPF pathobiology which has been in part enabled as a result of advances in single-cell approaches. The recent development and imminent application of single cell multiomics and spatial transcriptomics will continue to drive an ever greater appreciation of the complex interplay of the key transcriptional and signalling networks within the fibrotic niche. Ongoing improvements in pre-clinical in vitro, in vivo and ex-vivo models will continue to facilitate the exploration of new mechanistic hypotheses, as well as the identification of novel actionable drug targets. Finally, the continued exploration of a regenerative medicine-based therapeutic strategy to harness the reparative capacity of the lung, in combination with a precision medicine approach tailored to the individual patient, is likely to be transformative in our quest to discover therapeutic strategies which will not only halt disease progression but hold the potential to reverse fibrosis.

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Figure Legend

Figure 1. Pathogenesis of IPF.

IPF arises as a result of a highly abnormal wound healing response following repetitive epithelial injury in aged and genetically susceptible individuals. The pathological tissue injury response is characterized and perpetuated by the interplay between i) compromised epithelial repair as a result of stem cell failure in the distal lung leading to the appearance of transitional alveolar progenitor cells and aberrant basaloid cells which line the fibrotic foci, the sentinel lesions in IPF/UIP; ii) immune dysregulation dominated by pro-fibrotic macrophages and iii) uncontrolled fibrogenesis by highly activated fibroblasts and myofibroblasts under the influence of a network of proliferation and differentiation signals received from a homeostatically degenerate microenvironment

Table 1. Recent and ongoing clinical trials

	Drug	Trial	Outcome/status of trial
Targeting profibrotic signalling axes	TAS-115 - inhibitor of PDGFR, VEGFR, FMSR, HGFR	Exploratory phase 2 trial of TAS-115 in patients with IPF over 13 weeks, N=46 patients (JapicCTI-183898).	Acceptable safety and tolerability, with a trend towards decreased rate of FVC decline.
	KD025, an oral inhibitor of ROCK2	Phase 2 randomised, open-label, multi-centre trial in IPF patients over 24 weeks (NCT02688647).	Good safety and tolerability and reduction in lung function decline over 24 weeks.
	Saracatinib, src kinase inhibitor	Phase 1b/2a clinical trial (NCT04598919).	Recruiting.
	CC-9001, a JNK inhibitor	Phase 1b (NCT02510937), N=16.	Safe and well tolerated.
		Phase 2 multicentre, multi-national, randomised, double blind, placebo controlled trial in patients with IPF, N=138 (NCT03142191)	Put on hold– change in business objective.
	LPA pathway		

	Autotaxin inhibitors GLPG1690	 ISABELLA - 2 x phase 3 trials (NCT03711162 and NCT03733444)	 Discontinued due to toxicity.
	BBT-877	Phase 2 randomised, double-blind, placebo-controlled trial over 24 weeks (monotherapy or add on therapy) in patients with IPF (NCT05483907).	Recruiting.
	BLD-0409	Phase 2 randomised, double-blind, placebo-controlled trial (monotherapy or add on therapy) in patients with IPF – (NCT05373914).	Not yet recruiting.
	LPA1 receptor inhibitors		
	BMS-986020, a LPA1 receptor antagonist.	Phase 2, multicentre, randomized, double-blind, placebo-controlled trial over 26 weeks.	Reduction in rate of FVC decline, but increased hepatobiliary toxicity and study was terminated early.
	BMS-986278. -next - generation LPA1 receptor antagonist	A phase 2 randomised double blind, placebo-controlled trial (NCT04308681).	Recruiting.

	Galectin		
	GB0139 (formerly TD139) – small molecular inhibitor of galectin.	Phase 2b randomised double-blind, placebo controlled trial to assess effect on FVC decline at 52 weeks.	Finished recruiting.
	Integrins		
	BG00011 - αvβ6 humanised monoclonal antibody	SPIRIT - Phase 2b randomised double blind trial assessing safety in IPF (N=109)	Terminated early – increased mortality, exacerbations.
	PLN-74809 or Bexotegrast - αvβ6 and αvβ1 oral inhibitor	INTEGRIS-IPF – Phase 2a randomised double blind, placebo-controlled trial, N=120, NCT04396756.	Press release suggests favourable safety and tolerability profiles.
		The BEACON-IPF phase 2b trial (NCT04396756).	Commencing soon.
	Phosphodiesterase inhibitors		
	BI1015550 - PDE4B inhibitor	Phase 2 randomised, double-blind, placebo-controlled	

		parallel group study (monotherapy and in combination with anti-fibrotics) over 12 weeks, N=147 (NCT04419506).	Monotherapy or in combination with an antifibrotic agent, prevented lung function decline compared to placebo.
		Phase 3, double-blind, randomised controlled trial over 52 weeks in patients with IPF. (NCT05321069)	Recruiting.
		Phase 3, double blind, randomised, placebo-controlled trial over 52 weeks in patients with progressive fibrosing interstitial lung diseases (NCT05321082).	Recruiting.
	Sildenafil - PDE5 inhibitor	STEP-IPF - double-blind, randomised, placebo controlled trial in patients with IPF (N=180)	No effect on primary outcome – 6 minute walk distance.
		INSTAGE – 24 week, double – blind, randomised, parallel group – effect of sildenafil + nintedanib v nintedanib alone (N=274)	No superior effect on St Georges Respiratory Questionnaire scores of dual therapy compared to nintedanib alone.

	Prostacyclin analogue Inhaled Treprostinil	INCREASE – multicentre, randomised double-blind, placebo-controlled trial over 16 weeks in patients with ILD and pulmonary hypertension, N=326, NCT02630316. TETON - 2 x replicate randomised, double blind phase 3 trials over 52 weeks in patients with IPF (NCT04708782, NCT05255991)	Improvement in 6MWT distance at 4 months. Recruiting
Senolytics	Dasatinib and querceptin (D +Q)	Open label pilot study, N=14 IPF patients. No control group. (NCT02874989). A follow on confirmatory randomised placebo-controlled pilot trial. N=12, 1:1 ratio of IPF v control. 3 week intermittent treatment with 1 week follow up (NCT02874989).	No serious adverse events related to D+Q.
Gene therapy	TRK-250 - single stranded oligonucleotide releasing siRNA targeting TGFβ1 mRNA	Phase 1 placebo-controlled double blind trial - safety and tolerability of single and multiple inhaled doses in	Just completed recruitment.

		patient with IPF (N=34 recruited) (NCT03727802)	
Regenerative medicine	Autologous lung spheroid stem cells (intravenous)	HALT-IPF– Phase 1, randomised open label trial, N=24 IPF patients. Safety and efficacy outcomes (NCT04262167)	Recruiting
	Autologous lung stem cells (endobronchially administered)	Open label, non-randomised phase 1 trial, assessing safety and efficacy over 12 months, N=20 IPF patients, (NCT02745184).	Recruiting, estimated completion date October 2023.
	Allogenic bone marrow derived MSCs (intravenous)	AETHER trial (NCT02013700)- phase 1, N= 9 IPF patients	Safety and tolerability endpoints met at 60 weeks.

Pathogenesis of IPF