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Perturbational single-cell genomics to decode trajectories and interactions of regenerative intermediate cell states during lung fibrogenesis and anti-fibrotic drug treatments

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IMPLIES ALSO
A DUTY;
ONE MUST NOT
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TO BE TRUE.

Albert Einstein
1879 – 1955

1. Affidavit

I, Niklas Jonathan Lang, hereby declare, that the submitted thesis entitled:

"*Perturbational single-cell genomics to decode trajectories and interactions of regenerative intermediate cell states during lung fibrogenesis and anti-fibrotic drug treatments*"

is my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

In this context, I declare that I have utilized generative AI tools, specifically Large Language Model (LLM)-based agents, in accordance with the guidelines provided by my institution to improve and edit this thesis. These agents were solely used to enhance clarity, grammar, and style of this thesis. All outputs have been thoroughly reviewed by me.

I further declare that the dissertation presented here has not been submitted in the same or similar form to any other institution for the purpose of obtaining an academic degree.

Munich (GER), 23.12.2025 Niklas J. Lang

2. Confirmation of Congruency

I, Niklas Jonathan Lang, hereby declare, that the submitted thesis entitled:

"*Perturbational single-cell genomics to decode trajectories and interactions of regenerative intermediate cell states during lung fibrogenesis and anti-fibrotic drug treatments*"

is congruent with the printed version both in content and format.

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3. Abstract (English)

Pulmonary Fibrosis (PF) is the central pathomechanism shared across all fibrotic Interstitial Lung Diseases (fILD), a heterogeneous group of diseases with limited therapeutic options and poor outcomes. Fibrotic remodeling of the alveolar gas exchange unit due to impaired regeneration following injury is the common pathophysiologic feature of all fILDs. Recent studies probing the cellular underpinnings of these processes have highlighted the emergence of regenerative intermediate cell states (RICS) as transitional stages regulating whether injury leads to regeneration or fibrosis. Most of these studies are, however, focused on end stage disease, which by design provide only limited insights into the role and contributions of RICS during disease initiation and progression.

This thesis aims to investigate the emergence, interactions, and cellular trajectories of RICS underlying lung fibrogenesis and their modulation in response to anti-fibrotic drug treatments. To this end, it leverages single-cell RNA sequencing (scRNA-seq) in combination with both lineage tracing in mice and human precision-cut lung slices (hPCLS) treated with a pro-fibrotic cytokine mix to induce de novo fibrotic remodeling and study the cellular responses to pharmacological perturbations.

This thesis shows that hPCLS not only retain the full cellular repertoire of the human lung but also reveals that they can recapitulate the fibrogenic RICS observed in lungs from patients with PF upon stimulation with pro-fibrotic cytokines. During early disease alveolar type 2 cells differentiate into fibrogenic KRT17+/KRT5- basaloid cells, while capillary cells give rise to fibrogenic PLVAP/VWA1+ endothelial cells. Additionally, CTHRC1+ myofibroblasts arise from transcriptional convergence of activated stromal cell populations rather than from a single progenitor cell type. Micro-CT staged patient tissues and cell-cell communication analyses map the appearance and interactions of these fibrogenic RICS, validating their appearance during early disease stages. Finally, this work

explores the potential of hPCLS coupled to scRNA-seq for phenotypic drug testing directly in human lung tissue by providing a computational framework that accurately confirms known and identifies previously unrecognized mechanisms of the anti-fibrotic drug nintedanib.

In summary, this thesis introduces a novel framework to study lung fibrogenesis directly in human lung tissue, enabling spatiotemporal analysis at single-cell resolution. The results advance our understanding of the emergence, interactions, and lineage relationships of fibrogenic RICS during disease initiation, which could inform novel disease-modifying therapeutics. Ultimately, it showcases the potential of hPCLS for next generation, high-resolution drug testing directly in human lung tissue - the environment where effective drugs would have to unfold their antifibrotic properties - thereby opening new avenues for accelerated drug development and translation.

4. Abstract (Deutsch)

Die interstitielle Fibrose ist der zentrale gemeinsame Pathomechanismus aller fibrosierenden interstitiellen Lungenerkrankungen (engl. fILD), einer heterogenen Gruppe von Krankheiten mit schlechter Prognose und limitierten Therapieoptionen. Der fibrotische Umbau der im alveolären Septum lokalisierten Luft-Blut-Schranke in Folge einer fehlgeleiteten Regeneration einer Lungenschädigung ist das gemeinsame pathophysiologische Merkmal aller fILDs. Aktuelle Studien, die die zellulären Grundlagen dieser Prozesse untersuchen, weisen auf das Auftreten sogenannter Regenerative Intermediate Cell States (RICS) hin, die vorübergehend auftreten und regulieren, ob eine Lungenschädigung zu Regeneration oder Fibrose führt. Die Mehrzahl dieser Studien beschränken sich jedoch auf das Endstadium der Erkrankung und sind dahingehend limitiert, als dass sie das Auftreten und den Beitrag von RICS zur Krankheitsentstehung und -progression dementsprechend nur ungenügend abbilden können.

Diese Dissertation hat zum Ziel, die zelluläre Entstehung, Interaktionen, und Trajektorien von RICS, die der Entstehung der Lungenfibrose zugrunde liegen, sowie deren Modulation durch anti-fibrotische Medikamente zu untersuchen. Zu diesem Zweck kombiniert die Arbeit single-cell RNA-Sequenzierung (scRNA-seq) mit Lineage Tracing im Mausmodell und menschlichen Lungenschnitten (hPCLS), die mit pro-fibrotischen Zytokinen behandelt wurden, um den fibrotischen Umbau der bis dahin unversehrten Lunge zu induzieren und die zellulären Reaktionen auf pharmakologische Perturbationen zu untersuchen.

Die Arbeit zeigt, dass hPCLS das gesamte zelluläre Repertoire und die Architektur der menschlichen Lunge bewahren und sich fibrogene RICS induzieren lassen, welche sich in sehr ähnlicher Form in Lungen von Patienten mit Lungenfibrose finden. Die Arbeit liefert weiter Hinweise darauf, dass in der frühen Phase des fibrotischen Umbaus Alveoläre Typ 2 Zellen in fibrogene

KRT17+/KRT5- basaloide Zellen differenzieren, während Kapillarzellen in fibrogene PLVAP/VWA1+ Endothelzellen differenzieren. CTHRC1+ Myofibroblasten hingegen gehen nicht aus einem spezifischen Zelltyp hervor, sondern entstehen als Resultat einer transkriptionellen Konvergenz mehrerer, unterschiedlicher Zellpopulationen des Stromas der Lunge, die im Rahmen des fibrotischen Umbaus aktiviert werden. In menschlichen Gewebeproben von Lungenfibrose, denen mittels Computertomographie ein radiologisches Krankheitsstadium zugeordnet wurde, sowie mit Hilfe von Zell-Zell Kommunikations Analysen kartiert diese Arbeit darauf aufbauend das Auftreten und die Interaktion der oben genannte fibrogenen RICS und validiert ihr Auftreten bereits im frühen Krankheitsstadium. Abschließend untersucht diese Arbeit das Potenzial hPCLS mit scRNA-seq zu kombinieren, um phänotypische Medikamentenerprobung direkt in menschlichen Lungengewebe durchzuführen, indem wir eine bioinformatische Pipeline bereitstellen, die in korrekter Weise bereits bekannte Wirkmechanismen des klinisch zugelassenen anti-fibrotischen Medikaments Nintedanib erfasst, aber auch neue bisher unbekannte Wirkmechanismen identifiziert.

Zusammenfassend stellt diese Arbeit ein integriertes experimentelles und bioinformatisches Setup vor, das das Untersuchen der Entstehung von Fibrose direkt im menschlichen Lungengewebe ermöglicht, was die Analyse von zellulären Veränderungen in räumlicher und zeitlicher Auflösung ermöglicht. Die Arbeit verbessert unser Verständnis von der Entstehung potenzieller Vorläuferzellen fibrogener RICS während der Krankheitsentstehung, was zur Entwicklung neuer therapeutischer Ansätze führen könnte. Schließlich zeigt die Arbeit das Potenzial von hPCLS für hochauflösende Medikamententests direkt im menschlichen Lungengewebe auf – also genau dort, an dem ein wirksames Medikament seine antifibrotischen Eigenschaften entfalten müsste – und trägt so dazu bei, die Entwicklung und Translation neuer Medikamente zu beschleunigen.

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6. List of Abbreviations

A

aCap	Aerocyte capillary cells
ALAT	Latin American Thoracic Society
AT1	Alveolar epithelial cell type 1
AT2	Alveolar epithelial cell type 2
ATS	American Thoracic Society

C

cDNA	Complementary DNA
CHP	Chronic hypersensitivity pneumonitis
COL15A1	collagen type XV alpha 1 chain (gene/protein)
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
CT	Computed tomography
CTHRC1	collagen triple helix repeat containing 1 (gene/protein)

D

DNA	Desoxyribonucleic acid

E

EC	Endothelial cell
ECM	Extracellular matrix
ERS	European Respiratory Society

F

FC	Fibrotic cocktail
fILD	Fibrosing interstitial lung disease

G

gCap	General capillary cells
GWAS	Genome-wide association study

H

HRCT	High resolution computed tomography

I

ILD	Interstitial lung disease
IPF	Idiopathic pulmonary fibrosis

J

JRS	Japanese Respiratory Society

K

KRT	Keratin (gene/protein)

L

LPA	Lysophosphatidic acid

M

MDM	Monocyte-derived macrophage
mRNA	Messenger ribonucleic acid
MUC5B	Mucin 5B (gene)

P

PCLS	Precision-cut lung slices
PCR	Polymerase chain reaction
PDGF	Platelet-derived growth factor
PLVAP	Plasmalemma vesicle-associated protein (gene/protein)
PF	Pulmonary Fibrosis

R

RA-ILD	Rheumatoid arthritis with ILD
RICS	Regenerative intermediate cell states
RNA	Ribonucleic acid

S

scRNA-seq	Single-cell RNA sequencing
SFRP1	Secreted frizzled-related protein 1 (gene/protein)
SNP	Single nucleotide polymorphism
SPP1	Secreted phosphoprotein 1 or Osteopontin (gene/protein)

T

TGFβ	Transforming growth factor β (gene/protein)
TNFα	Tumor necrosis factor α (gene/protein)

U

UIP	Usual interstitial pneumonia

V

VEFG	Vascular endothelial growth factor (gene/protein)
VWA1	Von Willebrand factor type A domain (gene/protein)

7. List of Publications

The 'asterisk' symbol, i.e. *, indicates equal contribution. The 'dagger' symbol, i.e. †, is listed for co-senior authors.

Publications used in this thesis:

N J Lang*, J Gote-Schniering*, D Porras-Gonzalez, L Yang, L J De Sadeleer, R C Jentzsch, V A Shitov, S Zhou, M Ansari, A Agami, C H Mayr, B Hooshiar Kashani, Y Chen, L Heumos, J C Pestoni, E Sarolta Molnar, E Geeraerts, V Anquetil, L Saniere, M Wögrath, M Gerckens, M Lehmann, A Ö Yildirim, R Hatz, N Kneidinger, J Behr, W A Wuyts, M G Stoleriu, M D Luecken, F J Theis, G Burgstaller†, H B Schiller†. **Ex vivo tissue perturbations coupled to single-cell RNA-seq reveal multilineage cell circuit dynamics in human lung fibrogenesis.** *Science Translational Medicine* 15, eadh0908(2023).

C H Mayr*, A Sengupta*, S Asgharpour, M Ansari, J C Pestoni, P Ogar, I Angelidis, A Liontos, J Alberto Rodriguez-Castillo, **N J Lang**, M Strunz, D Porras-Gonzalez, M Gerckens, L J De Sadeleer, B Oehrle, V Viteri-Alvarez, IE Fernandez, M Tallquist, M Irmler, J Beckers, O Eickelberg, M G Stoleriu, J Behr, N Kneidinger, W A Wuyts, R M Wasnick, A Ö Yildirim, K Ahlbrecht, R E Morty, C Samakovlis, F J Theis, G Burgstaller†, H B Schiller†. **Sfrp1 inhibits lung fibroblast invasion during transition to injury-induced myofibroblasts.** *European Respiratory Journal* 63, 2301326 (2024).

Publications not used in this thesis:

L Heumos, P Ehmele, T Treis, [...], **N J Lang**, [...], R Eils, H B Schiller, A Hilgendorff, F J Theis. **An open-source framework for end-to-end analysis of electronic health record data.** *Nature Medicine* 30, 3369–3380 (2024).

G G Günsel*, T M Conlon*, A Jeridi*, R Kim, Z Ertüz, **N J Lang**, [...], H B Schiller, M Conrad, R Schneider, A Ö Yildirim. **The arginine methyltransferase PRMT7 promotes extravasation of monocytes resulting in tissue injury in COPD.** *Nature Communications* 13, 1303 (2022).

A Schröder*, L P Lunding*, U M Zissler, [...], **N J Lang**, H B Schiller, M A Mall, H Fehrenbach, C A Dinarello, M Wegmann. **IL-37 regulates allergic inflammation by counterbalancing pro-inflammatory IL-1 and IL-33.** *Allergy*, Volume 77, Issue 33, pp. 856-869 (2022).

D S Fischer*, M Ansari*, K I Wagner*, [...], **N J Lang**, [...], F J Theis, D H Busch, H B Schiller†, K Schober†. **Single-cell RNA sequencing reveals ex vivo signatures of SARS-CoV-2-reactive T cells through 'reverse phenotyping'.** *Nature Communications* 12, 4515 (2021).

8. Introduction

8.1 Pulmonary Fibrosis (PF)

Fibrosis is an ubiquitous pathological process characterised by excessive production of extracellular matrix (ECM) and consecutive destruction of tissue architecture. As such, it can occur in any organ as a result of failed regeneration after tissue injury and leads to organ dysfunction and ultimately organ failure[1,2]. In the developed world, fibrosis is responsible for more than 45% of deaths making it a leading cause of morbidity and mortality[2].

Pulmonary Fibrosis (PF) refers to the manifestation of fibrosis in the lung. PF is not a disease itself, but rather the shared pathomechanism common to all fibrotic lung diseases[1,3]. Fibrotic lung diseases are a heterogeneous group, comprising numerous conditions with different aetiologies, clinical presentations, imaging and pathological features, and varying prognosis. Since pathological abnormalities commonly found in this diverse group of diseases, such as inflammatory infiltrates or fibrosis, are typically located in the interstitial space of the alveolar septum, these conditions are collectively referred to as interstitial lung diseases (ILDs)[3].

Idiopathic Pulmonary Fibrosis (IPF) is the most prevalent and severe form of fibrotic ILDs[3]. IPF is an irreversible and progressive disease, typically leading to respiratory failure and death within a short period, which is reflected in a median survival time of 3.8 years[4,5]. The diagnosis of IPF relies on the characteristic radiographic and histopathological pattern of usual interstitial pneumonia (UIP) in the absence of known causes of ILDs or diseases associated with ILDs[3,4,6].

Non-IPF ILDs typically exhibit a lower degree of fibrosis but a higher presence of inflammatory infiltrates on pathological examination[3], which often corresponds to more favourable clinical outcomes. However, a subset of 13-40% of patients

with non-IPF ILDs develops a progressive fibrosing phenotype[7] with disease trajectories similar to those observed in patients with IPF[8,9].

For this reason, the focus of this introduction will be on IPF due to its status as the most prevalent and best-studied form of progressive PF, thereby serving as a representative prototype disease. Characteristics of other disease entities with PF will be discussed selectively based on significant deviations from IPF characteristics.

8.1.1 Epidemiology of Idiopathic Pulmonary Fibrosis

Fibrotic ILDs (fILDs) are a collection of rare diseases. Although individually rare, their cumulative prevalence amounts to 76.0 cases per 100,000 in Europe and 74.3 cases per 100,000 population in the United States[3]. The prevalence of IPF, the most common form of fibrotic ILDs, is estimated to be 8 to 60 cases per 100,000 people[10–12] in the United States but appears to be lower outside the US and Europe. Whether this observation reflects the geographical variation of intrinsic characteristics of the disease (such as genetics or exposure to environmental pollutants) or differences in diagnostic or reporting standards remains unclear[10–12].

Higher prevalences of IPF are observed in individuals of male sex (sex ratio 7:3) and older age. At the time of diagnosis, patients are typically older than 60 years[13]. Health registry data from the United Kingdom suggests that 85% of patients with IPF are older than 70 at the time of diagnosis[14]. This trend is mirrored in the observation that the prevalence of IPF grows exponentially with every decade after the age of 50[15]. Unlike IPF, non-IPF ILDs are characterized by a more balanced sex ratio and age distribution (20-60 years at the time of diagnosis)[3].

The prevalence of IPF is constantly increasing, which leads to an increase in hospital admissions and deaths due to IPF[16–18], emphasizing the growing healthcare impact of the disease.

8.1.2 Aetiology of Idiopathic Pulmonary Fibrosis

Although the term "idiopathic" implies the underlying cause of IPF is unknown, several genetic, environmental, cellular, and molecular mechanisms involved in developing the disease have been identified.

8.1.2.1 Genetics

Genome-wide association studies (GWAS) of IPF cohorts have identified several genetic loci associated with increased susceptibility to IPF[19].

A specific single-nucleotide polymorphism (SNP; rs35705950) in the promoter region of the gene MUC5B is the most common genetic variant associated with IPF and exhibits the strongest association with IPF[20]. It is present in 38% of IPF patients, compared to 9% of the general population, and is upregulated in IPF lungs[20]. MUC5B, which codes for mucin 5B, is expressed primarily by goblet cells residing in the airway epithelium[21] and plays a crucial role in mucosal immunity through mucociliary clearance. Paradoxically, the identical MUC5B SNP, which is associated with a 21-fold increased risk of developing IPF, is also associated with a better prognosis[22].

Further genomic regions conferring risk for IPF include cell adhesion genes (DSP, AKAP13, CTNNA, and DPP9)[23,24], genes regulating telomere length (TERT, TERC, RTEL1, and PARN)[25–27], and surfactant genes (SFTPC, SFTPA1, SFTPA2)[28].

Notably, many IPF-associated genetic variants also increase susceptibility to other forms of fILDs. For example, the MUC5B variant and mutations in genes regulating telomere length are also found in rheumatoid arthritis with ILD (RA-

ILD) and chronic hypersensitivity pneumonitis (CHP)[25,29,30], hinting at shared pathogenetic mechanisms across different entities of fibrotic lung diseases.

It should be noted, however, that although one in three IPF patients has a family history of PF[13], genetics alone insufficiently explain the complex pathogenesis of IPF[31].

8.1.2.2 Environmental factors

Due to its large surface that is directly connected to the external environment via the airways and oral cavity the lung is directly exposed to a variety of environmental agents throughout a lifetime. Many of these agents, typically classified as either organic, inorganic, or microbial, display risk factors for the development of IPF[32].

Besides male sex and older age, Tobacco smoke is considered the most important risk factor for IPF, with more than 70% of IPF patients being current or former smokers[33,34]. It is further associated with more severe disease and faster progression[35]. Occupational exposures to both organic (such as animal and wood dust) and inorganic (such as agricultural substances and metal dust) agents have been linked to higher risks of developing IPF[36]. While these risk factors are rather vague and descriptive terms, many non-IPF ILDs are explicitly linked to specific inciting agents, such as bird and fungal antigens in CHP[37] or asbestos and silica in Asbestosis and Silicosis, respectively[8]. While the underlying mechanisms remain unclear, it is hypothesised that exposure to environmental factors prompts the epigenetic changes frequently observed in IPF[28].

Additional risk factors for developing IPF include gastroesophageal reflux disease, obstructive sleep apnea syndrome, and viral infections[4]. Exposure to Epstein-Barr virus (EBV), cytomegalovirus (CMV), and human herpesviruses 7 and 8 (HHV-7/8) are also associated with an increased risk of IPF, implying a potential mechanistic link between latent viral infection and IPF[38].

8.1.3 Cellular Pathophysiology of Idiopathic Pulmonary Fibrosis

PF is a highly orchestrated, complex, multicellular process. The favoured pathogenetic model of IPF assumes that severe and/or repetitive injury of the alveolar epithelium (secondary to any cause) in a genetically susceptible ageing individual triggers a proinflammatory and profibrotic cascade causing alveolar remodelling and excessive deposition of ECM - often referred to as scars - in the interstitial space, ultimately leading to increased stiffness of the lungs and impaired oxygen exchange[4,39].

The smallest functional unit of the lung are the alveoli, which serve as the gas exchange unit and whose walls constitute the blood-air barrier. During homeostasis, the blood-air barrier comprises six cell types (see Figure 1). The alveolar side of the alveolar septum is lined by type 1 alveolar epithelial cells (AT1), which account for > 95 % of the alveolar surface. Type 2 alveolar epithelial cells (AT2), which produce and secrete surfactant proteins, serve as progenitors of AT1 cells. The vascular side of the alveolar septa are formed by capillary cells. While aerocyte capillary cells (aCap) contribute the majority of the capillary surface area and facilitate gas exchange, general capillary cells (gCap) serve as capillary stem cells that can differentiate into aCaps[40]. In the interstitial space - between alveolar and capillary cells - reside (alveolar) fibroblasts and pericytes, which serve as structural support cells[40].

Upon injury, the death of AT1 cells leads to aberrant alveolar regeneration. It is hypothesized that dysfunctional AT2 cells fail to replenish the pool of AT1 cells. As a result, the alveoli get populated by airway epithelial cells, a process referred to as bronchiolization, that ultimately leads to the formation of so-called honeycombing cysts lined with airway epithelium (see **Figure 1**).

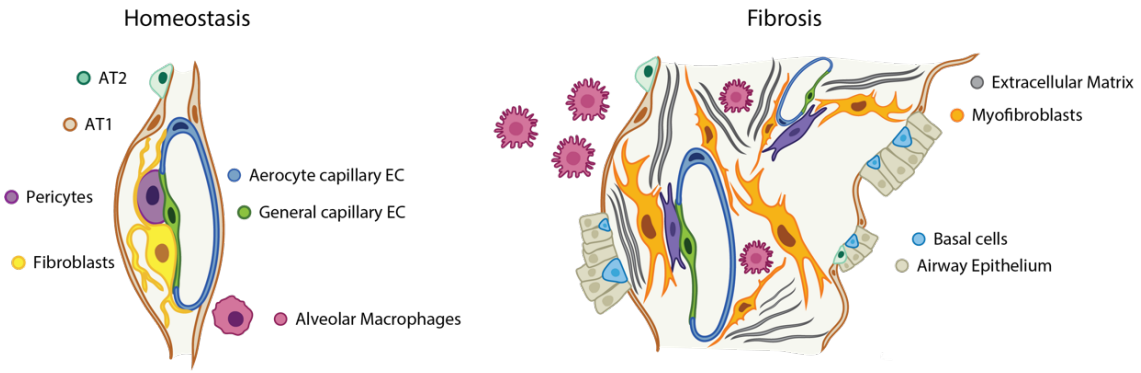

Figure 1. Schematic overview of the cellular remodelling of the alveolar gas exchange unit in Pulmonary Fibrosis. Alveoli in healthy lungs (left) are formed by 6 distinct structural cell types and are characterized by an extremely thin blood-gas barrier to facilitate efficient gas exchange. During Fibrosis (right), death of AT1 cells triggers an inflammatory response that triggers immune cell infiltration and differentiation of fibroblasts into myofibroblasts, leading to excessive production of extracellular matrix (ECM) and thickening of the interstitial space. Alveolar epithelial cells are replaced by non-specialized airway epithelial cells. These changes collectively significantly alter the structure of the gas exchange unit resulting in impaired gas exchange. Figure contains partially modified elements from Gillich et al. (Nature, 2020)[40], Buckley et al. (Nature Immunology, 2024)[41], and Bioicons[42].

Epithelial remodelling of the alveolar gas-exchange unit does not display an isolated event but occurs in a tightly co-regulated manner accompanied by an immune response and remodelling of the stromal compartment.

Damaged alveolar epithelial cells and resident alveolar macrophages secrete a collection of factors that promote fibroblast activation and differentiation towards ECM-producing myofibroblasts - the effector cells driving fibrotic remodeling of the lung (see Figure 1). These factors include Transforming Growth Factor β (TGFβ), platelet-derived growth factor (PDGF), connective-tissue growth factor (CTGF), matrix metalloproteinases (including MMP1 and MMP7), Tumor Necrosis Factor α (TNFα), as well as various chemokines[39]. As a consequence, myofibroblasts, which reside in the interstitial space of the alveolar septum, produce excessive amounts of ECM, primarily fibrillar collagens, fibronectin (FN1), tenascin (TNC). This process is thought to be primarily - but not exclusively - orchestrated by TGFβ [28]. The altered composition and amounts of ECM itself cause fibroblast activation, leading to a vicious circle of self-perpetuating fibrosis. The corresponding histopathological findings are

myofibroblast foci, agglomerations of proliferating fibroblasts, myofibroblasts, and ECM, which are indicative of active disease[39].

Stromal remodelling does not spare the vasculature. Indeed, fibrotic remodelling of the lung goes hand in hand with dysregulated angiogenesis. While myofibroblast foci are typically hypovascularized, the surrounding fibrotic regions are often characterized by increased vascularity[28,43]. It remains unclear, however, whether these vascular changes solely reflect adaptations to or causal processes of fibrotic remodeling[28].

The role of the immune system to the pathogenesis of IPF is poorly understood, but the most evidence points towards a central role of macrophages which undergo profound changes during fibrogenesis[28,39,44]. Factors secreted by alveolar epithelial cells following lung injury function as chemoattractants for monocytes, which differentiate into monocyte-derived macrophages (MDM). Depending on the context these MDMs can assume different phenotypes capable of either promoting or limiting fibrosis. A highly coordinated balance in space and time between these two opposing phenotypes is required for successful wound healing without scar formation. In IPF this balance is shifted towards profibrotic MDMs[45], which promote fibroblast activation and differentiation, angiogenesis, and ECM deposition through secretion of factors such as TGFβ, PDGFα, and vascular endothelial growth factor (VEGF)[28]. Increasing evidence also suggests a pathogenetic effect of T and B cells as well as circulating auto-antibodies, but the mechanisms through which they interfere with the remodeling of the tissue architecture and interact with epithelial and stromal cells remain unclear[39].

In summary, while the primary triggers and consecutive inflammatory responses in each non-IPF fibrotic ILD are distinct, it is assumed that the downstream mechanisms of fibrosis, including alveolar remodelling, ECM deposition, and distortion of tissue architecture are common to all fibrotic ILDs[3].

8.1.4 Clinical Presentation and Diagnostic Approaches

According to the official 2018 Clinical Practice Guidelines of the American Thoracic Society, European Society, Japanese Respiratory Society, and Latin American Thoracic Society (ATS/ERS/JRS/ALAT)[13] and its 2022 update[46], IPF should be considered in every adult individual with a history of unexplained chronic exertional dyspnea or cough or presenting with velcro-like crackles or digital clubbing on physical examination. Given that these symptoms are nonspecific and are also associated with other pulmonary and cardiovascular diseases, it is not uncommon for IPF patients to be initially diagnosed with heart failure or chronic obstructive pulmonary disease (COPD)[47], underlining the urgent need to advance our understanding of early disease to improve outcomes through early detection and accurate diagnosis.

Spirometry tests typically show a reduced diffusion capacity of the lung for carbon monoxide (DLCO), a reduced forced vital capacity (FVC), and a reduced total lung capacity (TLC) consistent with a restrictive lung-function pattern. However, normal spirometry by no means excludes IPF or other forms of fILD. Iterative spirometry tests are often utilized to monitor disease progression and response to therapy[3]. A steady decline of forced vital capacity (FVC) is the most accurate indicator of disease progression and predicts prognosis.

The ATS/ERS/JRS/ALAT Clinical Practice Guidelines further recommend taking a detailed history to exclude potential known causes of ILDs (such as environmental exposures and drug toxicity) and serological testing to rule out connective tissue disease as a potential cause of the ILDs[13].

In the absence of evidence for alternative underlying conditions, identification of the UIP pattern through imaging or histology is required to establish the diagnosis of IPF (see **Figure 2**)[13].

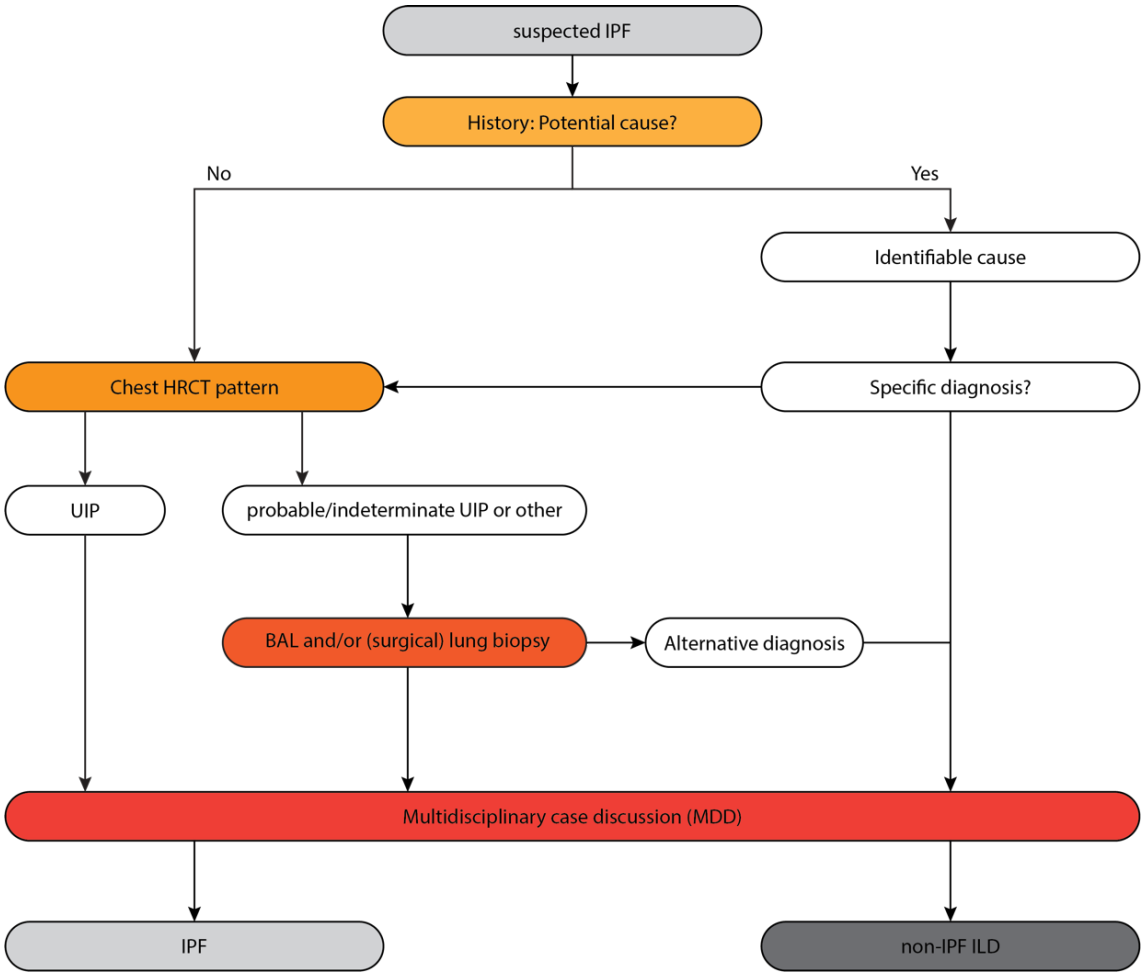

Figure 2. Diagnostic algorithm for IPF as outlined in the official Clinical Practice Guidelines of the American Thoracic Society, European Society, Japanese Respiratory Society, and Latin American Thoracic Society (ATS/ERS/JRS/ALAT). Based on Raghu et al. (AJRCCM, 2018 and AJRCCM, 2022)[13,46].

The first choice approach to identify the pattern of UIP is high-resolution computed tomography (HRCT)[13,39]. The radiographic UIP pattern is characterized by predominantly bilateral, basal, and subpleural reticulation, honeycombing, and traction bronchiectasis or bronchioloectasis. Honeycombing is the key radiographic feature of the UIP pattern, and its absence excludes a definite radiographic diagnosis of the UIP pattern[13,48] (see **Figure 3**).

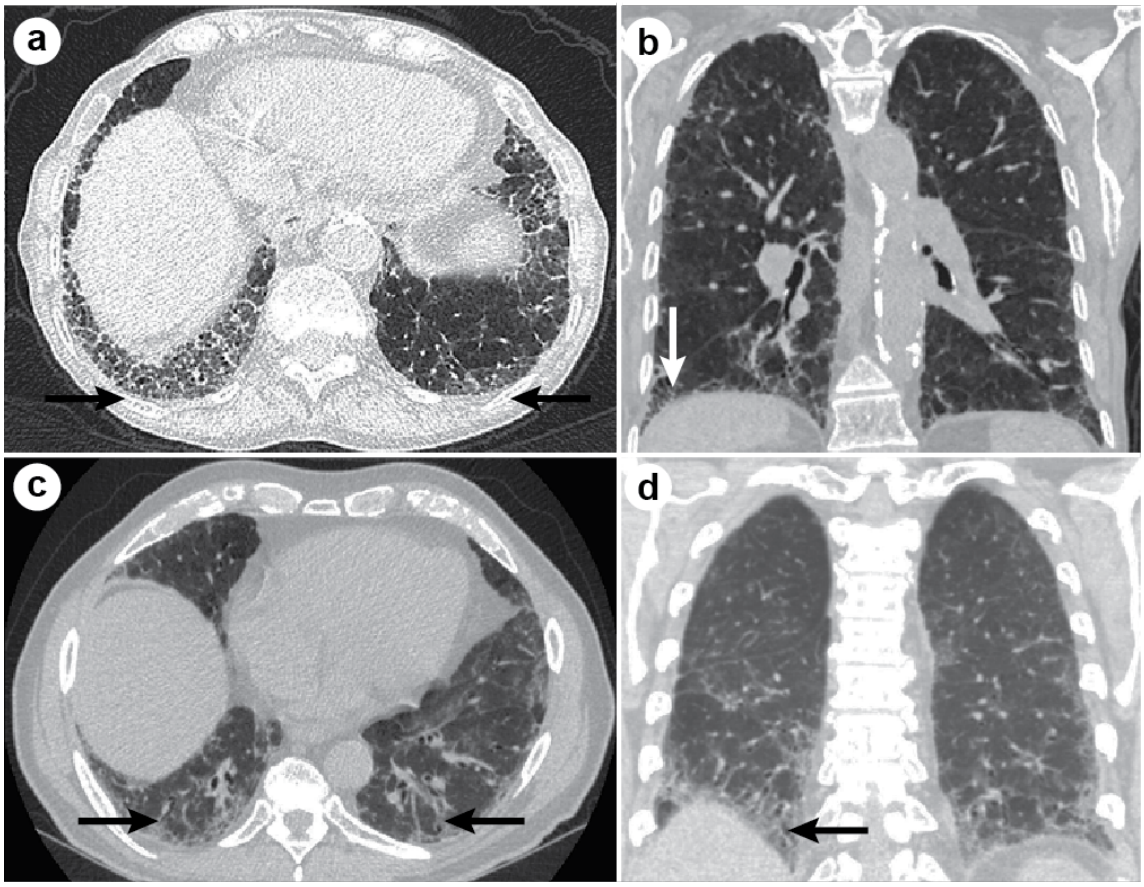

Figure 3. Radiographic features of the UIP pattern in HRCT images from individuals with IPF. Honeycombing pattern (a) in the transversal and (b) sagittal plane predominantly in the peripheral and basal regions of the lung (arrows). Reticular changes with traction bronchiectasis (c) in the transversal and (d) sagittal plane predominantly in the peripheral and basal regions of the lung (arrows). Reproduced with permission from Springer Nature from Martinez et al. (Nature Reviews Disease Primers, 2017).

In case HRCT is not sufficient to confidently identify the UIP pattern, guidelines recommend surgical lung biopsy or alternatively transbronchial lung cryobiopsy to obtain lung tissue for histopathological analysis (see **Figure 2**)[13,46]. However in clinical practice, surgical lung biopsy is not the first choice due to its associated risk of short-term mortality and perioperative complications[13,49]. Thus, transbronchial lung cryobiopsy is evolving as a less invasive alternative with similar risk profile and diagnostic yield[46].

Histopathological features of the UIP pattern include spatially heterogeneous interstitial fibrosis predominantly in the subpleural and paraseptal areas, destruction of the lung architecture, microscopic honeycombing, and myofibroblast foci, which are typically found between seemingly normal and

fibrotic lung parenchyma (see **Figure 4**). Myofibroblast foci are the distinguishing histopathological feature of the UIP pattern and must be present for a definite histopathological diagnosis of UIP[13,39].

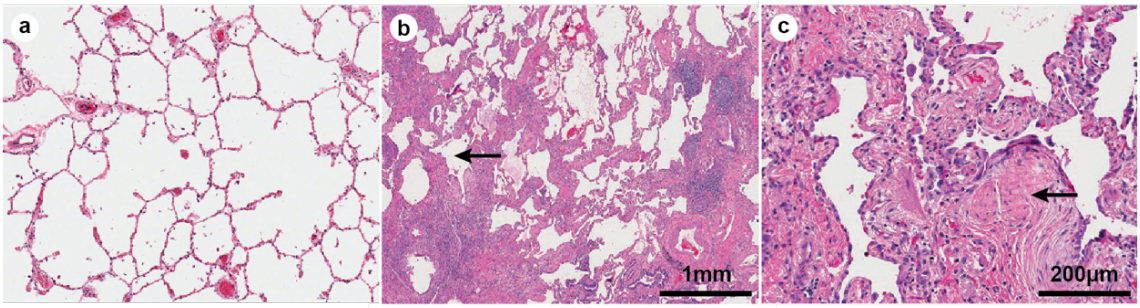

Figure 4. Histopathological features of the UIP pattern in haematoxylin and eosin (H&E)-stained sections obtained from surgical lung biopsy. (a) Normal lung histology. (b) Honey-combing pattern (arrow) (c) Myofibroblast foci (arrow). Reproduced with permission from Springer Nature from Martinez et al. (Nature Reviews Disease Primers, 2017).

The pattern of UIP is not exclusive to IPF, it also occurs in RA-ILD and CHP and is further found across progressive fibrotic ILDs of advanced stage[3,50].

If neither imaging nor histology enables the confident diagnosis of the UIP pattern, multidisciplinary discussions drawing from the expertise of pulmonologists, rheumatologists, radiologists, and pathologists should be used to jointly assess the diagnostic evidence in order to reach diagnostic consensus and identify the most promising therapeutic strategy[4,13,51].

8.1.5 Management and Prognosis

The treatment of patients with IPF encompasses two pillars: non-pharmacological and pharmacological interventions. These complementary strategies aim to improve the patient's quality of life through amelioration of symptoms, slow disease progression, and ideally improve survival and should take into account the individual course of the disease, potential drug side effects, and personal preferences[4,39].

8.1.5.1 Non-pharmacological Management

Non-pharmacological interventions can contribute significantly to the overall health and quality of life of patients with IPF[4]. Similar to other chronic lung diseases, smoking cessation for active smokers and administration of influenza, pneumococcal, and any age-appropriate or seasonal vaccines should be recommended in all patients with IPF[4]. Pulmonary Rehabilitation, structured programs aiming to improve the physical and psychological well-being of the patient, receives only limited endorsement by the ATS/ERS/JRS/ALAT guidelines[52]. Nevertheless, a recent systematic meta-analysis of randomized controlled trials has shown the benefits of such programs on 6-minute walk distance and quality of life in patients with IPF[53]. Administration of supplemental oxygen is strongly recommended by the ATS/ERS/JRS/ALAT guidelines and should be initiated if the oxyhemoglobin saturation is 88% or less during rest or sleep[4,52].

Lung transplantation is the only curative treatment for IPF to date. Although lung transplantation is frequently considered for patients with end-stage IPF, access to lung transplantation remains challenging due to the low number of available donor organs. Annually, over 2,000 lung transplants are performed in the United States and over 300 in Germany[54,55], the majority of which are due to ILDs. As a consequence, only a small fraction of patients with IPF can undergo lung transplantation[4]. Despite lung transplantation being considered a curative treatment, survival is only prolonged modestly with 5-year survival rates of around 50%[4,54]. Post-transplant complications, such as primary graft dysfunction, acute and chronic allograft rejection as well as infections and cancer (secondary to the immunosuppressive drug regimens required post transplantation) pose significant challenges that require structured surveillance and follow-up programs in experienced centers to optimise long-term survival and quality of life following lung transplantation[4].

8.1.5.2 Pharmacological Management

Over the past decade, two antifibrotic drugs have emerged as safe and effective treatment options: pirfenidone and nintedanib. Both therapeutic agents slowed the rate of FVC decline by approximately 50% within one year in placebo-controlled randomized trials[56,57] and are thus recommended by the ATS/ERS/JRS/ALAT guidelines for the treatment of IPF[58]. Encouragingly, there is accumulating evidence suggesting that both drugs may exert a favourable effect on the mortality of patients with IPF[59–62].

Pirfenidone is an anti-fibrotic and anti-inflammatory agent of unknown mode of action[4,39]. While the exact mechanism is poorly understood, pirfenidone has been shown to inhibit fibroblast proliferation and collagen synthesis on the cellular level and suppress TGFβ and TNFα signaling on the pathway level[63]. The most common side effects are anorexia, nausea, and vomiting, which may require the use of antacid and antiemetic therapeutics or even dose reduction in severely affected individuals[56].

Nintedanib is a tyrosine kinase inhibitor that inhibits platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF) signaling by targeting the respective receptors (PDGF-R, FGF-R1/2/3, VEGF-R1/2/3)[64]. Interfering with these pro-fibrotic signaling pathways reduces fibroblast activation, proliferation, and myofibroblast differentiation[64]. Diarrhea is the most frequent side effect during therapy with nintedanib and should be treated with antidiarrheal therapeutics to avoid discontinuation of the drug[57]. Nintedanib is further associated with a higher risk of bleeding and thromboembolic events[57]. Thus, it should be prescribed with great caution in patients with anticoagulant therapy and/or cardiovascular risk factors. In addition to IPF, nintedanib is also approved for the treatment of progressive fibrosing ILDs[65], supporting the idea that progressive PF depicts a conserved pathomechanism responsive to anti-fibrotic therapeutics that is shared across various ILDs of different etiologies.

In summary, two pharmacological treatment options are approved for treatment of IPF - pirfenidone and nintedanib - but neither exhibit disease modifying or even reversing capabilities but instead only slow disease progression. Due to a lack of head-to-head comparisons between these agents, it is challenging to recommend one over the other. Similarly, specific subgroups of patients with IPF that may derive superior benefit from either of the two have yet to be identified, warranting further clinical and translational investigations to elucidate the agents' precise mode of action and clinically relevant disease endotypes.

8.1.5.3 Prognosis

The irreversible and progressive nature of IPF is mirrored by the poor prognosis of the disease. Indeed, median transplant-free survival is quoted at 3-5 years in the literature[4,5], which is worse than many common forms of cancers and similar to lung cancer[66]. The most common cause of death in patients with IPF is chronic hypoxemic respiratory failure[4]. Acute exacerbations, i.e. rapid respiratory deterioration superimposed on the chronic course of the disease, are a characteristic feature of IPF that occur in 10-20% of patients per year[67]. These events are associated with an in-hospital mortality of 50% and thus pose a significant clinical challenge[68]. IPF often presents with a spectrum of comorbidities that have a profound impact on its clinical course and patient outcomes. The most frequent comorbidities are COPD, pulmonary hypertension, lung cancer, and cardiovascular diseases[69].

The prognosis of non-IPF ILDs depends on the underlying disease. In general, the prognosis is less severe than for IPF, particularly for those entities which do not exhibit a progressive fibrotic phenotype[3].

8.2 Single-cell RNA-sequencing (scRNA-seq)

Single-cell RNA-sequencing (scRNA-seq) allows the simultaneous profiling of the transcriptome of up to hundreds of thousands of individual cells[70]. Before the

advent of scRNA-seq it was only possible to collectively sequence the mRNA (messenger ribonucleic acid) of all cells contained in bulk tissue samples. As tissues are composed of a heterogeneous mixture of individual cells, these so-called bulk approaches measure average gene expression profiles, thereby masking differences between individual cells.

Cells constitute the fundamental organizing unit of each tissue[71]. As such, cellular composition, location, and interactions are crucial to maintaining tissue homeostasis. Hence, identifying alterations of the cellular composition and detecting aberrant, disease-specific cell states or signaling routes provides valuable cues to understand the mechanisms underlying disease initiation and progression[71]. These cellular changes are, however, obscured from bulk approaches. The emergence of scRNA-seq has provided the necessary tool to probe compositional and cell-intrinsic changes and thus map tissue remodeling during disease pathogenesis at unprecedented resolution[72].

Measuring and quantifying mRNA molecules in single cells can be achieved via numerous different scRNA-seq protocols[73]. While marked differences exist between these protocols, they all consist of 4 subsequent steps: (1) tissue dissociation, (2) single-cell isolation, (3) library preparation, and (4) sequencing[74,75]. First, the tissue that serves as the input material for the scRNA-seq experiment is dissociated into a single-cell suspension using digestive enzymes (such as collagenase or trypsin)[74]. The suspended cells are then isolated into isolated reaction chambers, which lays the foundation to sequence the mRNA of each cell separately[74]. Plate-based approaches isolate single cells into separate wells on a plate, whereas droplet-based approaches (such as the popular commercial 10X Chromium platform) use microfluidic chips to capture cells in nanoliter water droplets[75]. The obtained isolated reaction chambers, whether wells or droplets, contain the necessary reagents for cell lysis, mRNA capture, and library preparation. In addition, every mRNA molecule originating from one specific individual cell gets labeled with a molecular barcode. Library preparation

involves reverse transcription of mRNA into cDNA followed by polymerase-chain reaction (PCR). Ultimately, sequencing is performed jointly on all pooled single-cell libraries. The resulting mRNA sequence reads are then aligned against the matching reference genome and quantified on a per-cell level using the cellular barcodes to produce a cell-by-gene count matrix[76,77].

Owing to significant advances in scRNA-seq methods, both in accuracy and scale[70,78], since the first published transcriptome of a single cell in 2009[79], scRNA-seq has been widely adopted to study disease-associated cellular changes in large patient cohorts and has since revolutionized our understanding of disease pathogenesis at single-cell level[72].

8.3 Cellular Drivers of Pulmonary Fibrosis

Recently, numerous studies have generated detailed so-called single-cell atlases of the human lung in health and fibrosis that have revealed novel insights into the key cellular drivers of PF[80–84]. The term atlas describes curated datasets, obtained through systematic profiling of single cells from both fibrotic and healthy reference lungs, that present an unbiased comparison of the cellular repertoire of the lung in health and fibrosis on the transcriptomic level. As such, they provide a detailed overview of all distinct cell types, their abundance, and characteristic gene expression signatures, allowing researchers to query which cell types express a gene of interest, at which level, and how the expression of a gene or frequency of a cell type changes in disease[71].

For instance, scRNA-seq of normal and fibrotic lungs revealed CTHRC1+ (Collagen triple helix repeat containing 1) myofibroblasts as a unique feature of fibrotic lungs[80]. Interestingly, this subpopulation expressed the highest level of collagens across all cells and located to myofibroblastic foci, suggesting that CTRHC1+ myofibroblasts are indeed the effector cells of PF that drive ECM production and fibrotic remodeling. The progenitor cells that differentiate into

myofibroblasts as well as the molecular signals that drive the emergence of CTHRC1+ myofibroblasts are unknown.

Lung cell atlas projects have also led to the identification of a population of endothelial cells (EC) characterized by unique expression of Plasmalemma vesicle-associated protein (PLVAP), Von Willebrand Factor A Domain Containing 1 (VWA1), Collagen Type XV Alpha 1 Chain (COL15A1). These PLVAP+/VWA1+/COL15A1+ ECs transcriptionally resemble systemic venous ECs and, although present in healthy lungs, significantly increase in fibrotic lungs[81]. However, their cellular origin is unknown and whether this disease-associated cell state is perfused via the systemic or pulmonary circulation remains elusive.

In addition, several scRNA-seq studies independently described profibrotic macrophages which are exclusively found in the lungs of patients with PF and are characterized by the distinct expression of known profibrotic genes, such as Osteopontin (SPP1), matrix metalloproteinase-9 (MMP9), Osteonectin (SPARC), lipase A (LIPA), proto-oncogene tyrosine-protein kinase MER (MERTK), Lipoprotein-associated phospholipase A2 (PLA2G7), and chitinase-3 like protein (CHI3L1)[81,83–85].

The altered epithelial cell repertoire of fibrotic lungs is characterized by the loss of AT2 cells and the emergence of distinct Keratin 17 (KRT17)+/Keratin 5 (KRT5)- basaloid epithelial cells, which localize to the epithelial layer enveloping myofibroblastic foci[81,84] and express high levels of mesenchymal transition, cellular senescence, p53 activity, and other profibrotic genes. Comparative cross-species analysis with longitudinal scRNA-seq of lung injury has shown that KRT17+/KRT5- basaloid cells are most similar to an AT2-derived Keratin 8 positive (Krt8+) alveolar differentiation intermediate that differentiates into AT1 cells during lung regeneration[86]. A possible AT2 origin of KRT17+/KRT5- basaloid cells is supported by a recent finding that human transdifferentiating

AT2 cells in organoids subsequently upregulate KRT8 and KRT17[87]. However, the definitive cellular source of KRT17+/KRT5- cells and their pathogenic contributions to lung fibrogenesis remain unclear.

Of note, similar epithelial, stromal, endothelial, and myeloid cell states have recently been described in either lungs or bronchoalveolar lavage fluid of patients with severe or lethal COVID-19[88–93], advocating the concept that these cells depict Regenerative Intermediate Cell States (RICS) that emerge during acute or severe lung injury and, if persistent, drive fibrotic remodelling of the lung.

While these scRNA-seq studies have revolutionised our understanding of the cellular and molecular features of PF, their findings are intrinsically limited to end-stage disease. Most studies have profiled cells from dissociated explant lungs of deceased donors, which predominantly represent end-stage fibrosis[81,83,84]. Hence, inferences about early disease and disease progression are challenging, if not impossible. It remains unclear which molecular cues orchestrate the emergence of these fibrosis-associated RICS - in particular, their cellular origin, spatiotemporal relationships, and their cellular interactions that initiate, sustain, and propagate the profibrotic cascade that leads to the manifestation of PF. Studying these early fibrogenic events holds enormous potential for understanding and preventing deadly, progressive forms of PF through the development of targeted anti-fibrotic therapeutics with disease-modifying properties.

8.4 Precision-cut Lung Slices (PCLS)

Early stages of human PF have been challenging to study for two main reasons: (1) the difficulty of obtaining lung tissue from patients with early PF who are often asymptomatic at this point, and (2) the lack of experimental platforms that accurately recapitulate human lung fibrogenesis while preserving the cellular diversity and structural complexity of the human lung for systematic investigation of the early molecular events at cellular resolution.

Precision-cut lung slices (PCLS) have recently emerged as a promising new model to study early human lung fibrosis[94]. PCLS are typically generated by cutting 300-500 μm slices of fresh lung tissue obtained from histologically tumor-free specimens from surgical cancer or rejected organ donors and can be cultured for up to 14 days or longer[95–98]. When treated with a Fibrotic Cocktail (FC) consisting of the profibrotic cytokines transforming growth factor beta (TGFβ), platelet-derived growth factor subunit AB (PDGF-AB), tumor necrosis factor alpha (TNF-α), and lysophosphatidic acid (LPA), PCLS have been shown to undergo early fibrotic remodeling within just 6 days.

As PCLS preserve the cellular diversity and 3D architecture of the human lung while allowing precise manipulation of external factors similar to tissue culture, they allow for systematic interrogation of the early pathogenic mechanisms of human lung fibrogenesis at cellular resolution. Because sampling of PCLS is in principle possible at any point in time, this model enables researchers to study dynamic processes of the spatial and cellular architecture of the human lung through longitudinal examination, thereby providing insights into the spatiotemporal evolution of human lung fibrogenesis. Further, PCLS enable novel drug testing approaches directly within human lung tissue, which closely resembles the tissue niche in which a drug candidate has to unfold its anti-fibrotic effect in the patient, providing a level of realism unmatched by any other preclinical models. However, it is unclear which aspects of the pathogenesis of PF can be recapitulated in PCLS, and strategies to leverage the combined benefits of PCLS and scRNA-seq need yet to be explored.

9. Publication Summaries

9.1 Paper I: "Ex vivo tissue perturbations coupled to single-cell RNA-seq reveal multilineage cell circuit dynamics in human lung fibrogenesis"

N J Lang*, J Gote-Schniering*, D Porras-Gonzalez, L Yang, L J De Sadeleer, R C Jentzsch, V A Shitov, S Zhou, M Ansari, A Agami, C H Mayr, B Hooshiar Kashani, Y Chen, L Heumos, J C Pestoni, E Sarolta Molnar, E Geeraerts, V Anquetil, L Saniere, M Wögrath, M Gerckens, M Lehmann, A Ö Yildirim, R Hatz, N Kneidinger, J Behr, W A Wuyts, M G Stoleriu, M D Luecken, F J Theis, G Burgstaller†, H B Schiller† (* contributed equally, † co-supervision). **Ex vivo tissue perturbations coupled to single-cell RNA-seq reveal multilineage cell circuit dynamics in human lung fibrogenesis.** *Science Translational Medicine* 15, eadh0908 (2023). DOI: 10.1126/scitranslmed.adh0908

Link: https://www.science.org/doi/full/10.1126/scitranslmed.adh0908

The first paper included in this thesis "*Ex vivo tissue perturbations coupled to single-cell RNA-seq reveal multilineage cell circuit dynamics in human lung fibrogenesis*", was published in Science Translational Medicine in 2023. I am the shared first author of this publication alongside Janine Gote-Schniering.

Research problem

Pulmonary fibrosis is the central pathomechanism shared across all entities of fibrotic ILDs. While recent scRNA-seq atlassing studies have provided us with a detailed picture of the cellular aberrations that occur in lungs with pulmonary fibrosis, the tissue used for these studies is, however, typically obtained from lung explants of patients undergoing lung transplantation, thereby representing primarily end-stage disease. Our understanding of disease initiation and early

disease, and disease progression is limited due to the lack of organotypic experimental platforms that accurately recapitulate human lung fibrogenesis while preserving the cellular diversity and structural complexity of the human lung for systematic investigation of the early molecular events at cellular resolution.

Approach

To this end, we performed a systematic analysis of scRNA-seq data from both human PCLS treated with FC and anti-fibrotic drugs (*ex vivo*) and lungs from patients with PF (*in vivo*) in order to

(1) evaluate which aspects of *in vivo* PF pathogenesis are recapitulated in FC treated PCLS through systematic comparisons of *ex vivo* and in vivo scRNA-seq data

(2) utilize FC treated PCLS as a model of early disease to identify fibrogenic cell states that may drive early-stage PF

(3) validate the occurrence of these fibrogenic cell states in early-stage disease using microCT-staged patient tissues

(4) establish a computational framework for scalable drug testing and phenotypic treatment response assessment in PCLS

Results

We show that FC treatment of PCLS induces fibrogenic RICS *ex vivo* that are highly similar to key cellular features of lungs from patients with PF, including: CTRHC1+ myofibroblasts, KRT5+/KRT17- basaloid cells, PLVAP+/VWA1+ endothelial cells, and SPP1+ macrophages. We present evidence that KRT5+/KRT17- basaloid cells are a feature of early-stage disease and derived from AT2 cells. Similarly, we show that PLVAP+/VWA1+ endothelial cells replace capillary cells in the thickened alveolar septa in early-stage disease and computational trajectory analysis indicated PLVAP+/VWA1+ endothelial cells

develop from capillary endothelial cells. Finally, we show cell state specific drug effects including a potentially novel mode of action for the clinically approved drug nintedanib and provide a conceptual and computational framework to assess the disease-reversing drug effects on individual cell types.

Conclusion

In summary, we provide a framework for perturbational single cell genomics directly in native human lung tissue that enables spatiotemporal analysis of lung fibrogenesis and opens novel avenues for next-generation phenotypic drug testing at single-cell resolution. Our findings further underscore the therapeutic potential of targeting specific cell states and offer an avenue for scalable, high-resolution drug testing to accelerate antifibrotic drug development and translation.

Personal contribution

I am the shared first author of this publication alongside Janine Gote-Schniering. The paper represents an interdisciplinary team effort, to which I contributed the computational analysis. I wrote the code to process the data, performed the analysis and generated the figures as outlined below.

I planned and performed the pre-processing, quality control, processing, integration, and cell type annotation of the single-cell data generated in this manuscript. I conceptualized the strategy for the comparison of the ex vivo and in vivo single cell data. I retrieved, curated, and harmonized the publicly available datasets used for this comparison. I performed the cell type frequency and differential gene expression analysis, trajectory inference analysis, and cell-cell communication analysis of the ex vivo and in vivo single cell data as well as their comparisons, as presented in this manuscript in **Figure 1 b - g**, **Figure 2 a - h**, **Figure 4 a - h and k**, **Figure 5 a - i**, **Figure 7 a - f**, and **Figure 8 d – h**.

I also re-analysed publicly available bulk RNA sequencing data and performed the differential gene expression analysis and gene ontology term analysis, as presented in this manuscript in **Figure 1 j and k**.

Together with Herbert Schiller and Malte Luecken, I conceptualized the query-to-reference mapping-based approaches to assess the similarity of the cell states in hPCLS and the in vivo reference scRNA-seq datasets. I performed the analysis and visualization in collaboration with Vladimir Shitov, as seen **in Figure 1h and i**, and **Figure 8 b and c**.

With support from Meshal Ansari and Janine Gote-Schniering, I also designed the visual abstracts, as seen in **Figure 1a, 1h, and 8c**.

Additionally, I wrote the draft of the manuscript together with my co-first author Janine Gote-Schniering, and our supervisors edited the manuscript.

9.2 Paper II: "Sfrp1 inhibits lung fibrboblast invasion during transition to injury-induced myofibroblasts"

C H Mayr*, A Sengupta*, S Asgharpour, M Ansari, J C Pestoni, P Ogar, I Angelidis, A Liontos, J Alberto Rodriguez-Castillo, **N J Lang**, M Strunz, D Porras-Gonzalez, M Gerckens, L J De Sadeleer, B Oehrle, V Viteri-Alvarez, IE Fernandez, M Tallquist, M Irmler, J Beckers, O Eickelberg, M G Stoleriu, J Behr, N Kneidinger, W A Wuyts, R M Wasnick, A Ö Yildirim, K Ahlbrecht, R E Morty, C Samakovlis, F J Theis, G Burgstaller†, H B Schiller† (* contributed equally, † co-supervision). **Sfrp1 inhibits lung fibroblast invasion during transition to injury-induced myofibroblasts.** *European Respiratory Journal* 63, 2301326 (2024). DOI: 10.1183/13993003.01326-2023

Link: https://publications.ersnet.org/content/erj/63/2/2301326

The second paper included in this thesis "*Sfrp1 inhibits lung fibroblast invasion during transition to injury-induced myofibroblasts*" was published in the European Respiratory Journal in 2024. I am a co-author of this publication.

Research problem

Myofibroblasts are the ECM producing effector cells driving pathological lung remodeling during pulmonary fibrosis. Myofibroblasts are absent during homeostasis and only induced upon injury, hence constituting an attractive therapeutic target. The transcriptional changes governing the evolution of the naive, homeostatic fibroblast populations following injury are poorly understood and the cellular origins of myofibroblasts are currently unknown.

Approach

To profile the diversity and trajectories of lung fibroblast populations during lung fibrogenesis and resolution, we combine longitudinal scRNA-seq and spatial

transcriptomics with genetic lineage tracing in a mouse model of bleomycin induced lung fibrosis. Findings were then validated in lung tissue sections from patients with IPF and pathological lung fibroblasts populations were functionally characterized using cell differentiation and invasion essays.

Results

We identified three disease-associated fibroblast populations: SFRP1+ fibroblasts, SPP1+ myofibroblasts, and CTHRC1+ myofibroblasts. SFRP1+ fibroblasts are an early, noninvasive, transitional state that precede the emergence of CTRHC1+ myofibroblasts. Genetic and computational trajectory inference provided evidence that multiple homeostatic fibroblasts - instead of one specific fibroblast population - converge towards myofibroblasts via transitional SFRP1+ fibroblasts. Cell-cell communication analysis revealed potential regulatory ligands governing fibroblast fate transitions after injury. Mechanistically, TGFβ1 led to loss of SFRP1 in transitional SFRP1+ fibroblasts and differentiation towards invasive CTHRC1+ myofibroblasts.

Conclusion

Our study profiles the transcriptional and spatial heterogeneity of fibroblast populations during murine lung fibrosis and regeneration. We provide evidence that myofibroblasts arise through convergence of these spatially and transcriptionally distinct fibroblast populations. We identify SFRP1 as a key regulator of myofibroblast fate and function in response to TGFβ1, offering an attractive target for novel therapeutics aimed at inferring with myofibroblast function during early lung fibrogenesis.

Personal contribution

I am a co-author of this publication, contributing to the bioinformatics analysis of the scRNA-seq data generated for this manuscript.

Specifically, I performed the cell-cell communication analysis to predict upstream ligands regulating myofibroblast fate associated genes, as presented in the manuscript in **Figure 6a and b**. I wrote and implemented the analysis code, performed the analysis, and visualized the results.

I wrote the corresponding Results and Methods sections of the paper and assisted the main authors with interpreting the findings.

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