

Review

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Review

Predicting and Treating Pulmonary Fibrosis with Proteomic Biomarker Investigations

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Abstract

Idiopathic pulmonary fibrosis (IPF) is a chronic, rare and fatal disease that is the consequence of aberrant remodeling and defective repair mechanisms within the lung culminating in the loss of alveolar integrity. Although significant progress has been made in understanding the pathogenesis, it would be crucial to identify biomarkers for diagnosis, prognosis and prediction of therapy response to improve management of this challenging and debilitating disease. Omics technologies have profoundly advanced the understanding of disease mechanisms, presenting considerable potential for the identification of clinically relevant biomarkers. To date, specific molecular pathways have been implicated in the onset and progression of idiopathic pulmonary fibrosis, including abnormal wounding, fibroblast proliferation, inflammation, deposition of extracellular matrix, oxidative stress, endoplasmic reticulum stress and coagulation system. This review highlights the role of proteomics in identifying key biomarkers for IPF, focusing on their clinical relevance, including diagnosis, prognosis, disease progression and the identification of new therapeutic options, in light of the most recent technological advancements in mass spectrometry.

Keywords: mass spectrometry; IPF; biomarkers; proteomic analysis; machine learning

1. Introduction

Idiopathic pulmonary fibrosis (IPF) represents the most common form of idiopathic interstitial pneumonia (IIP) and is characterized by the progressive distortion of the lung parenchyma structure, aberrant extracellular matrix (ECM) deposition and irreversible scarring. It is a chronic and rare disease occurring in adults over 60 years old, with a median survival of 3 years after diagnosis, which is diagnosed according to multidisciplinary clinicopathological criteria, typically displaying the so called usual interstitial pneumonia (UIP) pattern [1].

Although the cause of IPF onset is unknown by definition, several risk factors have been identified that might increase the likelihood of developing this disease [2], [3] such as environmental exposure, radiation therapy, occupational exposures (e.g., asbestosis), smoking cigarettes and exposure to allergens.

Prognosis of IPF might be unpredictable, since patients can be characterized by progressive respiratory failure, often precipitated by acute events, namely, acute exacerbations (AE)[4] Despite the introduction of drugs such as Pirfenidone and Nintedanib, which can slow respiratory functional decline, IPF has still a poor survival times (20-40% after 5-year from diagnosis).

One of the most crucial clinical needs in the diagnosis and prognosis of this disease is the search for biomarkers capable of detecting disease onset, as well as assessing progression in terms of stage and rate [5]. Nowadays, the introduction of different omics techniques which refer to high-throughput technologies that provide comprehensive data at different levels and with unprecedented high resolution, can support both biomarker discovery and the identification of molecular mechanisms of disease onset and progression [6]. Different omics techniques involve Genomics aimed to analyze the entire genome (e.g., whole genome sequencing, SNP genotyping), Transcriptomics with the study of RNA transcripts (e.g., RNA-Seq), Proteomics based on large-scale study of proteins (e.g., mass spectrometry, 2D gel electrophoresis), Metabolomics with the analysis of small-molecule metabolites, Epigenomics as the study of epigenetic modifications (e.g., DNA methylation arrays, ChIP-Seq), Glycomics with the study of glycan structures and functions, Microbiomics, which studies the microbial communities (e.g., 16S rRNA sequencing) and pharmacogenomics, which studies the individual responses to drugs. Although genomics and transcriptomics have been considered the most used techniques of the past 20 years for the molecular profiling studies, they might have significant limitations since they do not analyze changes in posttranslational modifications (PTMs), including phosphorylation and protein degradation [7]. Thus, mass spectrometry (MS)-based proteomics through the identification and quantification of amino-acid sequences allows the analysis of protein expression and modifications under different conditions that mirror the complexity at protein level [8]. Then, mass spectrometry (MS)-based proteomics represents a crucial integrative analysis of multi-omics data that will allow the discovery and functional studies of complex human diseases. This approach has enabled to enter the era of so-called precision medicine, thus centering the decision-making process to the molecular profiling of patients.

This Review aims to provide a broad overview of the current MS-based proteomics workflow and techniques that might be used to understand the mechanism underlying the onset, progression, and and AE of pulmonary fibrosis, to support personalized diagnostics and treatment. Here, we describe applications in single-cell and spatial analysis, as well as in human body fluid profiling and finally, we discuss the potential transition of proteomics from basic research to clinical practice.

2. Diagnostic, Prognostic and therapeutic challenges of IPF

IPF is diagnosed by clinicopathological criteria, including the radiographic and/or histopathological hallmark pattern of UIP [9]. Histologically, the UIP pattern is characterized by a heterogeneous phenotype with the presence of fibroblastic foci (FF) interspersed with regions of relatively preserved parenchyma, as well as areas of inflammation and honeycombing. The High-resolution computed tomography (HRCT) of IPF patients shows a peripheral, subpleural and basal

distribution pattern characterized by reticular opacities, honeycombing and architectural distortion [10]. The definition of IPF requires the exclusion of other forms of interstitial pneumonia including other idiopathic interstitial pneumonias and ILD associated with environmental exposures, medications, or systemic diseases. Since the histological pattern is not exclusively associated with IPF but with other ILDs, a proven diagnosis of IPF may represent a challenge for physicians.

The pathogenesis of IPF is complex and far from being fully understood. Much evidence suggests that IPF is a consequence of multiple interacting genetic, and environmental risk factors such as cigarette smoking, metal and silica dust, or microbial agents, leading to repetitive local micro-injuries on alveolar epithelium and vascular endothelium, that cause aberrant epithelial–fibroblast functional activity, excessive ECM deposition, aberrant tissue repair process. These pathological mechanisms involve the activation, proliferation, and differentiation of fibroblasts into myofibroblasts and the secretion of pro-fibrotic, coagulant, and inflammatory cytokines, ultimately resulting in lung architectural distortion and progressive decline in respiratory function [11], [12]. In addition, some genetic variants have also been associated with IPF onset and progression such as variants in the mucin 5B (MUC5B) gene, which play a role in bronchoalveolar epithelial function, and in the TERT and TERC genes, which are involved in the telomere length maintenance [7]. Nevertheless, IPF remains a fatal disease, and the identification of different and new biomarkers indicative of progression represents an unmet clinical need that has to be addressed.

The natural course of IPF is variable and survival can be influenced by multiple clinical factors, including frequency and severity of AE, comorbidities (e.g., lung cancer, emphysema, pulmonary hypertension), and the rate of functional decline [4].

Therapeutic options remain a clinical challenge. Two antifibrotic agents, Pirfenidone and Nintedanib, have demonstrated efficacy in slowing FVC decline and disease progression, although neither has been shown to significantly reduce mortality.

Pirfenidone has antifibrotic, anti-inflammatory, and antioxidant effects, primarily through inhibition of transforming growth factor-beta (TGF- β) signalling. Common adverse effects include photosensitivity, gastrointestinal disturbances, and nausea. Nintedanib, a non-selective tyrosine kinase inhibitor targeting PDGF, VEGF, and FGF receptors, is also associated with gastrointestinal side effects, particularly diarrhea, nausea, and vomiting [13]. As a therapeutic strategy, the two antifibrotic drugs Pirfenidone and Nintedanib can slow the decline in the respiratory functional decline of IPF patients, and according to both the real-world data and randomized controlled trials show that mortality rates and survival times in IPF are highly heterogenous [14]. Emerging therapies offer potential hope. Recently, BI 1015550 (Nerandomilast), an oral selective phosphodiesterase 4B (PDE4B) inhibitor with antifibrotic and immunomodulatory properties, demonstrated efficacy in a Phase 2 trial by stabilizing lung function over a 12 weeks period. Furthermore, a Phase 3 randomized, double-blind, placebo-controlled trial reported that Nerandomilast significantly reduced the decline in FVC over 52 weeks compared to placebo [15].

Supportive measures, including long-term oxygen therapy in cases of hypoxemia and pulmonary rehabilitation in functionally disabled individuals, are essential components of comprehensive care.

3. Identification of the optimal biomarkers through mass spectrometry

3.1. Selection criteria of biomarkers and study design

The identification of sensitive and specific biomarkers should be based on a comprehensive comparative analysis of biological samples drawn from diagnosed cases and appropriately matched healthy controls. A proper comparison is essential to distinguish pathological changes from normal physiological variation, which depends on factors such as age, sex, lifestyle, genetic background, and environmental exposure [16], [17].

Typically, the biomarkers should be associated in a unique and definite manner with the disease under consideration, to minimize false positives and, in turn, enhance the overall diagnostic

precision. An ideal biomarker should show a dynamic response both to the disease progression and therapeutic intervention [18]. In general, various biofluids and tissues, including plasma, serum, urine, bronchoalveolar lavage fluid, and tissue biopsies, provide unique information regarding biological processes that lead to disease onset and progression but differ in accessibility, invasiveness, and suitability for high-throughput analysis. For ensuring biomarker assay validity, preanalytical conditions such as sample collection process, storage, and processing must be strictly controlled to avoid degradation, contamination, or other undesirable modifications [19].

3.2. Protein analysis through different type of mass spectrometry

A robust proteomic study must be based on the accurate definition of study cohorts and meticulous design of the experimental workflow.

The selection of an appropriate analytical strategy depends on multiple factors, including the biochemical properties of the target protein, the depth of proteome coverage required, and the need for absolute versus relative quantification. Different analysis platforms are being used in mass spectrometry research in order to quantitatively determine and identify specific proteins or organic compounds. In particular, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) are two core techniques employed in proteomics on a daily basis to identify and distinguish complex mixtures of proteins [20]. Although both methods are highly sensitive and specific, they play different roles in proteomics. For example, GC-MS is particularly useful for analyzing volatile and thermally stable molecules, and while it is less commonly applied in large scale proteomics, it has been employed in complementary analyses [21] such as identifying metabolic changes through volatile organic compounds (VOCs) in exhaled breath. Plantier et al. used GC-MS to analyze VOC profiles in IPF patients, patients with different ILDs, and healthy controls. Their study identified significantly higher concentrations of acetone and other VOCs in IPF patients, suggesting metabolic alterations associated to oxidative stress and inflammation. The results demonstrated that specific VOC patterns could differentiate IPF from other respiratory diseases, highlighting their potential role as early biomarkers for disease detection and progression monitoring. These findings support the use of VOC analysis as a non-invasive diagnostic tool for IPF [22]. LC-MS is more suitable for analyzing a wider range of biomolecules, including peptides and intact proteins [23]. In proteomics, protein identification can be achieved through a bottom-up approach (Figure 1A), where peptides derived from enzymatic digestion are analyzed, or by a top-down approach (Figure 1B), where intact proteins are examined directly. This provides more detailed sequence coverage and information about post-translational modifications (PTMs) [24] [25]. Top-down proteomics typically involves separating proteins by one- or two-dimensional liquid chromatography, followed by identification using high-resolution mass spectrometry, facilitating the detection of proteoforms resulting from PTMs, genetic mutations, RNA and histone splicing, and other multisite heterogeneities.

The main limitation of the top-down proteomic approach for wider application is related to the lower solubility of intact proteins compared to their peptide counterparts [26]. In contrast, bottom-up proteomic approaches are widely applied because peptides are easier to separate and analyze by LC coupled to tandem mass spectrometry (MS/MS). Since bottom-up proteomics involves protein digestion, it handles higher sample complexity, although this complexity may require various chromatographic separations before final analysis. With bottom-up analysis, a sequence coverage equivalent to 100% of the original protein can only rarely be achieved. This may lead to incomplete or incorrect evaluation of protein isoforms and combinatorial PTMs. The data obtained from the two working approaches top-down and bottom-up are complementary and interdependent [27]. The combined approach enabled a detailed analysis of both intact proteins and peptide fragments, enhancing the identification of PTMs and isoforms.

Moreover, when the goal is to achieve a more comprehensive understanding of protein structure and metabolic activity, nuclear magnetic resonance (NMR) spectroscopy becomes an invaluable tool. NMR provides a powerful and non-destructive approach for structural elucidation and metabolic

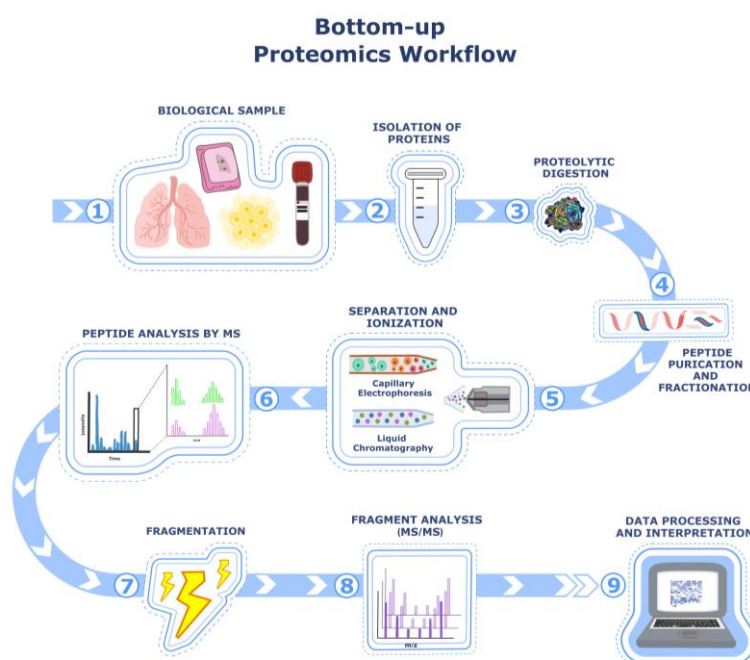
profiling. It enables detailed insights into molecular conformations and dynamic interactions without requiring extensive sample preparation.

In quantitative proteomics, isobaric tags for relative and absolute quantitation (iTRAQ) enable simultaneous comparison of multiple biological samples by labeling peptides with distinct isotopic tags, thereby allowing relative abundance measurements across experimental conditions [28]. In addition to MS methods, gel electrophoresis techniques such as two-dimensional gel electrophoresis (2D) and differential gel electrophoresis (DIGE) remain valuable for assessing differential protein expression patterns. These methods are able to resolve complex protein mixtures based on isoelectric point and molecular weight, with DIGE offering higher quantitative precision via fluorescent labeling, enabling direct comparison of protein samples on the same gel [29].

Furthermore, **high-resolution mass spectrometry (HRMS)** can employ **electrospray ionization (ESI)** or **matrix-assisted laser desorption ionization (MALDI)**. ESI is particularly suitable for analyzing proteins in solution due to its ability to generate high-quality ionized peptides with better fragmentation, while **MALDI allows for rapid analysis of complex tissue samples**, making it a valuable tool in pulmonary research [30]. **MALDI, as a diagnostic and prognostic tool**, might enable direct **spatial localization of ECM alterations** in different tissues. The ability to detect molecular changes at a histopathological level reinforces the importance of mass spectrometry-based approaches in understanding the mechanisms underlying **progressive fibrosis** and in identifying potential **biomarkers for disease stratification and therapeutic targeting**.

Thus, the growing spread of MS technique has changed the method of analysis in the proteomic field, increasing resolution, accuracy, and sensitivity. Combined use of advanced proteomic technologies, high-resolution mass spectrometry, quantitative proteomics, and bioinformatics analysis has enabled the characterization of the complex protein networks involved in rare lung diseases like IPF from different biological samples like blood serum and plasma, bronchoalveolar lavage fluid (BALF), fresh lung tissue, cells, and Formalin-fixed paraffin-embedded tissue (FFPE). In the next sections, we will summarize the biomarkers of IPF onset and progression identified through mass spectrometry from different biological samples.

A



B

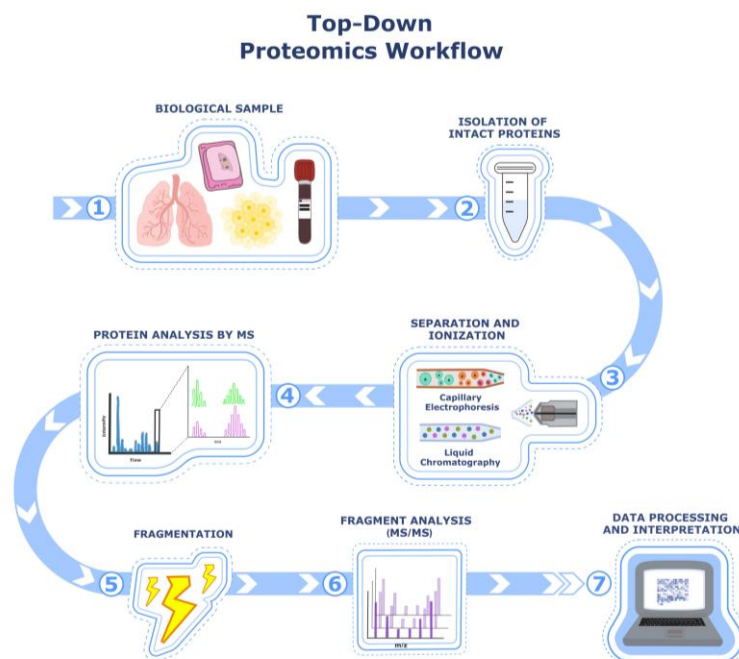


Figure 1. A Bottom-up proteomics is based on the indirect identification of proteins through their constituent peptides. The workflow starts with the acquisition of a biological sample (1) and extraction of total proteins (2), followed by enzymatic digestion, commonly with trypsin, to cleave proteins at defined sites and generate peptides suitable for MS analysis (3). Peptides are purified and, when needed, fractionated using strong cation exchange (SCX), high-pH reversed-phase LC, or other orthogonal techniques to reduce complexity (4). Separation by nanoLC or CE, and subsequent ionization by electrospray (5), enable MS1 analysis for peptide profiling and quantification (6). Selected precursor ions undergo fragmentation, typically via HCD or CID, to produce fragment ion spectra (7), which are analyzed by tandem MS (MS/MS) to determine peptide sequences (8). The resulting spectra are interpreted using database search engines and bioinformatics pipelines to identify peptides and infer the presence, abundance, and PTMs of the corresponding proteins (9). Although widely applicable and highly scalable, this approach does not preserve proteoform-level information and may obscure isoform- and PTM-specific distinctions. **B Top-down proteomics enables the direct analysis of intact proteoforms without enzymatic digestion.** The workflow begins with the collection of a biological sample (1), followed by the extraction of intact proteins under native or denaturing conditions (2). After protein separation, typically via reversed-phase liquid chromatography (RPLC) or capillary electrophoresis (CE), and electrospray ionization (3), intact protein ions are introduced into the mass spectrometer for high-resolution MS1 analysis to determine precursor masses and charge states (4). Gas-phase fragmentation techniques, such as electron capture dissociation (ECD), electron transfer dissociation (ETD), or higher-energy collisional dissociation (HCD), are then applied to generate fragment ions (5), which are analyzed by tandem MS to obtain sequence information (6). Computational tools subsequently perform spectral deconvolution, proteoform identification, and localization of post-translational modifications (PTMs) and sequence variants (7). This approach provides direct, site-resolved characterization of proteoforms, although it is currently limited by dynamic range and proteome coverage.

4. Mass spectrometry-based serum and plasma biomarkers in idiopathic pulmonary fibrosis

Since the diagnosis of IPF is often complex and invasive, identifying non-invasive markers could simplify the process and improve access to diagnosis for patients [31]. The identification of circulating biomarkers in the serum or plasma of patients affected by IPF offers noninvasive tools for diagnosis, outcome prediction, and treatment monitoring, aiming to prevent significant clinical and functional decline[32]. One of the most consistent and reproducible circulating biomarkers is matrix metalloproteinase-7 (MMP-7) [33]. MMP-7 belongs to the zinc-dependent endopeptidases and exerts

a pivotal role in degrading ECM components, such as collagens, fibronectin, and laminin [34]. The key role of the protein in fibrosis has been demonstrated in MMP-7 knockout mice, revealing a strong resistance to developing a fibrotic phenotype following intratracheal injection of bleomycin [35]. The key proteolytic activity in lung fibrosis and the elevated levels of MMP-7 in the plasma of patients affected by IPF, suggest MMP-7 as a valid marker capable to discriminate between healthy and diseased individuals and, at the same time, to enable differential diagnosis between IPF and other lung diseases such as chronic obstructive pulmonary disease (COPD), sarcoidosis and hypersensitivity pneumonitis [36].

Like MMP-7, other matrix remodeling proteins (such as MMP-1 and MMP-10) have been investigated and proposed as markers of disease [37]. Collagen production and the abnormal ECM deposition, typical events in fibrosis, are also linked to CC-chemokine 18 (CCL18), a chemokine mediating chemotactic and immunoregulatory functions. Literature reports evidence of elevated serum levels of CCL18 in patients with pulmonary fibrosis and a relationship between serum concentrations and the level of pulmonary fibrotic activity. CCL18 is associated with organ failure and, as predictor of survival and disease progression, it is positioned as a prognostic marker for IPF [38], [39]. Furthermore, high levels of periostin, involved in the remodeling of the ECM and linked to pulmonary function loss, were found in the serum of IPF patients [40]. Once secreted by fibroblast, epithelial and endothelial cells, it interacts with integrin receptor after stimulation by IL-4, IL-13, and TGF- β , sustaining fibrosis [41], [42]. Several studies have demonstrated that IPF-affected patients exhibit elevated surfactant proteins SP-A and SP-D expression profile in comparison with healthy individuals [32]. The elevated levels of these peculiar proteins, involved in maintaining tissue homeostasis and immune response in the serum of affected patients help the differential diagnosis and prognosis prediction [39]. Krebs von den Lungen-6 (KL-6) is a high-molecular-weight mucin-like glycoprotein produced by damaged and regenerating alveolar type II cells in the lungs [43]. KL-6 was proposed as a diagnostic tool for interstitial lung diseases (ILDs) and as a predictor of response to antifibrotic therapy.

In recent years, thanks to advanced protein-based techniques, new emerging blood-based biomarker panels for IPF have been proposed. For example, the new proteomic platform slow-off rate-modified aptamer (SOMAmer) SOMAscan panel was used to analyze plasma isolated from 60 IPF affected patients in 80 weeks of follow-up. The study identified a panel of six analytes associated with the disease. Specifically, disease progression was associated with sub-threshold levels of soluble vascular endothelial growth factor receptor 2, ficolin-2, legumain, and cathepsin, along with elevated levels of inducible T cell co-stimulator and trypsin-3 [44].

In addition, iTRAQ, which is able to simultaneously identify and quantify the peak intensities of reporter ions using tandem mass spectrometry (MS/MS), revealed the presence of four analytes associated with IPF. In the cohort of patients enrolled in the study, elevated levels of CRP and fibrinogen- α , along with decreased levels of haptoglobin and kininogen-1 were observed compared to healthy individuals. These biomarkers, identified through proteomic studies, provide insights into IPF pathogenesis and hold potential for improving diagnosis, prognosis, and treatment monitoring. However, further validation is needed before they can be routinely used in clinical practice [45]. Furthermore, Oldham et al. analyzed plasma samples from IPF patients using quantitative proteomics, found significant alterations in the expression of specific plasma proteins in progressive IPF compared to stable cases. Key biomarkers included increased levels of serum amyloid A1 (SAA1), haptoglobin (HP), and hemopexin (HPX), which were involved in inflammatory response, oxidative stress, and ECM remodeling—processes critical in IPF pathogenesis [46]. Blood biomarkers offer potential for diagnosing and managing IPF, but challenges such as lack of specificity and the need for standardization persist. Using biomarker panels may improve accuracy by capturing multiple disease aspects. Long-term monitoring is crucial to assess their predictive value, while integration with advanced study techniques could further enhance diagnosis and monitoring.

5. Biomarkers of IPF identified in BALF fluid through proteomics

BALF has been increasingly used to identify disease-associated protein signatures in IPF, reflecting the lung microenvironment offering insights into the local immune response and tissue remodeling processes, through minimal invasiveness compared to surgical biopsy. Advances in proteomic technologies and MS have enabled the identification of multiple proteins in BALF that are differentially expressed in IPF patients compared to healthy individuals or those with other interstitial lung diseases. Elevated levels of mediators of pulmonary fibrosis (osteopontin, MMP7, CXCL7, CCL18), eosinophil- and neutrophil-derived proteins, and proteins associated with fibroblast foci have been observed in BALF from IPF patients using gel-free quantitative proteomics (HDMSE) and targeted multiple reaction monitoring (MRM). Additionally, this study describes, for the first time, the upregulation of the pro-fibrotic cytokine, CCL24, in BALF of IPF patients [47]. Furthermore, the comparison of BALF protein profiles of IPF patients, never-smoker healthy control (nonsmoker control) and smoker control subjects using comparative two-dimensional gel electrophoresis (2D-PAGE) analysis, revealed transcriptional factors NF- κ B, PPAR γ , and c-MYC as a group of functional hubs, indicating the principal pathways involved in the progression and pathogenesis of IPF [48]. Comparative 2D-PAGE and MALDI analysis uncovered overexpressed proteins in BALF of patients with IPF, such as S100A9 compared to BALF of patients with other fibrotic diseases the importance of proteomics in identifying biomarkers that might discriminate different types of fibrotic interstitial pneumonia [49]. Then, the study of BALF protein composition from patients with IPF, sarcoidosis and pulmonary fibrosis associated with systemic sclerosis (SSc) analyzed by 2-DE showed that there were quantitative rather than qualitative differences in the protein profile between the three diseases, where IPF was characterized by a greater abundance in low molecular weight proteins compared to sarcoidosis and SSc. Additionally, cytokine-focused proteomic study further highlighted elevated levels of IL-1, IL-6, IL-8, TNF- α , IL-17A/F, alongside increased cellular counts of neutrophils, macrophages, and eosinophils in BALF of IPF patients. Furthermore, macrophage migration inhibitory factor (MIF) along with p23 and Calgranulin B, two calcium binding proteins, were also significantly increased in IPF [50]. Finally, proteomic profiling of BALF conducted using label-free quantitative methods (LFQ), through unsupervised cluster analyses identified protein expression profiles that were then analyzed to predict survival in patients with IIP, reinforcing the prognostic relevance of BALF biomarkers [51].

In summary, a wide array of BALF-derived biomarkers—identified through high-throughput proteomics and MS techniques including HDMSE, 2D-PAGE, MALDI-ToF/ToF, LC-MS/MS, and MRM—has elucidated key proteins involved in inflammation, ECM remodeling, cell signaling, and immune modulation in IPF. These findings underscore the potential of BALF proteomics to inform disease monitoring, prognostication, and development of personalized therapeutic strategies—pending validation in larger, multicenter studies.

6. Biomarkers of IPF identified in lung tissues through proteomics

Over the last few years, various attempts have been made to understand the molecular mechanism of IPF. Among them, tissue-based proteomics has had a particular impact, giving a comprehensive overview of molecular pathways active in the onset and progression of the disease. Given the rarity of the disease and the challenges associated with obtaining fresh samples such as surgical biopsies or cryobiopsies, FFPE tissue specimens from pathology archives offer a reliable and accessible alternative for proteomic investigations and biomarker discovery[52]. However, formalin fixation and paraffin embedding can affect protein quality, limiting the efficiency of proteomic analysis [53]. Despite these difficulties with FFPE samples, they have been successfully used for advanced proteomic analysis. In a study by Samarelli et al., FFPE lung tissue samples from patients with IPF were analyzed through LC-MS/MS and label-free quantitative analysis, leading to the identification of differentially expressed proteins, including those involved in the ECM signaling pathways, focal adhesion and transforming growth factor β (TGF- β) signaling pathways strongly associated with IPF onset and progression, compared with non-fibrotic lung as control samples. Moreover, five proteins were significantly overexpressed in the lung of IPF patients with either

advanced disease stage (Stage II) or impaired pulmonary function (FVC <75%, Diffusion Lung Carbon Monoxide DLCO <55%) compared to controls; these were lymphocyte cytosolic protein 1 (LCP1), peroxiredoxin-2 (PRDX2), transgelin 2 (TAGLN2), lumican (LUM) and mimecan (OGN) that might play a key role in the fibrogenic processes, representing potential biomarkers of disease progression [54]. Thus, the analysis of FFPE samples has the potential to identify key proteins that might be crucial for the IPF pathogenesis.

Several recent studies employed proteomic analysis on fresh or fresh-frozen lung tissue from IPF patients, identifying a more complex proteomic profiling compared to that from FFPE samples. Among these, **iTRAQ labeling combined with LC-MS/MS** to IPF fresh-frozen lung tissues revealed over 600 differentially expressed proteins, including both well-studied ECM components—such as **COL1A1, SCGB1A1, TAGLN, CTSB, SERPINB3**—and novel ECM-associated proteins such as **LGALS7, ASPN**, and HSP90AA1 and HSP90AB1 [55].

One large-scale quantitative proteomic study detected more than 1,500 differentially expressed proteins in IPF lungs compared to controls [56]. This study showed that although transcriptional signatures of IPF were mainly enriched in pathways associated with immune-mediated process and inflammatory responses, protein expression profiles of IPF lung tissues were related to production, deposition and remodeling of ECM. Intriguingly, two candidate proteins, BTNL9 crucial in the process of antigen presentation, and PLLP involved in epithelial cell development and differentiation, were down-regulated at the mRNA and protein levels in IPF lung tissue and bleomycin-induced mice, indicating that they have a protective effect by inhibiting ECM production and promoting wound repair in alveolar epithelial cells [56].

6.1. New insight in the biomarker's identification with spatial proteomic

Spatial proteomics has emerged recently as a powerful approach for dissecting the molecular complexity of IPF by mapping protein expression within histologically distinct regions of lung tissue. In particular, the spatially resolved proteomic studies have contributed to the identification of niche-specific biomarkers in IPF, providing novel insights into disease progression and potential region-targeted therapies.

Laser capture microdissection combined with mass spectrometry (LCM-MS) has been used to isolate specific fibrotic niches—such as fibroblast foci, fibrotic alveoli, and scarred regions—from IPF lungs. A study by Herrera et al. [57] used LCM-MS to analyze FF, adjacent mature scar, and adjacent alveoli in 6 fibrotic (UIP/IPF) specimens plus 6 nonfibrotic alveolar specimens as controls, founding that among 3000 proteins identified, the fibrotic alveoli protein signature was characterized by immune deregulation. Furthermore, FF were positive for both transforming growth factor beta 1 TGFβ1 and TGFβ3, whereas the aberrant basaloid cell lining of FF was predominantly positive for TGFβ2. A recent integrative study combining spatial proteomics with single-cell RNA sequencing (scRNA-seq) revealed that fibroblast foci are enriched in profibrotic proteins such as LTBP1 and fibronectin (FN1), confirming their active role in TGF-β signaling and ECM remodeling [58]. Furthermore, spatial proteomics of the basement membrane from distal bronchioles of IPF patients compared to control patients revealed the downregulation of cell junctional proteins, upregulation of epithelial-mesenchymal transition factors and dysregulated basement membrane matrix that induced epithelial desquamation in IPF distal bronchioles [59]. Finally, the study from Griesser et al. applied quantitative proteomics to whole-lung homogenates from an AAV-DTR mouse model of acute epithelial lung injury that might lead to IPF in patients, comparing bulk tissue data with spatially resolved proteomes from epithelial regions from the same animals. Injury induced upregulation in pathways related to interferon response, proliferation, DNA replication, and ECM deposition. Conversely, proteins associated with epithelial markers (SP-A, SP-C, Scgb1a1), cilium assembly, lipid metabolism, and redox balance were downregulated. This study based on a mouse model showed that the comparison between two proteomic methods, revealed both extensive overlap and notable differences, highlighting that while bulk proteomics is broadly informative, region-specific analyses add valuable resolution [60].

Thus, the advancement of the proteomic techniques has allowed the employment of mass spectrometry techniques for a broad range of tissue samples from FFPE to fresh or fresh-frozen lung tissue, to integrate the complex landscape of biomarkers that might be associated to the progression of IPF, identified through BALF and blood.

7. Biomarkers of IPF identified in pulmonary cell line and primary cells from IPF patients

Proteome analysis reflects functional protein expression, post-translational modifications, and secretion dynamics—especially at cellular resolution levels. Mass spectrometry-based proteomic studies of IPF lung fibroblast cell lines, LL97A and LL29, have revealed distinct biomarkers and molecular pathways implicated in fibrosis. In a label-free LC-MS/MS study comparing these IPF fibroblast cell lines with the normal lung fibroblast cell line (CCD19Lu), 80 proteins were found exclusively expressed in IPF cells, with additional 19 upregulated in LL97A and 10 in LL29. Furthermore, hub proteins identified via STRING network analysis were enriched in cell adhesion, integrin binding and hematopoietic cell lineage pathways, suggesting key roles in fibroblast activation and ECM remodeling [61].

Primary IPF patient-derived fibroblast proteomic profiling has provided detailed information on disease protein alterations that are potentially lost in non-disease or immortal models. Among these studies, comparative proteomics of IPF and systemic sclerosis (SSc) patients' primary fibroblasts, through the LC-MS on sequential extraction of cellular and ECM proteins processed with Tandem Mass Tag (TMT) reagents, revealed a shared ECM (matrisome) signature with overexpression of proteins such as PLOD2, LUM, POSTN, IGFBP5, GREM1, and SPARC [62], showing similar “fibrotic signatures” for IPF and SSc of activated myofibroblasts, mirroring the results of transcriptomic and miRNA data. Here, the matrisome proteomic profiles in IPF and SSc lung fibroblasts together with analyses of mRNA and miRNA, gave a crucial contribution to the comprehensive analysis of fibrotic signatures at both the gene and protein expression levels of IPF and SSc.

In a mouse model of IPF, LC-MS/MS and iTRAQ were applied to characterize proteomic changes in murine primary fibroblasts after fibrotic stimulation with bleomycin. In their study, Della Latta et al; identified key proteins involved in the transition from physiological to fibrotic states, including MMPs, OPN, CHI3L1, and CD44, with profound differences in soluble protein secretion and extracellular vesicle-derived factors compared to untreated fibroblasts [63].

To this purpose, in conjunction with cell and tissue-based proteomic approaches, extracellular vesicles (EVs) proteomics has also become a useful technology to study intercellular communication in IPF. Mass spectrometry-based proteomic profiling has recently been applied to IPF-derived fibroblast-secreted EVs for the identification and characterization of their protein cargo within the IPF lung microenvironment [64]. Proteomic analysis of EVs may serve as a valuable tool for both diagnostic and prognostic applications, while also reducing the need for invasive procedures and providing insight into the mechanisms driving IPF progression. The first shotgun proteomic investigation of EVs isolated from BALF of IPF patients characterized the proteome of the vesicular component of BALF. To do this, LC-MS/MS was performed on the total proteins isolated from EVs BALF compared to that of whole BALF in IPF patients, showing considerable differences between them. Analysis of protein content and protein functions evidenced that EV proteins are predominantly involved in cytoskeleton remodeling, adenosine signaling, adrenergic signaling, C-peptide signaling and lipid metabolism. Finally, their findings suggests the importance of the application of mass spectrometry as high sensitive technique for elucidating the pathogenesis of IPF analyzing the low-abundant proteins-mediated pathways. [65]. Moreover, proteomic analysis was performed through LC-MS/MS on plasma EVs in patients diagnosed with IPF, chronic hypersensitivity pneumonitis, nonspecific interstitial pneumonitis, and healthy subjects. Thus, five-protein signature was identified and validated in an independent cohort using ELISA, building a biomarker panel for differential diagnosis of IPF [66]. Finally, label-free proteomics of

bronchoalveolar lavage fluid EVs (BALF-EVs) collected from mice treated with bleomycin, identified 107 proteins enriched in fibrotic vesicles compared to that in control mice. Moreover, integrative omics analysis revealed fibroblasts as a major cellular source of BALF-EV cargo, which was enriched in secreted frizzled related protein 1 (SFRP1) that led to increased transitional cell markers, such as keratin 8, and WNT/ β -catenin signaling in primary alveolar type 2 cells. Then, Sfrp1 deficiency in fibroblast-derived EVs attenuates lung fibrosis in vivo. These findings suggest the role for **SFRP1-EVs as both biomarker of IPF and therapeutic target** [67].

7.1. New insights in biomarker discovery with single cell proteomic

Currently, several antibody-based methods for quantifying proteins in single cells have been introduced, including Cytometry by time of flight (CyTOF) [68], single-cell Western blots [69] and simultaneous multiplexed measurement of proteins in single cells using immune-assay coupled with PCR [70]. These techniques are able to quantify a few dozen of endogenous proteins recognized by specific antibodies. Of particular interest is the application of single-cell proteomics, an emerging frontier that enables protein quantification at near-single-cell resolution, aiming at a multi-layered characterization of the proteome signature compared to bulk MS. Analysis of protein content from individual cells can reveal cellular heterogeneity, providing insights into both normal and pathological physiology, but is currently limited to expression proteomics.

Single-cell proteomic is a challenging technique considering both the small quantity of protein in a single cell [7], [71], [8] and the evidence that proteomics cannot amplify the proteins quantity measured, although there is typically more than 100-fold more protein than transcript for every expressed gene. Nowadays, single-cell proteomics technology is based on advances in sample preparation, MS instrumentation and data acquisition that lead to an ultrasensitive single cell resolution. Specifically, it consists of lysis and proteolytic digestion of limited number of cells in nanoliter droplets, followed by direct loading onto the analytical column [72].

In particular, in a study using the Single Cell Proteomics by Mass Spectrometry (SCoPE) method, individually picked live cells were lysed by sonication and after the digestion with trypsin of the proteins in the lysates, the resulting peptides were labeled with TMT labels, combined and analyzed by LC-MS/MS, giving as readout about 1000 proteins for 100 analyzed tumor cells [73].

Nowadays, single-cell proteomics primarily exists as an emerging methodological technology and has not yet been directly applied to IPF tissues at single-cell resolution. However, the use of robust scRNA-seq and spatial proteomics provides highly relevant insights into the pathological mechanisms and target cell populations in IPF lungs. Then, integrating bulk tissue proteomics, single-cell platforms, EV profiling, and spatial proteomic are helping to delineate layered protein dysregulation and paracrine signaling networks that remain undetectable through transcriptomics alone. These high-resolution datasets are transforming our understanding of IPF pathogenesis from a generalized tissue response to a spatially resolved, functionally specialized process and are increasingly being leveraged to identify precision therapeutic targets.

8. AI-Powered Proteomics: A New Era in Translational Medicine

In complex and high-dimensional datasets of mass spectrometry-based proteomics, Artificial Intelligence (AI), particularly machine learning and deep learning, can improve peptide and protein identification enhancing quantification accuracy. AI-driven computational and predictive models can improve the proteomic workflows in biomarkers discovery identifying the protein expression profile that specifically discriminate between healthy and disease states in different medical fields such as cardiovascular diseases, cancer and neurodegenerative diseases [74], [75]. Deep learning can forecast experimental peptide measurements from amino acid sequences across huge proteomic datasets, resulting in higher reliability and consistency in MS workflows. Moreover, an AI technology named federated deep learning (e.g., ProCanFDL), allows the distribution of large datasets across several institutions without sharing confidential patient data [76]. AI facilitates the integration of proteomics with other omics techniques—such as genomics, transcriptomics, and metabolomics,

which is critical for identifying novel therapeutic targets and developing personalized treatment strategies [74].

AI and machine learning for analyzing mass spectrometry-derived proteomic data, have been applied in the research of IPF, for advancement in both biomarker identification and disease classification. To date, serum proteomics analyzed by DIA together with machine learning stratified IPF patients into distinct molecular subgroups associated with clinical outcomes, identifying age-related pathways and proteins such as LDHA and CCT6A. A machine-learned combinatorial biomarker panel distinguished IPF patients from healthy individuals [77]. Furthermore, plasma proteomes together with 1,600 patients with either IPF or connective tissue disease-associated interstitial lung disease employed recursive feature elimination and multiple classifiers to train classifiers such as support vector machine (SVM) and Least Absolute Shrinkage and Selection Operator (LASSO), which are suitable and reliable for high-dimensional proteomic data. This gave as outcome 37-protein classifier (PC37) that with high sensitivity and specificity were able to distinguish IPF from other ILDs [78]. Additionally, integrated analyses of lung transcriptomic and plasma proteomic supported by an AI-based analytic approach identified a set of 34 differentially expressed analytes in IPF samples compared to healthy controls. Indeed, IPF samples showed strong enrichment of chemotaxis, tumor infiltration and mast cell migration pathways and downregulated ECM degradation together with mucosal (CCL25 and CCL28) and Th2 (CCL17 and CCL22) chemokines upregulated in IPF and highly correlated within the subjects [79]. Finally, direct application of deep learning models to raw MS spectra—for proteomics—offer the potential for automated feature discovery and improved interpretability in IPF proteomic research facilitating the translation to the clinic.

9. Clinical Translation of proteomics from bench to bedside in IPF

Biological markers that might be identified with aforementioned techniques of mass spectrometry are essential elements for forecasting the course and fatality of IPF.

Biomarkers, when paired with medical evaluations, allow for the creation of predictive models that estimate individual risks related to disease progression and mortality[32]. Patients with IPF have different rates of disease progression and treatment response, making the condition inherently heterogeneous. Clinicians can use biomarkers to detect progressive phenotypes, which is essential for adjusting therapeutic interventions and disease management to each patient's needs [5]. In addition to helping with risk stratification, biomarkers may be early warning signs for acute exacerbations, a dangerous and frequently fatal side effect of IPF [4], [80]. A more precise and personalised prognosis is made possible by a multifaceted approach layered risk assessment in IPF, which integrates imaging results and omics data with clinician-based evaluations. This integrative approach can help determine whether a more conservative treatment plan or more aggressive therapeutic approaches are necessary based on individual risk profiles [81]. The effectiveness of treatment and patient standard of care in IPF can be thus improved by combining this information to create a full, tailored treatment programme according to the collected data [82].

Notwithstanding, rigorous validation is necessary for biomarkers to cross the research-to-clinical divide [83]. Research into clinical utility examines the effects of biomarkers on patient management and therapeutic decisions; confirmatory studies evaluate the efficacy and reliability of biomarkers in wider patient cohorts; initial exploratory studies identify potential biomarkers; and, lastly, biomarkers must gain legislative endorsement before being included in standard clinical protocols for the diagnosis, prognosis and treatment of IPF [84].

To guarantee uniformity and comparability across various studies and research facilities, standardized protocols for the collection and storage of breath samples must be established. For widespread clinical adoption, it is essential to develop analytical methods for measuring breath biomarkers that are sensitive, specific, and economical. Taken together, extensive and multicenter studies are necessary to prove the accuracy and consistency of respiration-based biomarkers as tools for diagnosis or prognosis [85].

To guarantee the scalability and feasibility for clinical application of biomarkers and the improvement of patient prognosis in such conditions as idiopathic pulmonary fibrosis, cooperation between academic, clinical, and industry partners is essential, as is adherence to regulatory guidelines. The use of biomarkers in IPF management appears to have a bright future with the introduction of new technologies in the fields of single-cell sequencing and proteomic, genomic profiling, and spatial transcriptomics and proteomic that will advance our knowledge of the illness and help identify new biomarkers. For IPF patients, combining several biomarkers with clinical data could significantly advance personalized medicine by revolutionizing risk assessment and treatment selection.

9.1. Current limitations to the clinical translation of proteomics

Cost remains an important issue for the widespread use of proteomics in clinical protocols and settings. Thus, it is critical to advance technologies that are both effective and economically sustainable with the implementation of biochemical protocols that will allow high-throughput analyses of several samples adapted to the demands of routine clinical practice. Another concern is related to the limited awareness and integration of mass spectrometry technologies within the clinical community, as their use has been confined to basic and preclinical research contexts rather than certified clinical diagnostic laboratories, whose validation is mandatory before their implementation in the routine patient care [86].

Thus, the transition of LC-MS techniques from research laboratories into clinical laboratories requires the alignment with Clinical Laboratory Improvement Amendments (CLIA) that implies adequate laboratory space and infrastructure, staff training, competency and quality management [87]. For this reason, a limited number of FDA-approved diagnostic assays, such as MALDI-TOF MS, are available. Nowadays, MS-based diagnostic tests and device that either measure or identify biomarkers are not currently used in clinical practice [88], whilst few biomarkers identified through mass spectrometry and validated with orthogonal assays in one or several independent cohorts, have been translated into clinical settings [89], [90]. Then, if a panel of different biomarkers identified needs to be validated, a subsequent computational model based on AI, [91] must to be employed for precise quantification with additional costs, staff training and competency.

10. Conclusion

Different MS approaches—from GC-MS analysis of volatile organic compounds in exhaled breath to innovative LC-MS/MS applications on plasma, bronchoalveolar lavage fluid, lung tissue, cell line, primary cells from lung niche and extracellular vesicles—have uncovered a broad spectrum of IPF biomarkers associated with crucial molecular pathways such as ECM remodeling, immune regulation, oxidative stress, inflammation, and fibrotic signaling. Furthermore, several key proteins such as MMPs, CCL18, periostin, and surfactant proteins, summarized in Table 1, consistently emerged across multiple sample types and studies, underscoring their potential as reliable biomarkers of disease progression and therapeutic targets. Finally, emerging spatial proteomics and single cell-based techniques have enhanced resolution at tissue and cellular levels, providing novel insights into IPF pathogenesis. Developing minimally invasive biomarker assays from accessible samples like blood or exhaled breath will facilitate early diagnosis and real-time disease monitoring.

Taken together, these findings reveal the potential of proteomic mass spectrometry to deepen our understanding of IPF for the development of diagnostic and prognostic tools along with new therapeutic target therapy. The integration of advanced mass spectrometry technologies with multi-omics data and large-scale validation studies is fundamental for translating proteomic biomarkers into clinical practice for IPF. Emerging approaches such as single-cell and spatial proteomics offer new opportunities to characterize cellular heterogeneity and microenvironmental factors driving fibrosis, potentially unveiling new therapeutic targets. Standardization of sample collection, processing, and analytical protocols across studies will be critical to ensure reproducibility and comparability of results. Additionally, interdisciplinary collaboration among researchers, clinicians,

and industry will accelerate the translation of these proteomic insights into personalized medicine frameworks, ultimately improving patient outcomes and quality of life.

Table 1. Mass Spectrometry-Based Biomarker Discovery in IPF and Related Pathways.

Mass Spectrometry Technique	Identified Biomarkers	Activated Molecular Pathway	Sample Type	Reference(s)
GC-MS	Acetone, other VOCs	Oxidative stress, inflammation	Exhaled breath (VOCs)	[23]
LC-MS/MS	MMP-7, MMP-1, MMP-10	ECM remodeling	Plasma/Serum	[34–38]
LC-MS/MS	CCL18	Inflammation, immunoregulation	Plasma/Serum	[39–40]
LC-MS/MS	Periostin	TGF- β , IL-4, IL-13 signaling	Plasma/Serum	[41–43]
LC-MS/MS	SP-A, SP-D, KL-6	Immune response, alveolar regeneration	Serum	[33, 40, 44]
SOMAscan (aptamer-based)	VEGFR2, Ficolin-2, Legumain, Cathepsin, ICOS, Trypsin-3	IPF progression (not specified)	Plasma	[45]
iTRAQ + LC-MS/MS	CRP, Fibrinogen- α , Haptoglobin, Kininogen-1	Systemic inflammation	Plasma	[46]
Quantitative proteomics	SAA1, Haptoglobin, Hemopexin	Inflammation, oxidative stress, ECM	Plasma	[47]
HDMSE, MRM, 2D-PAGE, MALDI-ToF	MMP-7, CXCL7, CCL18, S100A9, ILs, MIF, Calgranulin B, CCL24	Inflammation, ECM remodeling, cellular signaling	BALF	[48–52]
LC-MS/MS (FFPE tissue)	LCPI, PRDX2, TAGLN2, LUM, OGN	TGF- β , cell adhesion, ECM	Lung tissue (FFPE)	[55]
iTRAQ + LC-MS/MS	COL1A1, SCGB1A1, HSP90AA1/AB1, LGALS7, ASPN	ECM production and remodeling	Fresh lung tissue	[56]
LCM-MS, Spatial Proteomics	TGF- β 1/2/3, LTBP1, FN1, SFRP1	TGF- β signaling, ECM remodeling, EMT	Laser-captured lung tissue sections	[58–61]
Label-free LC-MS/MS	>80 proteins (e.g., POSTN, IGFBP5, SPARC)	Matrisome, cell adhesion, ECM signaling	Primary cell lines (fibroblasts)	[62–63]
LC-MS/MS (EVs from BALF & plasma)	SFRP1, signaling proteins, cytokines, cytoskeletal proteins	WNT/ β -catenin, cell–cell communication	EVs from BALF and plasma	[64–68]

Abbreviations

The following abbreviations are used in this manuscript:

2D Two-Dimensional

2D-PAGE Two-Dimensional Polyacrylamide Gel Electrophoresis

16S rRNA 16S Ribosomal Ribonucleic Acid

AAV DTR Adeno-Associated Virus Diphtheria Toxin Receptor

AI Artificial Intelligence

ASPN Asporin

ATS American Thoracic Society

BALF Bronchoalveolar Lavage Fluid

BALF-EVs Extracellular Vesicles Isolated from Bronchoalveolar Lavage Fluid

BTNL9 Butyrophilin Like 9

CCL CC-chemokine Ligand

CCL17, 22, 25, 28 Chemokine (C-C motif) Ligands

CCL18 Chemokine (C-C motif) Ligand 18

CCD19Lu Normal Human Lung Fibroblast Cell Line

CD44 Cluster of Differentiation 44

ChIP-Seq Chromatin Immunoprecipitation Sequencing

CLIA Clinical Laboratory Improvement Amendments

COL1A1 Collagen Type I Alpha 1 Chain

COPD Chronic Obstructive Pulmonary Disease

CRP C-Reactive Protein

CyTOF Cytometry by Time of Flight

CCT6A Chaperonin Containing TCP1 Subunit 6A
 DIA Data-Independent Acquisition
 DIGE Differential Gel Electrophoresis
 DLCO Diffusion Lung Carbon Monoxide
 ECM Extracellular Matrix
 ELISA Enzyme-Linked Immunosorbent Assay
 ERS European Respiratory Society
 ESI Electrospray Ionization
 FDA Food and Drug Administration
 FF Fibroblastic Foci
 FFPE Formalin-Fixed Paraffin-Embedded (Tissue)
 FGF Fibroblast Growth Factor
 FVC Forced Vital Capacity
 GC-MS Gas Chromatography–Mass Spectrometry
 GREM1 Gremlin 1
 HDMSE High Definition Mass Spectrometry with Data-Dependent Acquisition
 HP Haptoglobin
 HPX Hemopexin
 HRCT High-Resolution Computed Tomography
 HRMS High-Resolution Mass Spectrometry
 HSP90AA1 Heat Shock Protein 90 Alpha Family Class A Member 1
 HSP90AB1 Heat Shock Protein 90 Alpha Family Class B Member 1
 IIP Idiopathic Interstitial Pneumonia
 IGFBP5 Insulin-Like Growth Factor Binding Protein 5
 IL Interleukin
 ILD Interstitial Lung Disease
 IPF Idiopathic Pulmonary Fibrosis
 iTRAQ Isobaric Tags for Relative and Absolute Quantitation
 KL-6 Krebs von den Lungen-6
 LASSO Least Absolute Shrinkage and Selection Operator
 LC-MS Liquid Chromatography–Mass Spectrometry
 LC-MS/MS Liquid Chromatography–Tandem Mass Spectrometry
 LCP1 Lymphocyte Cytosolic Protein 1
 LDHA Lactate Dehydrogenase A
 LGALS7 Galectin-7
 LL29, LL97A IPF-derived Human Lung Fibroblast Cell Lines
 LFQ Label-Free Quantification
 LTBP1 Latent Transforming Growth Factor Beta Binding Protein 1
 LUM Lumican
 MALDI Matrix-Assisted Laser Desorption/Ionization
 MALDI-TOF MS Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry
 MALDI-ToF/ToF Matrix-Assisted Laser Desorption/Ionization Time-of-Flight/Time-of-Flight Mass Spectrometry
 MIF Macrophage Migration Inhibitory Factor
 MIMECAN (OGN) Mimecan
 MMP Matrix Metalloproteinase
 MMPs Matrix Metalloproteinases
 MS Mass Spectrometry
 MS/MS Tandem Mass Spectrometry
 MUC5B Mucin 5B

NF- κ B Nuclear Factor Kappa-light-chain-enhancer of Activated B Cells
 NMR Nuclear Magnetic Resonance
 OGN Mimecan
 OPN Osteopontin
 PC37 37-Protein Classifier
 PCR Polymerase Chain Reaction
 PDE4B Phosphodiesterase 4B
 PLOD2 Procollagen-Lysine, 2-Oxoglutarate 5-Dioxygenase 2
 POSTN Periostin
 PPAR γ Peroxisome Proliferator-Activated Receptor Gamma
 PRDX2 Peroxiredoxin-2
 ProCanFDL Proteomics Cancer Federated Deep Learning
 PTMs Post-Translational Modifications
 RNA-Seq RNA Sequencing
 RCT Randomized Controlled Trial
 SAA1 Serum Amyloid A1
 SCGB1A1 Secretoglobin Family 1A Member 1
 SCoPE Single Cell Proteomics by Mass Spectrometry
 SERPINB3 Serpin Family B Member 3
 SFRP1 Secreted Frizzled-Related Protein 1
 SNP Single Nucleotide Polymorphism
 SOMAmer Slow-Off Rate-Modified Aptamer
 SP-A Surfactant Protein A
 SP-D Surfactant Protein D
 SPARC Secreted Protein Acidic and Rich in Cysteine
 STRING Search Tool for the Retrieval of Interacting Genes/Proteins
 SSc Systemic Sclerosis
 SVM Support Vector Machine
 TAGLN2 Transgelin 2
 TERC Telomerase RNA Component
 TERT Telomerase Reverse Transcriptase
 TGF- β Transforming Growth Factor Beta
 Th2 T Helper Type 2
 TMT Tandem Mass Tag
 TNF- α Tumor Necrosis Factor Alpha
 UIP Usual Interstitial Pneumonia
 VEGF Vascular Endothelial Growth Factor
 VOCs Volatile Organic Compounds

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