Precision medicine advances in idiopathic pulmonary fibrosis

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Summary

Idiopathic pulmonary fibrosis (IPF) is a highly heterogeneous, unpredictable and ultimately lethal chronic lung disease. Over the last decade, two anti-fibrotic agents have been shown to slow disease progression, however, both drugs are administered uniformly with minimal consideration of disease severity and inter-individual molecular, genetic, and genomic differences. Advances in biological understanding of disease endotyping and the emergence of precision medicine have shown that "a one-size-fits-all approach" to the management of chronic lung diseases is no longer appropriate. While precision medicine approaches have revolutionized the management of other diseases such as lung cancer and asthma, the implementation of precision medicine in IPF clinical practice remains an unmet need despite several reports demonstrating a large number of diagnostic, prognostic and theragnostic biomarker candidates in IPF. This review article aims to summarize our current knowledge of precision medicine in IPF and highlight barriers to translate these research findings into clinical practice.

Keywords: Idiopathic pulmonary fibrosis; Precision medicine; Theragnostic biomarkers; Personalized medicine

Idiopathic pulmonary fibrosis (IPF) is a chronic, debilitating lung disease with increasing prevalence, characterized by a complex interplay of genetic, epigenetic, immunologic and environmental factors.[1,2] The disease course is highly heterogeneous and unpredictable. Three distinct patterns of disease progression have been suggested including slowly progressive, rapidly progressive and relatively stable disease interposed by acute exacerbations.[3–5] The last years have seen the emergence of use of two anti-fibrotic agents able to slow disease progression,[6,7] however, both drugs are administered uniformly with minimal consideration of differences in molecular subphenotypes associated with disease progression.[8,9]

Research advances in our understanding of pulmonary fibrosis pathogenesis and progression and the emergence of precision medicine have shown that "a one-size-fits-all approach" to the management of this group of diseases is probably not appropriate.[8] Tailored therapies based on precision medicine can improve treatment outcomes and concomitantly be cost-effective through the avoidance of unnecessary exposure of patients to ineffective treatment regimens. In addition to treatment response prediction, precision medicine could also have a major role in the identification of individuals with disease susceptibility and pave the way for early diagnosis. Importantly, precision medicine can discriminate individuals at risk for progressive disease even before progression occurs, providing a window for early intervention or alternative treatment regimens.

Precision medicine approaches have revolutionized management of lung cancer due to the fact that prognosis and treatment in lung cancer is largely based on patients' molecular profile.[10,11] Recently, a plethora of novel biologic therapies have been approved for severe asthma, such as anti-IL-5/anti-IL-5R and anti-IL4 for patients with eosinophilic predominant severe asthma and anti-IgE for allergic predominant severe asthma with increased IgE.[12] Implementation of precision medicine in clinical practice for patients with IPF remains an unmet need. This review article aims to summarize current knowledge for precision medicine in IPF and highlight barriers to overcome for the implementation of these findings in clinical practice (Fig. 1).

Disease susceptibility

Recent studies implicate that IPF is a highly polygenic disease with multiple variants associated with disease susceptibility.[13,14] The variant showing the strongest association with pulmonary fibrosis development and pathogenesis is a polymorphism in the promoter region of *MUC5B* (rs35705950), found using a genome-wide linkage scan in a large-scale study.[15–19] This variant leads to higher *MUC5B* expression, deregulated mucosal host defense and ultimately increased risk of IPF development almost by 6-fold.[16,17,20] Patients with the *MUC5B* risk allele are less likely to present with telomere-gene mutations.[21] Despite the fact that the variant rs35705950 is more common in IPF [38% vs 9% in the general population[16]],

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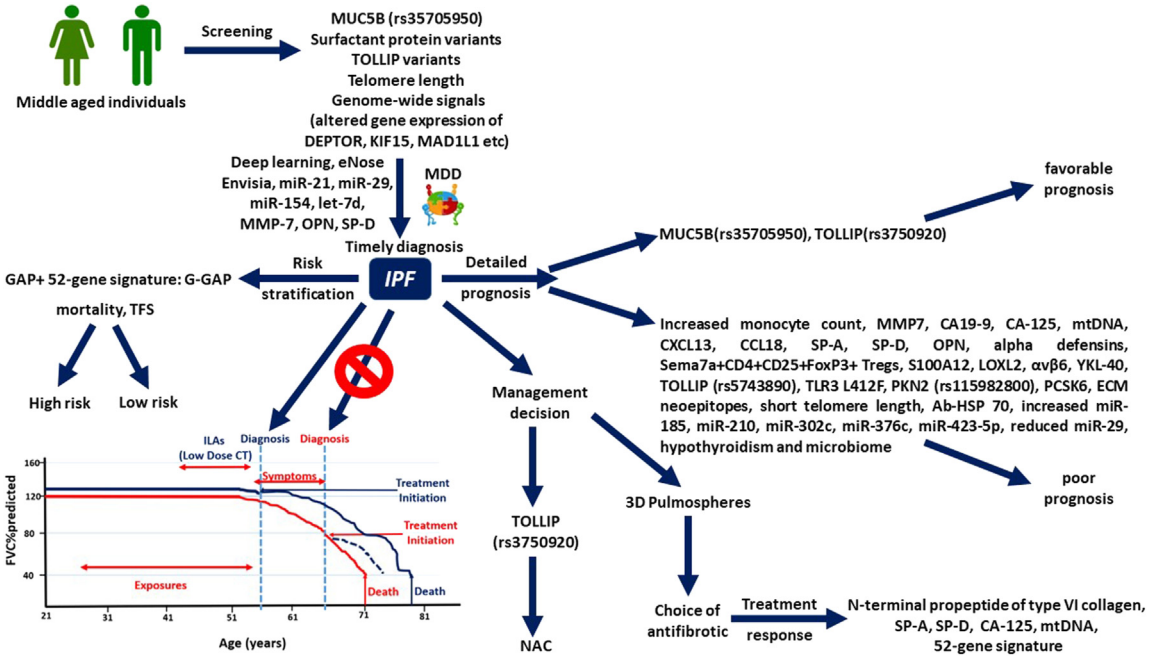

Fig. 1: Schematic representation of personalized medicine approaches that could be implemented in future clinical practice for patients with IPF. Early screening could lead to timely diagnosis, alter the nature course of the disease (red demarcation) and improve outcomes (blue demarcation). Implementation of the 52-gene signature could considerably improve the prognostic performance of GAP index. A plethora of other biomarkers could have prognostic and/or theragnostic role.

it is present in less than half of the cases. Recent data implicated that *MUC5AC* may have a role in IPF susceptibility as well, further corroborating the role of mucins in IPF.[22] Variants in toll-interacting protein (*TOLLIP*) leading to reduced expression and a variant of SPPL2C have been also associated with IPF susceptibility in a three-stage Genome-wide association study (GWAS) study.[23] On the contrary, another variant of *TOLLIP*, rs5743890, seemed to be protective against the development of pulmonary fibrosis.[23,24]

Moreover, large GWAS have demonstrated 20 frequent single nucleotide polymorphisms (SNPs) related to IPF with minor allele frequency above 5%, highlighting the association between disease susceptibility with impaired host defense, cell-to-cell adhesion, signaling and telomere maintenance.[13,14,18,23,25,26] In particular, a recent study validated genome-wide significant associations with disease susceptibility for 11 out of the 17 previously published SNPs (7q22.1, *AKAP13*, *ATP11A*, *DPP9*, *DSP*, *FAM13A*, *IVD*, *KANSL1*, *MUC5B*, *TERC* and *TERT*).[18] The same study identified and replicated three novel genome-wide significant SNPs (with associated altered gene expression of *DEPTOR*, *KIF15* and *MAD1L1*) related to IPF susceptibility.[18] DEPTOR inhibits mTOR kinase activity being part of mTORC1 and mTORC2 protein complexes, while TGFb-induced DEPTOR suppression stimulated collagen synthesis.[18] Thus, association of decreased *DEPTOR* expression with increased IPF susceptibility corroborates evidence supporting the cardinal role of mammalian target of rapamycin (mTOR) signaling in pulmonary fibrosis.[27] With regards to *MAD1L1*, it is noteworthy that its homolog MAD1 inhibits TERT activity, which implies that *MAD1L1* might increase disease susceptibility via reduced telomerase activity.[28,29] Moreover, novel signals of *KIF15*, *SPDL1* and *MAD1L1* derived from this work and/or other elegant studies might imply a potentially important role of mitotic spindle-assembly genes in disease susceptibility.[18,30,31] An updated meta-analysis of the aforementioned work including five studies demonstrated five robust novel signals (an intergenic variant in 10q25.1, variants in introns of RTEL1, STMN3, KNL1 and NPRL3) further implicating telomere maintenance, mTOR signaling and spindle assembly genes in IPF susceptibility.[13] Finally, a large recent meta-analysis including patients from 6 ancestries identified 7 novel IPF loci (index variant gene: GPR157, DNAJB4-GIPC2, RAPGEF2, FKBP5, RP11-286H14.4, PSKH1, FUT6) with 4 of them being driven by non-European ancestry, highlighting thus the differences in terms of genetic background across the world.[14]

Polymorphisms in several other genes such as transforming growth factor beta-1 (*TGFB1*), *HLA DRB1*1501*, interleukin-1 receptor alpha (*IL1RN*) and *IL8* have been suggested as candidate genes for IPF susceptibility; yet, further larger studies are needed to determine their exact role in disease susceptibility.[32–36]

Finally, except variants, polymorphisms and GWAS signals, telomere length has a major role in disease susceptibility. Short telomere length is a frequent finding in patients with IPF compared to aged-matched healthy individuals,[37] while interstitial lung abnormalities (ILAs) have been recently associated with decreased mean telomere length.[38]

Genetic mutations in familial pulmonary fibrosis

While variants (altered genome that could contain one or more mutations and presents with distinct characteristics) have been implicated in sporadic IPF, some mutations (single change in genome that could lead or not to distinct characteristics) have been implicated in familial pulmonary fibrosis. Telomerase complex mutations are more common in familial forms of pulmonary fibrosis and might not be necessarily specific to individual ILD entities.[37,39–41] Accordingly, surfactant protein (SP) mutations including SP-A1, SP-A2 and SP-C[42–44] have been linked to development of Familial Pulmonary Fibrosis, whereas these variants are rarely encountered in sporadic IPF.[45–47] Genome-wide analysis of six families from Finland with Familial Pulmonary Fibrosis suggested *ELMOD2*, a gene associated with cell migration and phagocytosis of apoptotic cells, as a candidate gene for IPF susceptibility. This gene was expressed in alveolar epithelial cells II and alveolar macrophages. Mutations in *ELMOD2* led to reduced ELMOD2 mRNA expression in the pulmonary parenchyma of patients with IPF compared to healthy individuals.[48]

Taken together, testing for disease susceptibility genes could help personalize the radiological follow-up of individuals at risk for IPF and the treatment of patients with IPF or ILAs. For example, if patients with ILAs have SNPs in the common genes associated with IPF risk, these patients should have more frequent radiologic follow-up compared to patients with ILAs and no genetic variations in IPF susceptibility genes. Further investigation toward this direction is needed.

Diagnosis

Establishing an accurate diagnosis in patients with interstitial lung diseases (ILDs) is often challenging despite the improved quality of High-Resolution Computed Tomography, the advent of deep learning and the multidisciplinary discussion of clinical, laboratory and radiographic data.[49–51] An ideal diagnostic biomarker could reduce rates of misdiagnosis following multidisciplinary discussion and concomitantly provide mechanistic insights for the origin of the disease. The potential of several molecular biomarkers has been investigated in an effort to discriminate patients with IPF from healthy individuals or patients with other ILDs.[47,52] Despite an exponential increase in our knowledge regarding IPF pathogenesis, the lack of diagnostic accuracy, disease specificity, applicability and cost-effectiveness of individual biomarkers has been insufficient to justify their incorporation into clinical practice, especially in a setting with limited resources.[51] In addition, given the recent guidelines for IPF and Progressive Pulmonary Fibrosis (PPF) and the trend towards lumping rather than splitting, diagnostic biomarkers might be the least pressing need with regards to IPF. However, ruling out other fibrotic ILDs in need of immunosuppressive therapy is still of paramount importance.

A study investigating a panel of 35 extracellular matrix (ECM), ECM-related and lung-specific analytes in plasma showed that matrix metalloproteinase (MMP)-7 > 1.75 ng/ml, SP-D >31 ng/ml and osteopontin >6 ng/ml were able to discriminate patients with IPF from patients with alternative idiopathic interstitial pneumonias, both if used individually and as a combined index.[53] However, the same index could not distinguish patients with IPF from patients with rheumatoid arthritis-ILD.[53] Data analysis from the IPF-PRO registry showed that patients with IPF have significantly different levels in 551 proteins compared to controls.[54] The glycoproteins thrombospondin 1, von Willebrand factor, as well as C–C motif chemokine ligand 17 and bactericidal permeability-increasing protein were among the proteins with the more pronounced difference between IPF and controls[54] suggesting coagulation as an important mechanism required for IPF pathogenesis. A targeted proteomic approach in a study with acceptable sample size resulted in a protein signature that included IGFBP-1, MMP-1, MMP-7, MMP-8 and TNFRSA1F and was able to discriminate patients with IPF from control subjects with a sensitivity of 98.6% and a specificity of 98.1%.[55] Such approaches with combined indexes increase considerably the diagnostic accuracy of all the above biomarkers compared to results yielded when these biomarkers were investigated individually.[53,56–59]

Other biomarkers related to epithelial cells, innate immunity and aging have been investigated mainly on individual basis and not as part of multidimensional indexes. MicroRNAs have also been largely studied as biomarkers for IPF diagnosis. Circulating caspase-cleaved cytokeratin-18, an alveolar epithelial cell apoptosis biomarker, was increased in IPF compared to control subjects.[60] The epithelium derived glycoprotein Krebs von den Lungen-6 (KL6)/mucin 1 (MUC1) has been reportedly increased in serum and BAL of patients with IPF[47,61,62]; yet, high quality studies adjusting for age, smoking and comparing patients with IPF and non-IPF ILDs are still needed. Other studies focusing on the diagnostic potential of epithelium derived proteins demonstrated that SP-A and C-pro-SP-B serum levels were increased in patients with IPF compared to non-IPF ILDs and other pulmonary diseases, respectively.[63,64] Investigations of immune deregulation in IPF led to the report that BAL levels of toll-like receptor 7 were higher in IPF compared to controls.[24,65] A recent study focusing on biomarkers of aging reported that

increased plasma concentration of growth differentiation factor 15 (GDF15), IL-6, tumor necrosis factor α receptor II and C-reactive protein was linked to presence of ILAs.[66] Results for GDF15 were validated in a different cohort.[66] With regards to miRNAs, miR-29 and let-7d were among the most downregulated, while miR-21 and miR-154 were upregulated in patients with IPF compared to controls.[67–71] Finally, the diagnostic potential of several other biomarkers including secreted phosphoprotein 1 (SPP1), FK506-binding protein 11 and chitinase-3-like protein 1 (YKL-40) has been investigated.[72–75] None of the biomarkers described in this paragraph are currently used in clinical practice but they may have potential for such use with further refinement and development.

To this end, the potential for clinical applicability of most of the aforementioned biomarkers is limited, especially if used individually. Thus, the last years have seen an extensive research effort to investigate the IPF lung tissue, where the disease is identified. Transcriptomic analysis of lung tissue from patients with IPF and Hypersensitivity Pneumonitis showed that genes associated with epithelial development and collagen catabolism were upregulated in both diseases, while immune-response related genes were specifically upregulated in patients with Hypersensitivity Pneumonitis.[76] Genomic analysis of lung tissue from transbronchial biopsies resulted in a commercially available biomarker, denominated Envisia Genomic Classifier, with sustained accuracy and high reproducibility for the detection of histopathologic features of usual interstitial pneumonia (UIP).[77–79] Envisia Genomic Classifier might be helpful as a surrogate of histopathology that could improve the diagnostic accuracy in IPF without the need of surgical lung biopsy, if used in a multidisciplinary setting.[79] Of course, a major limitation is that identification of UIP through the Envisia Genomic classifier does not necessarily mean IPF. Association of genomic UIP with PPF might substantially increase the clinical applicability of this biomarker: yet, to this end, genomic UIP has not been associated with progression free survival or longitudinal FVC decline.[80]

Disease severity, risk stratification & outcome prediction

Estimation of disease severity and risk stratification in IPF is still based almost exclusively on functional and physiological indices such as Forced vital capacity (FVC), diffusion capacity for carbon monoxide (DLCO) and 6-min walking test (6MWT) given that computed tomographic biomarkers (deep learning algorithms, e-Lung) are in their infancy.[81–87] Composite physiologic index (CPI) and GAP (Gender, Age and Physiology) index represent two of the most widely used indexes for risk-stratification.[88,89] Significant caveats of these indices including the effect of emphysema on FVC, technical variabilities affecting DLCO and the impact of heart-related, myoskeletal disorders on 6MWD highlight the pressing need for disease specific biomarkers.[83,90–92]

Gene variations, microRNAs and telomere shortening have all been shown to predict IPF mortality (Table 1). With regards to gene variations, the same *MUC5B* polymorphism (rs35705950) that led to IPF susceptibility, was paradoxically associated with decreased mortality in IPF.[15,131] However, a recent report showed that this finding might be a source of index event bias, a phenomenon observed if subjects are selected based on disease status without accounting for other common causes of incidence and prognosis.[132] In terms of other gene variations, the presence of a functional variant of *TOLLIP*, rs5743890, was associated with reduced survival in IPF.[23,133] Similarly, the identification of a TLR3 functional variant (Leu412Phe, TLR3 L412F) in patients with IPF has also been suggested as a marker of progressive disease.[125] A recent staged genome-wide association study identified a novel variant, named PCSK6, that reached genome-wide significance.[118] PCSK6 which encodes a calcium-dependent serine endoprotease and is mainly expressed in airway epithelial cells, lymphatic endothelial cells and adventitial fibroblasts was associated with increased mortality.[118] Another recently identified variant found in an antisense RNA gene of the Rho and Rac effector protein, named protein kinase N2, PKN2, (rs115982800) demonstrated genome-wide significant association with rapid FVC decline[119] in IPF. In addition to gene variations, five microRNAs (miR-185, miR-210, miR-302c, miR-376c and miR-423-5p) were increased in IPF lung tissue of rapid compared to slow progressors.[111] In terms of peripheral blood, reduced miR-29 expression in serum and plasma was associated with increased mortality in two cohorts of patients with IPF.[110] Regarding telomere shortening, shorter telomere length was associated with increased risk of mortality in patients with IPF in independent patient cohorts.[122] Additionally, mutations in genes related to telomere maintenance (*TERT*, *TERC*, *PARN* and *RTEL1*) can be indicative of the PPF phenotype and reduced survival.[123]

In addition to genetics and epigenetic variations, changes in gene expression, particularly in peripheral blood, have also been shown to predict IPF mortality. In particular, one of the studies that provided a significant advance in precision medicine in ILD was the identification of a 52-gene signature in peripheral blood able to predict mortality in IPF in six independent cohorts.[9,130] A genomic risk scoring system denominated Scoring Algorithm for Molecular Subphenotypes (SAMS) based on this 52-gene signature was able to discriminate IPF patients into high and low-risk mortality subgroups after adjusting for clinical covariates. The combination of the 52-gene signature risk profiles and GAP index, improved substantially the prognostic accuracy of the GAP index.[9] Interestingly, this signature also predicted

Biomarker	Disease susceptibility	Diagnosis	Prognosis	Treatment response/theragnostic	References
Alpha defensins			+		56
CA 19-9			+		59
CA-125			+	+	59
CCL18			+		93
cCK18		+			60
CXCL13			+		94
ECM neoepitopes			+		95
eNose		+		+	96–98
Envisia Genomic Classifier		+			77–79
Genome-wide signals (altered gene expression of DEPTOR, KIF15, MAD1L1 etc)	+				18
KL-6/MUC1		+	+	+	47,99–105
LOXL2			+		106
MMP7		+	+		53,59,107,108
Microbiome			+		109
miR-21, miR-154, let-7d		+			67–71
miR-29		+	+		110
miR-185, miR-210, miR-302c, miR-376c and miR-423-5p			+		111
Mitochondrial DNA			+	+	112
Monocyte count			+		113–115
MUC5B	+		+		15–19
N-terminal propeptide of type VI collagen				+	116
Osteopontin		+	+		53,117
PCSK6	+	+	+		118
PKN2			+		119
S100A12			+		120,121
Surfactant proteins	+	+	+	+	45–47,53,59
Telomere length/telomerase	+		+		37,39,122,123
Thyroid hormone			+		124
TLR3			+		125
TOLLIP	+			+	23,24,126
Tregs			+		127,128
3D pulmospheres				+	129
52-gene signature			+	+	9,130

Abbreviations: cCK18, caspase-cleaved; CCL18, chemokine ligand 18; CXCL13, CXC-motif ligand 13; ECM, extracellular matrix; KL-6, Krebs von den Lungen-6; LOXL2, lysyl oxidase like-protein-2; MUC, mucin; PKN2, protein kinase 2; SP, surfactant protein; TLR, toll-like receptor; TOLLIP, toll-interacting protein.

Table 1: **Most important biomarkers studied in IPF and their potential clinical utility.**

mortality in COVID-19 suggesting the presence of a profibrotic subphenotype of COVID-19 patients with severe disease.[134] Cellular deconvolution of gene expression data not only demonstrated that monocytes are the cellular source of the high-risk profile based on the 52-gene signature but also contributed to the identification of a high monocyte count as predictor of mortality in IPF and other fibrotic disorders.[113,134] Large-scale studies have shown that increased monocyte count was linked to increased risk of disease progression, hospitalization, and mortality in IPF.[113–115]

Besides monocytes, other peripheral blood and BAL immune cells and biomarkers have been associated with IPF progression and mortality. For example, higher serum levels of chemokine (C-X-C motif) ligand 13 and chemokine ligand 18 (CCL18) were shown to predict disease progression in IPF.[93,130,135] Lower CD4 T cell counts and low expression of T-cell co-stimulatory genes are associated with decreased IPF survival.[9,130] In terms of regulatory T cells, while BAL Treg proliferative response and IL-4 release were negatively correlated with lung function, Semaphorin 7a^{+}CD4^{+}CD25^{+}FoxP3^{+} Tregs were increased in the circulation of patients with progressive disease[127,128] suggesting immune dysregulation as a mechanism associated with IPF progression. Altered microbiome has been proposed as responsible for immune dysregulation in multiple diseases and could be responsible for the changes seen in IPF.[136] For example, increased bacterial burden and specific pathogens in BAL of patients with IPF are predictive of functional decline and death in IPF.[109] This could also explain increased expression of the cationic antimicrobial peptides alpha defensins that are mainly expressed in alveolar type II cells and form an essential

element of innate immunity, which have been suggested as a marker of acute exacerbation in patients with IPF.[56] This finding further highlighted that factors associated with immune deregulation and alveolar epithelial cell injury can be both relevant to IPF pathogenesis and provide prognostic information.

Several studies have looked at the identification of peripheral blood biomarkers reflective of alveolar epithelial cell injury. The PROFILE study, a large prospective study of treatment-naive patients with IPF, focused on epithelium-derived proteins and identified four serum biomarkers (CA19-9, CA-125, MMP-7, SP-D) that had considerable prognostic potential and were suitable for replication.[59] Baseline values of SP-D and CA19-9 were higher in patients with the progressive phenotype compared to patients with stability. Increased concentrations of CA-125 over three months were predictive of mortality.[59] Increased concentrations of MMP7 were associated with worse survival.[59] The aforementioned results were in line with other studies showing the negative prognostic of higher SP-D, SP-A, MMP-7 and other metalloproteinases in IPF.[59,137–140] Higher levels of osteopontin, another protein mainly expressed in alveolar epithelial cells, might be a marker of acute exacerbations in patients with IPF.[56,117] ELISA obtained values of osteopontin, MMP-7, periostin and ICAM1 led also to a progression index able to discriminate patients with stable and progressive disease.[141] Increased KL-6, which is also a protein reflecting injury of alveolar epithelial cells type II, has been suggested as a marker of disease progression and mortality[47,99]; yet, results were not reproducible in other studies.[47,100,142] Serial measurements of KL-6 might be the key for the optimization of the prognostic value of this biomarker.[101,102] Most recently, increased KL-6 was demonstrated as a prognosticator of acute exacerbations.[100,103,104]

Investigations related with the metabolic state of alveolar epithelial cells yielded also important findings. The evidence that hypothyroidism predicted mortality in patients with IPF coupled with experimental data showing that thyroid hormone improved epithelial mitochondrial function.[124,143] Further research effort to obtain prognostic information through studying metabolic derangements showed that increased mitochondrial DNA correlated with poor survival in two cohorts of patients with IPF.[112] Studies focusing on biomarkers relevant to collagen demonstrated also interesting results. Another report analyzing data from the PROFILE study investigated longitudinal change in collagen degradation biomarkers and showed that extracellular matrix neoepitopes were associated with disease progression.[95] Higher serum levels of lysyl oxidase-like 2, a protein promoting collagen accumulation, have been associated with IPF progression[106]; yet, findings require validation. Several other non-disease specific biomarkers, including periostin, YKL-40, S100A12, αvβ6 integrin and anti-heat shock protein 70 have been suggested as prognostic markers in IPF[3,47,52,62,120,121,133,144–148] (Table 1). Further studies focusing on the implementation of these prognostic biomarkers are required to monitor disease progression, timing for lung transplant referrals and treatment decisions.

Prediction of treatment response—theragnostic biomarkers

There is still a pressing need of biomarkers able to predict and measure treatment response in IPF (Table 2). One of the first studies demonstrating the applicability of theragnostic biomarkers in IPF showed that TOLLIP CC genotype and TT genotype were associated with worst and better N-Acetylcysteine (NAC) response, respectively.[126] The main limitations of this study are 1) that genetic data were available for a small number of patients in this study and 2) that NAC is not currently FDA approved to be used as a treatment for patients with IPF. Results from the PRECISIONS trial which seeks to address whether NAC has a differential effect on lung fibrosis progression depending on TOLLIP gene variants are greatly anticipated.[154] Currently, only two drugs, nintedanib and pirfenidone are FDA approved to slow lung function decline in IPF.

In terms of theragnostic biomarkers able to measure treatment response to nintedanib and pirfenidone in IPF, the N-terminal propeptide of type VI collagen and SP-D may be adequate theragnostic biomarkers given that nintedanib significantly reduced levels of both biomarkers as early as week 4.[116] CA-125 holds potential as a marker of response to nintedanib and further data are greatly anticipated.[59] Serum SP-D might be a biomarker for pirfenidone effectiveness as well[152] as a potential pharmacodynamic biomarker, especially when measured serially.[153] Change in mitochondrial DNA following 3 months of treatment correlated with pirfenidone response after 1 year.[112] Finally, KL-6 and CCL18 were able to predict disease progression and mortality in IPF; yet, more studies are needed to address if KL-6 can be used as a theragnostic biomarker, while CCL-18 failed to predict treatment response.[105,147,155,156]

Other studies have investigated the theragnostic potential of certain biomarkers in IPF. For example, a study limited by sample size suggested that SP-A might have theragnostic potential both for nintedanib and pirfenidone.[151] Analysis of the INMARK trial with regards to the change of biomarkers following treatment with nintedanib, showed that ECM turnover biomarkers such as collagen 1 degraded by MMP (C1M), collagen 6 degraded by MMP-2/9 (C6M), collagen 5 degraded by MMP-2/9 (C5M) and C reactive protein degraded by MMP-1/8 (CRPM) might be theragnostic biomarkers; yet, further investigation is needed.[150] Importantly, a novel study suggested a novel way to simulate lung microenvironment through 3D pulmospheres, which are spheroids consisting of cells from primary lung

Biomarker	Compounds	Exact role	References
CA-125	Nintedanib	CA-125 may be a biomarker of response to nintedanib Adjusted mean CA-125 levels decreased with nintedanib vs placebo from week 4	59,149
ECM turnover biomarkers	Nintedanib	Potential as theragnostic biomarkers	150
eNose	Both antifibrotics	eNose technology may predict treatment response	96
KL-6/MUC1	Pirfenidone	Levels might correlate with pirfenidone response; further studies are needed	105
Mitochondrial DNA	Pirfenidone	Change following 3 months of treatment correlated with pirfenidone response after 1 year	112
N-terminal propeptide of type VI collagen	Nintedanib	Nintedanib reduced levels as early as week 4	116
SP-A	Both antifibrotics	Decrease baseline to 3 months and 6 months predicted outcomes at 6 months; larger studies are needed	151
SP-D	Both antifibrotics	Nintedanib reduced levels as early as week 4; may be a pharmacodynamic biomarker of pirfenidone	116,152,153
TOLLIP TT genotype	NAC	Better response in NAC	126
3D pulmospheres	Both antifibrotics	Invasiveness predicted antifibrotic responsiveness	129
52-gene signature	Both antifibrotics	Genomic risk profiles shifted their trends over time following antifibrotic treatment	9,130

Abbreviations: cCK18, caspase-cleaved; CCL18, chemokine ligand 18; CXCL13, CXC-motif ligand 13; ECM, extracellular matrix; KL-6, Krebs von den Lungen-6; LOXL2, lysyl oxidase like-protein-2; MUC, mucin; PKN2, protein kinase 2; SP, surfactant protein; TLR, toll-like receptor; TOLLIP, toll-interacting protein.

Table 2: **Biomarkers able to predict or measure treatment response in pulmonary fibrosis.**

biopsy. Quantification of 3D pulmospheres invasiveness, defined as the zone of invasion percentage, was a reliable way to assess responsiveness in antifibrotics and thus this approach holds promise for the guidance of treatment decision toward the antifibrotic that is more likely to confer benefit to each individual. A major caveat hampering the clinical applicability of that work is that pulmospheres were obtained via video-assisted thoracic surgery.[129] Forming 3D pulmospheres with tissue derived from less invasive methods such as cryobiopsy might be the key to implement such personalized medicine approaches in the future clinical practice. Recent data showed that eNose technology may predict treatment response to antifibrotics before treatment initiation.[96–98] A clinical molecular signature of CA-125, CXCL13, MMP-7, OPN and YKL-40 that predicted differential transplant free survival in untreated patients with IPF, was able to retain its prognostic accuracy (but at higher thresholds) in patients receiving antifibrotics.[94] Finally, the 52-gene signature that was predictive of mortality in the peripheral blood in IPF, showed also potential as a biomarker of treatment response, given that genomic risk profiles shifted their trends over time after initiation of antifibrotic therapy (Fig. 2).

Future perspectives and concluding remarks

During the last decade, we have witnessed a scientific explosion leading to several biomarkers and two antifibrotic compounds able to slow IPF progression (Tables 1, 2, and 3, Fig. 1). The new challenge is the translation of biomarkers that have acceptable cost and are able to provide actionable information to clinicians. A really important biomarker should alter clinicians' decision i.e. by leading to early diagnosis, providing information regarding disease activity or the need for treatment modification. This could be achieved through single biomarkers, multidimensional indexes or polygenic risk scores (scores that identify individual's risk based on the combination of different gene abnormalities associated to pulmonary fibrosis) like the one recently presented in the form of an abstract.[157]

Biomarkers that correlate with disease activity could be a major tool to identify the time to intervene. These biomarkers could have a major role for patients ILAs and mild functional impairment, given that in these cases antifibrotic treatment is sometimes delayed. Moreover, biomarkers that can lead to identification of specific endotypes will be important.[15,122,158,159] Classifying pulmonary fibrosis as high-risk genomic PF, *MUC5B*-PF or telomeropathy-PF instead of using the word "idiopathic" might be a better approach. The use of precision medicine and endotyping could pave the way to pharmacogenetic approaches and guide treatment decisions. Treating endotypes with targeted therapies based on the expression of specific biomarkers could maximize effectiveness of future therapies and concomitantly spare adverse events. Current examples of this are 1) the clinical trial testing the synthetic androgen danazol for patients with short telomeres based on previous reports that androgens can restore telomerase activity in IPF and 2) the PRECISIONS trial for NAC based on TOLLIP gene variants.[160–162] Moving from a uniform approach to a patient-centered approach is critical.[163,164] Trials of theragnostic biomarkers along with weight-based dosage of antifibrotics or trials of lower doses in patients with ILAs might confer benefit and concomitantly spare the adverse events that lead to treatment discontinuation. Studies aiming to manage symptoms, in a personalized fashion, should be strongly encouraged. For example, the extended-release form of nalbuphine, a dual-acting k opioid receptor agonist/μ opioid receptor antagonist holds promise as a compound able to reduce chronic cough in patients with IPF.[165]

Taken together, considerable progress has been made in the area of precision medicine in IPF. Nonetheless,

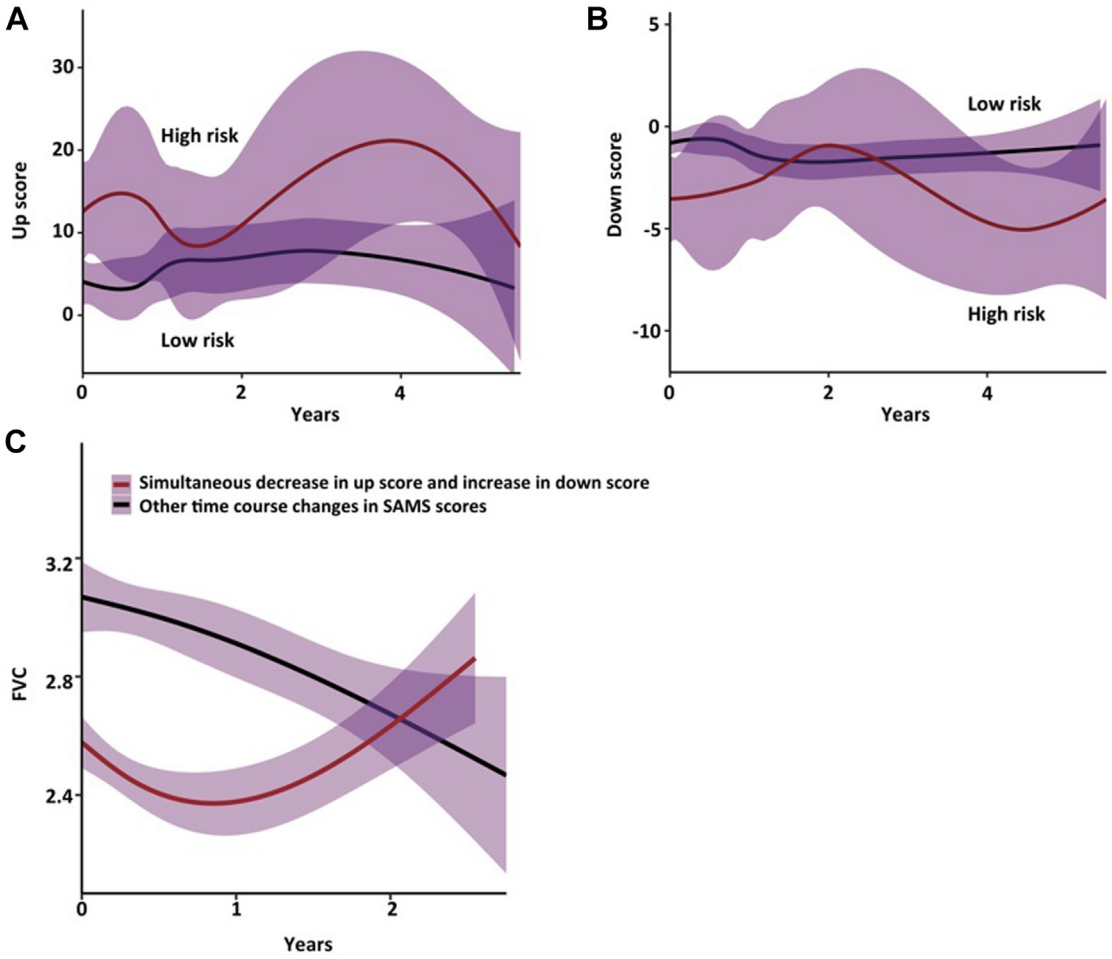

Fig. 2: 52-gene signature trends in high-risk patients with IPF shift after anti-fibrotic therapy initiation. Panel A and B show up and down scores derived from SAMS respectively. Scores shift their trends over time in high (continuous red line) vs low (continuous black line) risk groups after antifibrotic initiation. Panel C shows FVC trends of treated patients. A simultaneous reduction in up score and increase in down score is shown with black line, while other score changes are shown with red lines. (Modified from the article of Herazo-Maya et al, Lancet Respiratory Medicine 2017 with permission.)

there is a need for high-quality, implementation research to bring these biomarkers into daily clinical practice. Of course, only a substantial minority of the aforementioned biomarkers will ultimately be applied in the clinical practice. Probably these biomarkers will be cost-effective and able to provide actionable information. Future clinical trials for new compounds should focus on disease endotypes and pharmacogenetics. They should also include disease severity and theragnostic biomarkers. Such approaches will require significant investment but will lead to improvement in quality of life and better patient outcomes.

Mechanistic pathway	Biomarkers
Alveolar epithelial dysfunction	Alpha defensins, CA19-9, CA-125, CK18, KL-6/MUC1, MUC5B, PCSK6, osteopontin, SP-A, SP-C, SP-D, telomeres, telomerase
Immune dysregulation	Alpha defensins, CXCL13, CCL18, HSP70, microbiome, monocyte count, S100A12, TLR3, *TOLLIP*, Tregs, 52-gene signature
Extracellular matrix remodeling	Collagen degradation biomarkers, ECM neoepitopes, LOXL2, MMP7, PCSK6
Epigenetic markers	miR-29, let-7d, miR-21, miR-154, miR-302c, miR-423, miR-210, miR-376c, miR-185
Metabolism	Thyroid hormone, mitochondrial DNA

Abbreviations: ECM, extracellular matrix; KL-6, Krebs von den Lungen-6; MUC, mucin; SP, surfactant protein; TOLLIP, toll-interacting protein.

Table 3: **Main mechanistic pathways related to biomarkers in IPF.**

Outstanding questions

1. Should we classify fibrotic ILDs based on their endotype using terms such as *MUC5B*-PF or telomeropathy-PF instead of using the word "idiopathic"?
2. Could biomarker-based clinical trials lead to the approval of novel compounds for specific subpopulations?
3. Should we treat ILAs with lower doses of antifibrotics in order to slow disease progression and reduce the likelihood of adverse events?

Search strategy and selection criteria

Data for this Review were identified by searches of MEDLINE, PubMed and references from relevant articles using the search terms "precision medicine in IPF", "personalized medicine in IPF", "pathogenesis of pulmonary fibrosis", "biomarkers in pulmonary fibrosis", "epithelial cells in pulmonary fibrosis", "extracellular matrix", "metabolism in pulmonary fibrosis", "genetics and epigenetics in pulmonary fibrosis", "immunity in pulmonary fibrosis", "diagnostic biomarkers in pulmonary fibrosis", "prognostic biomarkers in pulmonary fibrosis" and "theragnostic biomarkers in pulmonary fibrosis". Abstracts and reports from meetings were included only when they related directly to previously published work. Only articles published in English between 2000 and 2023 were included.

Contributors

Theodoros Karampitsakos: conceptualisation, investigation, methodology, visualisation, writing—original draft.

Brenda M. Juan-Guardela: investigation, methodology, visualisation, writing—review & editing.

Argyris Tzouvelekis: conceptualisation, investigation, methodology, visualisation, project administration, supervision, writing—original draft.

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All authors approved final form and offered significant intellectual contribution.

Declaration of interests

None to declare.

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